Kafrelsheikh Vet. Med. J. Vol. 12 No. 1 (2014) (195-211)

EFFECT OF PROPYLENE GLYCOL IN THE DIET OF CLOSE UP COW ON SUBCLINICAL KETOSIS

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ABSTRACT

The objective of this study was to determine if use of propylene glycol in high producing dairy cows diet during the transition period, consisting of the 3 wk before and 3 wake after calving, and continued for 6 to 8 weeks post partum,could reduce the risk of SCK after calving, and to investigate the difference form use of propylene glycol on the concentrations of the different metabolities in milk and blood and to evaluate the effect of metabolities concentrations in the detection of subclinical ketosis in two groups of Holstien cows. First group have propylene glycole as feed additives in its diet second group have no feed additives in its diet.Cows were defined subclinically ketotic when their concentration of blood β-hydroxybutyrate was over 1200μmol/L. Determination ofβ-hydroxybutyrate in the milk via an enzymatic analysis or via the Ketolac strip test provided valuable results, with threshold concentrations of 70 and 100μmol/L, respectively. The simplicity of use of the Ketolac strip test makes it a valuable way to investigate subclinical ketosis.

INTRODUCTION

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows. They include the fatty liver syndrome, ketosis, oxidative stress, laminitis, mastitis, milk fever, retained placenta, metritis, and infertility ***(Oetzel, 2004; Jóźwik et al. 2012)***.

Ketosis and fatty liver are closely linked and responsible for severe economic losses in dairy farms due to declining milk yield ***(Gustafsson et al., 1993)*** and reproductive performance ***(Andersson and Emanuelson, 1985)***, decreased nonspecific immunity ***(Sartorelli et al., 2000)***, increasing culling rates and increased frequency of left displaced abomasum ***(Geishauser et al., 1997)***.

Ketosis is a common disease in high producing dairy cows. It is caused by a negative energy balance. Dairy cattle experience a remarkable shift in metabolism after calving, after which milk production typically increases so rapidly that feed intake alone cannot meet energy requirements ***(Bauman and Currie, 1980; Baird, 1982)***. As a result of negative energy balance (NEB), a high mobilization of lipids from body fat reserve and hypoglycaemia in early lactation ***(Veenhuizen et al., 1991; Djoković et al., 2007)*** are generated. The main blood indicators of lipomobilization in ruminants are beta-hydroxybutyrate (BHB), the most important and abundant ketone body, and non esterified fatty acids (NEFA) ***(Oetzel, 2004; Civelek et al., 2011; Gonzales et al., 2011)***. NEFA are preferentially and greatly accumulated as triglycerides (TG) in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes ***(Herdt et al. 1983; Sevinc et al. 2003)***.

Fatty liver infiltration and hepatocyte degeneration involve cell membrane damage and hepatocyte destruction coupled with the release of cytoplasm enzymes (aspartate transaminase (AST), gamma glutamic transferase, lactate dehydrogenase) ***(Pechova, et al., 1997; Lubojacka et al., 2005; Jóźwik et al., 2012)***. Diagnosing liver lipidosis and susceptibility to ketosis in dairy cows may include liver biopsy or echography, but a less invasive and more economical analytical method may be the measurement of blood biochemical indicators ***(Baird, 1982; Bobe et al., 2004)***.

Based on blood biochemical indicators, ketosis in cows may be diagnosed when the following values match both the clinical signs (BHB >1.2 mmol/l, glucose <2.5 mmol/l, and TG <0.12 mmol/l) and blood values of NEFA >0.7 mmol/l and AST activity above 100 IU/l, which is indicative of hepatic lipidosis ***(Sevinc et al., 1998; Oetzel, 2004; Xu et al., 2008, Gonzales et al., 2011)***.

Clinical and sub clinical ketosis both result in increased concentrations of ketone bodies in tissues and milk of the cows. Blood BHBA concentration has often been used for this detection, and several authors used a cut-off point of 1200µmol/L to discriminate between healthy cows and animals with subclinical ketosis ***(Duffield et al., 1997, 1998; Geishauser et al., 1998;Jorritsma et al., 1998)***. However, blood sampling is not easy for farmers, and determination of ketone bodies in milk can make sampling easier. Moreover, semiquantitative cow-side tests for determination of milk BHBA (MBHBA) recently became available ***(Geishauser et al.,1998)*** and can provide an immediate result. Evaluations of these cow-side tests have already been published ***(Geishauser et al., 1998; Jorritsma et al., 1998)***, but the literature does not provide extensive data for comparing concentrations of ketone bodies in milk and blood or for comparing epidemiological interest of the different ketone bodies.

It has been observed by ***Emery et al., (1964), Hooven et al., (1969), and Von Kedenburg and Mulling (1970)*** that the daily supplementation of 250 g of propylene glycol (PG) to the ration of high-yielding cows in early lactation reduced the level of ketones in blood and milk, increased blood glucose levels and improve milk yield.

Hooven et al. (1969) concluded that the addition of PG to the ration of cows during the first few weeks of lactation reduced the incidence of ketosis but failed to give any economic advantage for its use in terms of increased milk yield. In a preliminary study, ***Fisher et al. (I971a)*** demonstrated that PG exhibited antiketogenic properties that resulted in altered pro-portions of rumen volatile fatty acids, and lowered levels of ketones in the blood.

Propylene glycol is a glycogenic precursor .PG is not fermented in the rumen, but is cnverted to pyruvate, which eventually converted to glucose ***(Van soest,1994; Moore and Ishler, 1997)***. Glucose affect the rate of milk lactose synthesis, and hence milk volume, through an osmotic association ***(Kronfeld,1982)***. This research aimed to investigate the effect of propylene glycol (PG) in close up die and fresh diet on subclinical ketosis. Also, it evaluated milk parameters as a simple tool for detection of subclinical ketosis

MATERIALS AND METHODS

2.1. Animals and diets

20 Holstien cows in close up period were divided into two equal groups. The first group was the control one which received the basal diet. The other group was supplemented with propylene glycol (300 g per cow per day). The experiment and the dietary treatment lasted for 2 months post partum. Body condition scores (BCS) were recorded using the 1~5 scale according to ***Ferguson et al., (1994)***, with 1 meaning = too thin and 5 =too fatty. BCS were 3.7±0.1 in the control group and 3.65±0.16 in the treated group. All the cows were kept in tie-stall barns. Diet and the housing facilities were adapted to research purposes, with diet suited to the energy required by close up and early lactation cows. Dietary

nutrient contents for dairy cows in close up and early lactation are given in Table 1. The chemical analysis of the feed was performed according to AOAC (2010). Energy balance was calculated by NRC recommendation ***(NRC 2001)***. All cows were sampled 10 prepartium for NEFA estimation and 30 days postpartum for serum NEFA, BHBA, urea, triglycride, AST and ALT. Milk samples were collected to measure BHB and milk composition.

Table (1): Physical compostion of close up ration of both group.

Ingredients %	Control	Treated	Ingredients %	Control	Treated
Alfalfa	8.71	21.87	Salt	0.33	0.41
Corn silage	46.35	15.02	Ca soapy of FA	0.38	0.63
Sorghum	---	21.87	Yeast	0.07	0.24
Corn grains	17.98	14.74	Acid buffer	0.4	0.32
Soya bean meal (SBM)	11.88	12.33	Anti myctopxin	0.13	0.12
Wheat breane	7.51	---	Organic Zinc	---	0.08
Rice grain	---	10.66	Mg	0.49	---
Sugar beat bulb	3.68	.....	Lime stone	0.38	---
Cacl	0.98	1.22	Mineral Vit.premix	0.73	0.49

Table (2): Chemical composition of close up ration of both group.

Ingredients %	Control	Treated	Ingredients %	Control	Treated
CP	13.9	14.1	DCAD	74	-15
NDF	36.4	34.3	Na	---	0.17
FNDE	33.4	27.8	CL	---	0.92
ADF	24.7	19.2	K	---	1.28
NFC	37.6	40.3	Mg	---	0.3
TDN	64	68	Se	---	3
Ca	1.2	1.1	EE	---	---
P	0.3	0.4			

Table (3): Physical copostion of fresh cow ration.

Ingredients %	Control	Treated	Ingredients %	Control	Treated
Alfalfa	19.29	28.5	Salt	0.27	0.27
Corn silage	26.47	20.93	Ca soapy of FA	---	2.6
Corn grains	25.39	15.2	Yeast	0.06	0.28
Soya bean meal (SBM)	19.33	15.47	Acid buffer	---	0.41
Wheat breane	6.08	---	Anti myctopxin	0.15	0.14
Rice grain	---	14.26	Organic Zinc	---	0.05
Cacl	1.6	0.27	Na. bicarbonate	1.02	0.89
Mineral Vit. premix	0.34	0.4	Optgin	---	0.45

Table (4): Chemical composition of fresh cow ration.

Ingredients %	Control	Treated	Ingredients %	Control	Treated
CP	16.6	17.6	DCAD	284	274
NDF	29.6	28	Na	0.42	0.39
FNDE	20.9	22.9	CL	0.45	0.45
ADF	18.1	19	K	1.41	1.4
NFC	43.5	41.2	Mg	0.23	0.23
TDN	72	71	Se	0.21	---
Ca	0.8	1.1	EE	3.9	4.4
P	0.4	0.4			

2.2. Samples and Analyses

For each cow, coccygeal blood samples were taken before the afternoon milking into 10-ml glass tubes. These tubes were strongly stirred after blood sampling then were refrigerated at 4°C in the ice pox, and within 2 h were centrifuged at 3000 rpm for10 min. The collected sera were frozen at−30°C until analysis.

At the time of testing, sera were defrosted and the following biochemical blood components were measured by different colorimetric techniques using a spectrophotometer (Genesys ¦Thermo, USA): NEFA levels, AST and ALT were measured using a kit from Randox (Ireland), and triglycerides were measured using kits from Spinreact (Spain), glucose were measured using ACCU-CHEK ACTIVE set used in human (Germany), blood urea nitrogen and triglycride were measured using kits used for Human (Germany).

About 100 ml of milk was sampled during the beginning of the afternoon milking. A semiquantitative determination of milk BHBA concentration with the Ketolac BHB strip was performed immediately. Results were denoted according to the milk BHBA concentration (μmol/L). the results were scored according to the following table.

Milk BHBA concentration	Results
0 µmol/l - 99 µmol/l	Normal (-)
100 µmol/l - 199 µmol/l	Faire (+/-)
200 µmol/l- 499 µmol/l	Positive (+)
500 µmol/l and more	Strong positive (++)

Cow side blood BHBA testing with a hand held ketometer. Our understanding of subclinical ketosis (SCK) and the ability of veterinarians and dairy producers to diagnose ketosis has been greatly enhanced by the availability of a rapid and accurate cow side test for blood BHBA. The ketometer (Abbott Laboratories) was used to measure either whole blood BHBA or whole blood glucose. The ketone test strip contains the enzyme β-hydroxybutyrate dehydrogenase which oxidizes BHBA to acetoacetate. This reduces nicotinamide adenine dinucleotide (NAD+) to NADH. The NADH is then reoxidized to NAD+ by an electron transfer mediator molecule. The electrical current generated by this conversion is measured by the meter and is directly proportional to the BHBA concentration.

2.3. Statistical analyses

All the data were analyzed using SPSS statistical analysis software ***(version 16)***. The data were analyzed by independent samples t test. Differences detected at the 0.05 level or less were considered statistically significant.

RESULTS AND DISCUSSION

At the beginning of the experiment, the body condition score of the both groups were nearly similar (Table 5). Post-partum, although there was no significant difference between the two groups in body condition

score, the treated group had a numerical higher body condition score than the control one. ***Friggens et al. (2004)*** suggested that lipid mobilization is a natural phenomenon common to all mammals and is part of an orchestrated pattern of bodyweight change to support lactation. Propylene glycol acts to reduce adipose tissue lipolysis, which can be viewed as an orchestrated metabolic adjustment by the cow to support parturition and lactation.

Table (5): Effect of propylene glycol in close up ration on post-parturient body condition score and milk yield

Item	Control	Treated
Body condition score pre-partum[1]	3.7±0.1	3.65±0.16
Body condition score post-partum	3.2±0.06	2.8±0.09

1 scoring of cows `varies from 1-5:1, thin cows; 5, fat cows.

Milk yield of cows supplemented with propylene glycol was dramatically higher than the cows of the control group (Table 6). This is consistent with higher blood glucose level. Propylene glycol is fermented in the rumen into propionic acid. Then, it is metabolized in the liver into glucose which is the precursor of milk lactose. Increasing synthesis of milk lactose increases milk yield. Also, milk protein% was significantly higher for the treated group than the control one. This is compatible with ***Sutton, 1989*** who stated that milk protein concentration is positively correlated with the energy concentration of the diet. The low milk protein percent in the control group may be due to subclinical ketosis ***(Miettenan and Setala, 1993).*** It could be denoted that propylene glycol supplementation in close up period increases milk yield and milk protein concentration. Although there was no significant effect of propylene glycol on milk fat % and milk lactose %, the daily yield of both fat and lactose were higher in the supplemented group. There were no significant

differences in total solids, solids not fat and somatic cell count between the two groups. No studies have reported the effect of PG on cows with SCK except ***McArt et al. (2011)*** who found that early detection and treatment of SCK with propylene glycol (300 ml orally once daily until the ketosis resolved) improve milk production by about 1.5 lbs of daily milk.

Table (6): Effect of propylene glycol in close up ration on post-parturient milk composition and quality

Item	Control	Treated
Milk yield(kg/day)	31^{a} ±1.35	41^{b} ±1.35
Fat %	4.4±0.73	4.05±0.36
Protein %	1.81^{a} ±0.47	2.57^{b} ±0.14
Lactose %	3.35±0.34	3.67±0.24
Total solids %	10.4±1.04	10.98±0.15
Solids not fat %	5.98±0.39	6.94±0.38
Somatic cell count	722250±412	680750±347

Means in this raw with different letters are significantly different.

Results of the parameters of ketosis detection are shown in Table 7. Serum NEFA and blood BHBA did not show a significant difference in detection of ketosis between the two groups. Subclinical ketosis may be diagnosed when serum BHBA concentration is above 1.2 mmol/l, while clinical ketosis is associated with BHBA concentration above 2.6 mmol/l ***(Duffied 2000; Oetzel, 2004)***. NEFA concentrations > 0.40 mmol/l indicate problems with energy balance and subsequent intensive lipomobilization ***(Oetzel, 2004)***. Numerically, this indicated that the cows of the control group were suffered from subclinical ketosis. Our results showed an important significant difference in milk BHBA in which the treated group had lower level than the control one. Milk BHBA is easily detected, rapid, economic and reliable test for detection of subclinical ketosis.

Table (7): Effect of propylene glycol in close up ration on ketosis parameters 1 NEFA, non esterified free fatty acids.

Item (mmol/L)	Control	Treated
Pre-partum NEFA[1]	0.42±0.4	0.3±0.02
Post-parturient NEFA	0.41±0.07	0.34±0.03
Blood BHBA[2]	1.16±0.23	0.73±0.12
Milk BHBA	220[a] ±34	115[b] ±15

Means in this same raw with different numbers and letters are significantly different. There was significant difference pre partum NEFA, post partum NEFA, and milk BHBA.

Results of serum biochemical metabolites are shown in Table 8. Although there was non significant difference in serum triglycerides, it is consistent with serum NEFA. The treated group had low NEFA, high triglycerides levels and this is in-contrast to the control group. Also there was no significant difference in serum urea level. This indicated that the amino acid metabolism in the liver was within normal and could not be reliable in the diagnosis of subclinical ketosis. On the other hand, serum glucose level was significantly higher in the treated group and could be used as an important reliable parameter in the diagnosis of subclinical ketosis. Subclinical ketosis is diagnosed at serum glucose lower than 45mg/dl. The serum glucose result indicates that the cows of the control group were suffered from ketosis but the cows of the treated group were healthy. In our study, the results of serum glucose level, milk yield and milk protein level are consistent, in which increasing serum glucose level increases milk yield and milk protein. The use of propylene glycol to treat clinical ketosis is not novel ***(Johnson, 1954; Maplesden, 1954)***; it is known to be anti-ketogenic by increasing plasma glucose concentrations through decreased peripheral tissue glucose demand ***(Kristensen and Raun, 2007)*** and lowering NEFA and liver triglyceride levels, resulting in a decrease in plasma BHBA concentrations ***(Sauer et al., 1973; Grummer et al., 1994; Chung et al., 2009).***

Table (8): Effect of propylene glycol in close up ration on serum biochemical metabolites.

Item	Control	Treated
Urea	35.5±2.7	28.55±3.1
Triglycerides	0.84±0.45	1.37±0.33
Glucose	30^{a} ±6.1	57.8^{b} ±1.1

Means in this same raw with different letters are significantly different.

As shown in Table 9, there was no significant difference in liver enzymes (AST or ALT) between the two groups. The AST level was within the normal level in both two groups because AST activity higher than 100 IU/L is indicative of hepatic lesions **(Gonzales *et al.* 2011)** . This indicates that the hepatocytes were not injured too much to release their enzymes into the serum. This is consistent with the result of blood urea level.

Table (9): Effect of propylene glycol in close up ration on liver enzymes.

Item	Control	Treated
Aspartate aminotransferase	51±5.1	42.7±11.5
Alanine aminotransferase	94.2±6.8	83.5±6.2

Effect of propylene glycol on first estrus and services per conception are shown in Table 10. Cows supplemented with propylene glycol came in first estrus more rapidly than the cows of the control one. This may be due to the high glucose, insulin and insulin like growth factors which have a positive effect on reproductive performance. Although there was no significant difference in services per conception, the treated group had a numerical better value than the control one. It has also been postulated that the decrease in fat mobilization and hepatic ketogenesis after PG administration may have beneficial effects on reproduction ***(Nielsen and Ingvartsen, 2004)***.

Table (10): Effect of propylene glycol in close up ration on reproductive performance.

Item	Control	Treated
First estrus (days)	57.0[a] ±9	35.3[b]±2.99
Service per conception	2.6±0.51	2±0.26

Means in the same raw with different letters are significantly different.

CONCLUSIONS

Dairy cattle experience a remarkable shift in metabolism after calving, after which milk production typically increases so rapidly that feed intake alone cannot meet energy requirements. As a result of negative energy balance (NEB), a high mobilization of lipids from body fat reserve and hypoglycaemia in early lactation. So high producing dairy cow in transition period very need to substances easly digested, highly energetic, rabidly absorbed, economic, and highly converted to glucose. Results indicated that propylene glycol may be a convenient management tool for the dairy producer. Propylene glycol maintained energy related blood metabolites, reduced the occurrence of subclinical ketosis, increase milk yield, and improve cow health in the early lactation.

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