Research Article

Relationship between Dietary Energy Level, Silage Butyric Acid and Body Condition Score with Subclinical Ketosis Incidence in Dairy Cows

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Abstract | Subclinical ketosis is one of the most prevalent metabolic disorders that usually occurs in cows during the first weeks of lactation. This study investigated the relationship between energy level, body condition score and silage butyric acid with incidence of subclinical ketosis (SCK) in dairy cows during the first month of postpartum. Fifty healthy pregnant Holstein cows from 10 commercial dairy farms were studied. Whole blood β-hydroxybutyrate (BHBA) concentration equal or more than 1,400 μmol/L, at least in two successive blood samplings was considered as SCK. The mean plasma β-hydroxybutyrate concentration of SCK cows was 1,932 μmol/L whereas that for the healthy cows was 770 μmol/L. Diet nutrient was significantly affected by the farms studied. Crude protein, net energy for lactation, and non-fiber carbohydrates contents of the diets were lower, whereas that of neutral detergent fiber was higher than those recommended for lactating cows. The effect of farm on pH, lactic acid, propionic acid and lactic acid to acetic acid ratio of corn silage was significant. Effect of net energy for lactation was significant on SCK incidence. The incidence of SCK was not affected by body condition score, butyric silage, crude protein and non-fiber carbohydrates of the diets. Results show that the best way to prevent or minimize the incidence of SCK in dairy farms under commercial farming conditions as that of the present study would be to provide sufficient dietary energy to meet the needs of the cows, especially during the first month of postpartum when the SCK prevalence is normally high.

Keywords | Body condition score, Butyrate, Cow, Dietary energy, Suclinical ketosis

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | May 20, 2015; **Revised** | May 23, 2015; **Accepted** | May 24, 2015; **Published** | May 25, 2015
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Citation | Samiei A, Liang JB, Ghorbani GR, Hirooka H, Ansari-Mahyari S, Sadri H, Tufarelli V (2015). Relationship between dietary energy level, silage butyric acid and body condition score with subclinical ketosis incidence in dairy cows. Adv. Anim. Vet. Sci. 3(6): 354-361.
DOI | http://dx.doi.org/10.14737/journal.aavs/2015/3.6.354.361
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Generally, high producing dairy cows are challenged postpartum with large metabolic demands caused by the sudden increase in energy requirements due to the start of the lactation, which cannot be met by feed intake alone. It has been reported that the requirements for glucose and metabolizable energy increased two- to three-fold from 21 d before to 21 d after parturition (Drackley, 2001). As a result, during early postpartum, high producing cows, experience a negative energy balance (NEB) because of low energy intake relative to energy required for maintenance and milk production. In addition, because milk yield has been increased substantially in dairy breeds through improved nutrition, management, and genetic selection, an exacerbation of NEB is very likely to take place during this period

(Nocek, 1997). Cows mobilize body fat to compensate for this energy deficit. The extensive body fat mobilization and high energy demands predispose them to fatty liver and ketosis because of an inability to dispose of fatty acids via β-oxidation or the limited capacity to export triacylglycerides (TAG) in the form of very low density lipoproteins (VLDL) from liver (Bell, 1995). Ketosis is associated with an increased incidence of transition cow diseases, and reduced productive and reproductive performance (McLaren et al., 2006; Ospina et al., 2010; Chapinal et al., 2012; Dubuc et al., 2012).

On the other hand, excess body condition during close-up (4 weeks before calving) increases losses in body weight (BW) and body condition during lactation and decreases dry matter intake (DMI) and milk production (Treacher et al., 1986). In addition, obesity at calving contributes to the development of metabolic diseases such as fat cow syndrome, mastitis, and fatty liver. Excess body condition score (BCS) prior to calving is an important risk factor for subsequent development of subclinical ketosis (SCK) during lactation (Duffield et al., 1999). Gillund et al. (2001) reported that cows with BCS ≥ 4.0 were at the highest risk, and had the highest β-hydroxybutyrate (BHBA) concentrations in plasma as compared to healthy and underweight cows prior to calving. In other word, the ketotic cows had higher BCS at calving and during the first weeks postpartum than the healthy cows, and significantly lost more body condition score over a prolonged period of time compared to the non-diseased cows (Gillund et al., 2001). Recently, Akbar et al. (2015) reported that cows with high BCS at calving had greater concentrations of BHBA and hepatic expression of genes related to ketogenesis postpartum compared to cows with low BCS at calving.

Some herds have persistent ketosis problems caused by feeding ketogenic silages (Tveit et al., 1992). Hay crop silages chopped too wet (insufficient wilting time or direct-cut silages) or those low in water-soluble carbohydrates favour the growth of *Clostridium sp.* bacteria. *Clostridium sp.* bacteria convert sugars or lactic acid to butyric acid. Butyric acid concentrations of more than 0.1% of DM in corn silage could result in its loss of DM and energy (51% and 18%, respectively) (Muck, 2006). Oetzel (2007) reviewed the risk factors causing ketosis including silage butyric acid and suggested that daily doses of over 50 to 100 g of butyric acid can result in ketosis, and doses over 200 g of butyric acid may induce severe ketosis. He also concluded that about 450 to 950 g of butyric acid constantly induced severe ketosis in nearly any early lactation cows.

Butyric silage risk increased in areas with high rainfall because high moisture promotes the growth of clostridium bacteria in silage. In the previous study (Samiei et al., 2013), we investigated prevalence of ketosis among 1,002 Iranian Holstein cows from days 5 to 50 post calving in various parity and lactation stages in 13 regions of Iran. The results showed that prevalence of ketosis was higher in Golestan province, Shahrekord and Sari than the other regions. Golestan province is located near the Caspian Sea with high rainfall (between 560 to 680 mm) and thus the high incidence of keosis reported previously (Samiei et al., 2013) in this province may be because cows are exposed to high incidence of butyric silage feeding, and to the poor nutritional management of the local farmers.

Therefore, the aim of this study was to investigate the relationship between energy level, BCS and butyric silage with SCK in dairy cows during the first month after calving in selected farms in the Golestan Province, Iran.

MATERIALS AND METHODS

Experimental Design

Fifty healthy pregnant Iranian Holstein cows were selected from 10 commercial dairy farms (average herd size was about 100 cows/farm) in Golestan province of Iran (Table 1). The cows were selected based on the computer database and the advice of the veterinarians of the respective farms. The following criteria were taken into consideration for the selection of the cows: (i) health status (including free of laminitis, milk fever, acidosis and fatty liver), (ii) cows between third and sixth parities, (iii) milk yield of above the average yield of the farm (7,650 kg/cow for 305 days lactation), and (iv) body weights of between 534 to 808 kg and BCS of between 3.25 to 3.75.

Table 1: Average milk yield, body condition score (BCS), body weight (BW), and parity in the farms studied

Farm	Milk yield[1]	BCS[2]	BW[3]	Parity
1	10,065	3.40	716	3.8
2	10,522	3.65	790	4.0
3	11,000	3.90	667	4.0
4	9,800	3.42	729	4.4
5	10,500	3.05	633	4.0
6	9,607	3.25	664	4.4
7	10,300	3.35	638	4.0
8	11,100	3.50	657	3.6
9	10,600	4.25	740	3.6
10	9,950	3.95	704	4.4

[1] Average milk yield during 305 days; [2] Average BCS at calving; [3] Average BW at calving

Blood Sampling and Analysis

Blood samples (about 10 mL) were collected from coccygeal vein in EDTA coated tubes on 3, 7, 10, 14, 17, 21, 24 and 28 day in milk and were immediately transported to the laboratory in a cooler box with ice packs and processed within 1 h after collection. Blood samples were centrifuged at 2,000 × g for 20 min and the plasma was stored at -20°C for later analysis. Blood samples were analyzed for BHBA using Ranbut D-3-hydroxybutyrate kit on the JENMAY Spectrophotometer (6105 UV) analyzer. Plasma BHBA was measured during the first milking month and those with plasma BHBA concentration of ≥ 1400 µmol/L, at least in two successive blood samplings (3 d interval), were considered subclinically ketotic (Oetzel, 2004; McArt et al., 2012).

Corn Silage and Total Mixed Ration (TMR) Sampling

The corn forage variety used was hybrid-704. Corn forage was cultivated in the month May and harvested in the month September. Generally, Iranian farmers harvest corn forage with a high moisture content (DM less than 26%) which is lower than the preferred 30 to 35% DM to achieve 95% DM in the final silage (Kung and Shaver, 2001). Corn silage was sampled in five replicates from each of the 10 farms. Within farm, corn silage was taken from 10 to 15 locations of the respective silage bunker, mixed thoroughly and a sub-sample of 0.5 kg was transferred to the laboratory at the Animal Science Research Institute, Tehran, Iran and stored at -20°C pending chemical analysis including pH and fatty acids profile. Fatty acids were measured by a Gas Chromatography (GC, Agilent Technologies, 6890N, Abbott Co. USA).

In this study, the TMR included barley grain, corn grain, soybean meal, canola meal, beet sugar pulp, wheat barn, vitamin and mineral premix, salt, NaHCO3, alfalfa hay, corn silage and wheat straw. The TMR samples were taken in eight replicates from each farm. Each replicate was taken from 10 locations from the distributed feed in the feed-bunk of fresh cows, mixed thoroughly and 0.5 kg was transferred to the laboratory and stored at -20°C for further analysis. Nutrient contents of the TMR were determined at the Animal Science Research Centre of Iran. Dry matter was analyzed by drying the samples in a conventional oven at 55°C for 24 h, ash by igniting the samples in duplicate at 600°C for 2 h in a muffle furnace (Method 942.05; AOAC, 1995), ether extract (EE, Method 920.39; AOAC, 1995), N (Method 984.13; AOAC, 1995), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Analysis of Ca and P were conducted using a Thermo Jarrell Ash IRIS Advantage inductively coupled with a plasma radial spectrometer (Model ICAP 61, Thermo Jarrell Ash, Ithaca, USA). Net energy for lactation (NE_L) and NFC of TMR were calculated using the following equations (NRC, 2001):

$$NE_L = [0.866 - (0.0077 \times ADF)] \times 2.2$$
$$NFC = 100 - (\%\ CP + \%\ EE + \%\ Ash + \%\ NDF)$$

NE_L and NFC of corn silage were calculated using the following formulas (NRC, 2001):

$$NE_L = [1.044 \times (0.0132 \times ADF)] \times 2.2$$
$$NFC = 100 - (\%CP + \%EE + \%Ash + \%NDF)$$

Determination of BW and BCS

Body condition score of the selected cows was determined using the scale of 1 to 5 (NRC, 2001). On a five-point scale, a score of 1 denotes a very thin cow, while 5 denotes an excessively fat cow. At the close-up period as well as on days 3, 14 and 28 postpartum BW was estimated using a Dalton meter that measured around chest and calculated BW.

Statistical Analysis

Statistical analysis was conducted using SAS (2003) to assess the data. Dependent variables (BCS and BW) were analyzed to examine the farm effect as a fixed independent variable. As observations were repeated, the farm effect on dependent variables was also evaluated using repeated measures analysis. Correlation coefficients between BCS before calving and 3, 14 and 28 days postpartum were calculated. In order to find the effective factors on the incidence of ketosis, other parameters such as NEL, CP, NFC, BCS, milk yield and silage butyric acid concentration were considered in the study as independent factors. The models were submitted using GENMODE procedure in SAS (2003). One-way completely randomized design was used to examine the farm effect on DM, pH, lactic acid, propionic acid, butyric acid and lactic acid to acetic acid ratio of silage.

RESULTS

The overall mean plasma BHBA concentration, sampled on 3, 7, 10, 14, 17, 21, 24 and 28 day in milk was 1,234 µmol/L. Fifty eight percent (58%) of the cows which had plasma BHBA concentration ≥ 1,400 µmol/L, at least in two successive blood samplings (3 d interval), were considered as suffering from SCK. The mean plasma BHBA concentration of SCK cows was 1,932 µmol/L while that for the healthy cows was 770 µmol/L.

Mean DM and nutrient contents of TMR in the ten farms are shown in Table 2. The nutrient contents of the TMR used were different among the farms. Overall, the above values suggested that CP, NE_L, NFC contents of the TMR were lower while that of NDF was higher than those recommended for lactating cows (NRC, 2001). The average DM in corn silage was less than 30% (ranged from 22 to 28%). Crude protein of corn silage ranged from 7 to 8% while the NDF content was higher than 50%. The NE_L was often less than 1 Mcal/kg. Mean NFC of corn silage in the 10 dairy farms studied was 28% DM (Table 3). The average pH of the corn silage ranged from 3.50 to 4.05 (Table 4).

Table 2: Dry matter and chemical composition (on DM basis) of total mixed ration in the farms studied

Item[1]	Dairy farms									
	1	2	3	4	5	6	7	8	9	10
DM	57.0 ± 5.54^{b}	49.8 ± 2.12^{bc}	54.3 ± 2.90^{b}	52.6 ± 0.75^{bc}	52.0 ± 1.73^{bc}	54.7 ± 0.79^{b}	87.3 ± 1.95^{a}	45.1 ± 1.25^{c}	49.3 ± 1.14^{bc}	56.0 ± 0.91^{b}
CP	14.9 ± 0.58^{a}	13.9 ± 0.36^{ab}	11.6 ± 0.58^{cd}	13.9 ± 0.29^{ab}	14.6 ± 0.62^{ab}	13.0 ± 0.49^{bc}	15.5 ± 0.95^{a}	13.2 ± 0.28^{bc}	10.9 ± 0.24^{d}	15.3 ± 0.16^{a}
NE_L	1.58 ± 0.02^{ab}	1.50 ± 0.04^{bc}	1.47 ± 0.06^{c}	1.53 ± 0.02^{abc}	1.53 ± 0.06^{abc}	1.56 ± 0.01^{abc}	1.47 ± 0.03^{c}	1.50 ± 0.09^{bc}	1.48 ± 0.01^{c}	1.59 ± 0.06^{a}
NDF	44.4 ± 1.83^{a}	44.9 ± 2.33^{a}	48.9 ± 2.88^{a}	44.7 ± 2.05^{a}	45.2 ± 0.41^{a}	44.1 ± 0.8^{a}	43.4 ± 2.64^{a}	47.9 ± 0.65^{a}	45.5 ± 2.03^{a}	35.2 ± 0.51^{b}
ADF	29.1 ± 1.71^{bc}	23.3 ± 2.57^{abc}	25.5 ± 3.89^{a}	21.8 ± 1.48^{abc}	21.8 ± 0.39^{abc}	20.4 ± 0.62^{abc}	25.2 ± 2.17^{a}	23.8 ± 0.55^{ab}	25.2 ± 0.69^{a}	18.3 ± 0.30^{c}
NFC	30.5 ± 2.31^{b}	30.0 ± 2.39^{b}	32.4 ± 3.66^{b}	31.5 ± 1.95^{b}	30.5 ± 1.09^{b}	35.8 ± 1.08^{ab}	30.8 ± 2.35^{b}	29.7 ± 0.78^{b}	34.8 ± 1.91^{b}	41.2 ± 0.52^{a}
EE	2.84 ± 0.52^{a}	2.5 7± 0.31^{a}	1.36 ± 0.29^{cd}	1.11 ± 0.09^{d}	1.51 ± 0.11^{cd}	1.25 ± 0.08^{d}	2.22 ± 0.18^{abc}	2.22 ± 0.35^{abc}	1.61 ± 0.19^{bcd}	2.42 ± 0.09^{ab}
Ash	7.31 ± 0.31^{bc}	8.53 ± 0.73^{ab}	5.76 ± 0.61^{d}	8.88 ± 0.26^{a}	8.18 ± 0.53^{ab}	5.88 ± 0.13^{cd}	8.06 ± 0.26^{ab}	7.03 ± 0.21^{bcd}	7.30 ± 0.34^{bc}	5.94 ± 0.18^{cd}
Ca	0.72 ± 0.12^{ab}	0.65 ± 0.05^{bc}	0.44 ± 0.08^{c}	0.80 ± 0.08^{ab}	0.82 ± 0.05^{ab}	0.76 ± 0.06^{ab}	0.76 ± 0.03^{ab}	0.73 ± 0.04^{ab}	0.80 ± 0.02^{ab}	0.91 ± 0.03^{a}
P	0.45 ± 0.03^{ab}	0.30 ± 0.03^{cd}	0.25 ± 0.03^{d}	0.32 ± 0.03^{cd}	0.35 ± 0.03^{cd}	0.37 ± 0.01^{bc}	0.31 ± 0.038^{cd}	0.31 ± 0.02^{cd}	0.38 ± 0.02^{abc}	0.47 ± 0.01^{a}

[1]**DM** = dry matter; **CP** = crude protein; **NE_L** = net energy for lactation, Mcal/kg; **NDF** = neutral detergent fiber; **ADF** = acid detergent fiber; **NFC** = non-fiber carbohydrates; **EE** = ether extract; $^{a-d}$ Different superscripts within a row indicate statistical significance at $P < 0.05$.

Table 3: Dry matter and chemical composition of corn silage in the farms studied

Item[1]	Dairy farms									
	1	2	3	4	5	6	7	8	9	10
DM	23.2 ± 1.35^{b}	22.6 ± 0.72^{b}	28.9 ± 0.50^{a}	24.1 ± 0.8^{b}	28.1 ± 2.30^{a}	28.0 ± 0.33^{a}	25.6 ± 1.15^{ab}	24.7 ± 0.67^{ab}	25.7 ± 0.67^{ab}	28.6 ± 0.40^{a}
CP	7.86 ± 0.21^{abc}	7.56 ± 0.19^{abc}	6.92 ± 0.10^{c}	7.78 ± 0.19^{abc}	7.86 ± 0.36^{abc}	8.25 ± 0.07^{ab}	7.37 ± 0.16^{bc}	7.47 ± 0.16^{bc}	8.53 ± 0.58^{a}	7.86 ± 0.18^{abc}
NE_L	0.97 ± 0.02^{ab}	0.91 ± 0.01^{abcd}	0.83 ± 0.02^{cd}	1.0 2 ± 0.08^{a}	0.82 ± 0.04^{d}	0.85 ± 0.01^{bcd}	0.96 ± 0.09^{abc}	0.94 ± 0.09^{abc}	0.92 ± 0.03^{abcd}	0.91 ± 0.02^{abcd}
NDF	58.3 ± 0.68^{ab}	51.6 ± 3.02^{b}	54.1 ± 1.10^{ab}	62.1 ± 3.24^{a}	51.9 ± 1.8^{b}	53.1 ± 0.93^{ab}	54.3 ± 5.30^{ab}	53.3 ± 5.30^{ab}	56.5 ± 0.43^{ab}	56.0 ± 0.48^{ab}
ADF	32.2 ± 0.75^{ab}	30.3 ± 0.47^{abcd}	27.7 ± 0.9^{cd}	34.0 ± 2.74^{a}	27.3 ± 1.5^{d}	28.1 ± 0.30^{bcd}	31.8 ± 0.30^{abc}	30.8 ± 0.30^{abc}	30.5 ±1.16^{abcd}	30.1 ± 0.69^{abcd}
NFC	24.1 ± 0.59^{c}	33.7 ± 2.89^{a}	30.6 ± 0.89^{ab}	18.4 ± 3.10^{c}	32.3 ± 2.32^{ab}	31.7 ± 0.90^{ab}	29.2 ± 4.99^{ab}	26.3 ±0.50^{abc}	26.4 ± 0.55^{abc}	27.4 ± 0.09^{ab}
EE	1.47 ± 0.22	1.82 ± 0.10	1.43 ± 0.04	1.74 ± 0.21	1.63 ± 0.02	1.45 ± 0.05	1.45 ± 0.15	1.54 ± 0.10	1.58 ± 0.10	1.67 ± 0.07
Ash	8.15 ± 0.44^{ab}	5.32 ± 0.33^{b}	7.00 ± 0.36^{b}	10.1 ± 2.07^{a}	6.32 ± 0.39^{b}	5.53 ± 0.12^{b}	7.77 ± 0.29^{ab}	7.67 ± 0.29^{ab}	7.02 ± 0.42^{b}	7.06 ± 0.37^{b}
Ca	1.30 ± 0.14^{a}	0.78 ± 0.10^{b}	0.90 ± 0.11^{ab}	0.98 ± 0.17^{ab}	0.95 ± 0.04^{ab}	1.27 ± 0.24^{a}	1.19 ± 0.12^{ab}	0.86 ± 0.05^{b}	0.83 ± 0.06^{b}	0.89 ± 0.06^{ab}
P	0.18 ± 0.008^{dc}	0.14 ± 0.007^{d}	0.20 ± 0.01^{bc}	0.25 ± 0.01^{ab}	0.22 ± 0.008^{abc}	0.26 ± 0.04^{a}	0.20 ± 0.008^{bc}	0.22 ± 0.01^{abc}	0.23 ± 0.01^{abc}	0.24 ± 0.005^{ab}

[1]**DM** = dry matter; **CP** = crude protein; **NE_L** = net energy for lactation, Mcal/kg; **NDF** = neutral detergent fiber; **ADF** = acid detergent fiber; **NFC** = non-fiber carbohydrates; **EE** = ether extract; $^{a-d}$ Different superscripts within a row indicate statistical significance at $P < 0.05$.

Table 4: Volatile fatty acids profile of corn silages in the farms studied

Item	Dairy farms									
	1	2	3	4	5	6	7	8	9	10
pH	3.71 ± 0.01[b]	3.57 ± 0.03[b]	3.55 ± 0.03[b]	4.04 ± 0.10[a]	3.80 ± 0.05[ab]	3.78 ± 0.05[ab]	3.67 ± 0.05[b]	3.69 ± 0.09[b]	3.74 ± 0.04[b]	3.56 ± 0.03[b]
Lactic acid (L)	3.68 ± 0.36[bc]	3.11 ± 0.31[c]	8.06 ± 2.60[a]	6.38 ± 0.98[ab]	4.01 ± 0.57[bc]	3.67 ± 0.50[bc]	4.33 ± 0.47[bc]	3.78 ± 0.35[bc]	5.04 ± 0.47[bc]	8.70 ± 2.22[a]
Acetic acid (A)	4.09 ± 0.67	3.70 ± 0.27	3.86 ± 0.29	3.45 ± 0.26	4.39 ± 1.08	4.05 ± 0.31	3.52 ± 0.37	3.78 ± 0.40	4.72 ± 0.10	3.68 ± 0.45
Propionic acid	0.38 ± 0.02[cd]	0.56 ± 0.09[bc]	1.47 ± 0.23[a]	0.26 ± 0.01[cd]	0.23 ± 0.05[d]	-	0.82 ± 0.16[b]	0.72 ± 0.16[b]	0.34 ± 0.02[cd]	1.45 ± 0.2[a]
Butyric acid	-	0.38 ± 0.07[b]	-	1.01 ± 0.31[a]	0.53 ± 0.09[ab]	-	-	0.95 ± 0.07[ab]	-	-
L:A[1]	1.02 ± 0.1[b]	0.85 ± 0.1[b]	2.07 ± 0.59[a]	1.89 ± 0.32[a]	0.97 ± 0.06[b]	0.94 ± 15[b]	1.23 ± 0.15[b]	1.03 ± 0.12[b]	1.06 ± 0.08[b]	2.30 ± 0.32[a]

[1]Lactic acid to acetic acid ratio; [a-d] Different superscripts within a row indicate statistical significance at P < 0.05

Table 5: Spearman's correlation coefficients between fatty acids profile of corn silages

	pH	DM	Lactic acid (L)	Acetic acid (A)	Pro-pionic acid	Bu-tyric acid	L :A[1]
pH	1	-	-	-	-	-	-
DM	-0.20	1	-	-	-	-	-
L	-0.02	0.22	1	-	-	-	-
A	0.02	-0.19	0.09	1	-	-	-
P	-0.28	0.33*	0.45**	0.12	1	-	-
B	0.47*	-0.02	0.06	0.03	0.05	1	-
L:A	0.07	0.27	0.88**	0.33	0.44**	0.96	1

[1]Lactic acid to acetic acid ratio; *Significant at P < 0.05; **Significant at P < 0.01

Table 6: Body condition score (BCS) of cows during postpartum period in the farms studied

Farms	Day 1	Day 3	Day 14	Day 28
1	3.35 ± 0.38[a]	3.10 ± 0.38[ab]	2.70 ± 0.33[bc]	2.35 ± 0.29[c]
2	3.05 ± 0.21[a]	2.80 ± 0.21[ab]	2.55 ± 0.21[bc]	2.40 ± 0.14[c]
3	3.65 ± 0.34[a]	3.40 ± 0.34[ab]	3.15 ± 0.34[ab]	2.90 ± 0.42[b]
4	3.70 ± 0.37[a]	3.45 ± 0.37[a]	2.85 ± 0.22[b]	2.25 ± 0.25[c]
5	3.50 ± 0.73	3.25 ± 0.73	2.95 ± 0.78	2.65 ± 0.74
6	3.40 ± 0.38[a]	3.25 ± 0.38[ab]	2.75 ± 0.31[c]	2.50 ± 0.25[c]
7	3.25 ± 0.35[a]	3.05 ± 0.33[ab]	2.70 ± 0.33[bc]	2.40 ± 0.29[c]
8	3.90 ± 0.22[a]	3.65 ± 0.22[a]	3.25 ± 0.25[b]	2.90 ± 0.42[b]
9	4.25 ± 0.25[a]	4.00 ± 0.25[a]	3.50 ± 0.18[b]	3.10 ± 0.29[c]
10	3.90 ± 0.22[a]	3.70 ± 0.21[a]	3.40 ± 0.14[b]	3.25 ± 0.18[b]

[a-c] Different superscripts within a row indicate statistical significance at P < 0.05

Only one farm had silage lactic acid concentration of more than 6%, and the silage acetic acid content in all the farms was more than 3%. Propionic acid concentration in all the silage samples studied was more than 0.25%. In four farms, silage butyric acid concentration was higher than that of normal corn silage (less than 0.1%). The ratio of lactic acid to acetic acid in silage was often less than 2 (Table 4). Butyric acid concentration had a significant positive correlation with silage pH in the corn silage ($p<0.05$; Table 5). In addition, there was a significant correlation between propionic acid and lactic acid concentrations in corn silage ($p<0.01$). A significant correlation was observed between lactic acid to acetic acid ratio with propionic acid and lactic acid values in the corn silage ($p<0.01$). Mean BCS at calving ranged from 3.25 to 4.25 (Table 6). The minimum and maximum BCS before calving were 2.75 and 4.5, respectively. The BCS declined at least 0.6 unit and at most, 1.16 unit during the first month after calving. The mean change in BCS during one month after calving was more than 0.5 unit in all the farms studied. The average BW loss ranged from 37 to 83 kg during the same period. Body condition score at calving had a high correlation with BCS during 3, 14 and 28 days post calving ($p<0.01$) (Table 7). In addition, BCS on days 3, 14 and 28 after calving were also significantly correlated ($p<0.01$).

Table 7: Spearman's Correlation coefficients for BCS at calving and 3, 14 and 28 days in milk

	At calving	d 3	d 14	d 28
At Calving	1	-	-	-
d 3	0.87**	1	-	-
d 14	0.77**	0.92**	1	-
28	0.71**	0.81**	0.90**	1

*Significant at P < 0.05; **Significant at P < 0.01

The averaged NE_L, BCS at calving, silage butyric acid, CP, NFC and milk yield were 1.48 Mcal/kg, 3.65, 0.34% DM, 13.43% DM, 30.55% DM and 30.03 kg/d in SCK cows. On the other hand, the above values for healthy cows were 1.57 Mcal/kg, 3.5, 0.21% DM, 14.02% DM, 34.05% DM and 33.71 kg/d, respectively. Effect of NE_L was significant

(p<0.05) on SCK incidence, while BCS, butyric silage, CP and NFC had no significant effect on SCK incidence (Table 8). The effects of milk yield tended to be significant for incidence of SCK (Table 8).

Table 8: Effect of silage butyric acid, net energy for lactation (NE_L), body condition score (BCS), crude protein (CP), non-fiber carbohydrates (NFC), and milk yield of subclinical ketotic cows

Parameter	Estimate	Standard Error	Chi-Square
Intercept	-32.5690	12.6824	6.59*
Butyric silage	0.7481	0.7481	0.78^{ns}
NE_L	21.9917	9.3095	5.58*
BCS	-0.6073	0.8483	0.51^{ns}
CP	-0.1773	0.2117	0.70^{ns}
NFC	-0.0493	0.1045	0.22^{ns}
Milk yield	0.1357	0.0796	2.90^{ns}

*Significant at P < 0.05

DISCUSSION

Postpartum high-yielding dairy cows are typically in a state of negative energy balance (NEB) because the amount of energy required for maintenance of body tissue functions and milk production exceeds the amount of energy the cows can consume. The degree of NEB in the early postpartum period and the recovery rate are critical for both, the health status and productivity of cows. Insufficient energy supply postpartum may result in a higher risk for metabolic disorders including ketosis (Andersson, 1988). The deleterious effects of ketosis on animal health, and productivity are well documented in the literature (McLaren et al., 2006; Ospina et al., 2010; Chapinal et al., 2012; Dubuc et al., 2012). The recommended NEL and CP requirements for fresh cows by NRC (2001) are 1.73 Mcal/kg and 16.5 to 17.5%, respectively, but the above nutrient contents in the TMR used in the farms in this study (averaged 1.53 Mcal/kg and 13.7%, respectively; Table 2) were lower than the recommended values by NRC (2001). In addition, the NDF content of the TMR (average 44.3%) was higher than the recommended NDF content (25 to 33%) for fresh cows (NRC 2001). In lactating cows, intake of high-fiber diets may limit DM intake due to rumen fill and the inability to degrade fiber fast enough to flow out of the rumen to make space for additional feed consumption. This would lead to insufficient energy consumption to meet the requirements (Grummer et al., 2004). The low NEL and CP contents of the TMR in farms recorded in this study further decreased the availability of these nutrients required by the cows, resulting in a more severe NEB condition.

It has been suggested that increasing energy density of the diet by increasing NFC during the transition period has some benefits to the cows (Grummer, 1995; Vandehaar et al., 1999; Roche et al., 2013). Feeding diets containing higher NFC content has been suggested for the transition period in order to promote the development of ruminal papillae (Dirksen et al., 1985), facilitate acclimatisation of the rumen microorganism to the higher postpartum NFC levels, as well as increase insulin secretion and thereby suppressing lipolysis and reducing the influx of fatty acid into the liver (Lee and Hossner, 2002; Lafontan et al., 2009). For instance, cows fed high NFC (40–42%) diets consumed more DM during the prepartum period, had lower plasma non-esterified fatty acid (NEFA), reduced BHBA level and liver triglyceride (Minor et al., 1996). However, in a review of published studies, Overton and Waldron (2004) concluded that, in the majority of these studies, changes in carbohydrate source were confounded by energy intake. In the current study the NFC content in the fresh cow (4 weeks after calving) rations was very low (on average 32.75 %; Table 1). Furthermore, the effect of NEL on SCK incidence was significant for fresh cows, but NFC and CP contents in diets had no significant effect on the incidence of SCK.

The amount of DM in normal corn silage should contain between 30 to 35% (Kung and Shaver, 2001), however, the average DM measured in the corn silage sampled from the different dairy farms studied was low (less than 30%), especially those of the butyric silage (25%). Hay crop silages chopped too wet (due to insufficient wilting time or direct-cut silages) or that low in water-soluble carbohydrates favour the growth of Clostridium sp. bacteria. These bacteria ferment some of the carbohydrates to butyric acid instead of the desired lactic acid (Oetzel, 2007). Lactic acid concentration should be as much as 4 to 7% DM and at least between 65 to 70% of the total acids in silage. On the other hand, in a good corn silage, acetic acid should be less than 3% DM, giving a lactic acid to acetic acid ratio of more than 3 (Kung and Shaver, 2001). High acetic acid silage depresses DM intake in ruminants (Kung and Shaver, 2001). Results obtained in this study showed that acetic acid concentration was more than 3% DM and lactic acid to acetic acid ratio was lower than 3 in all the corn silage samples (on average 4% and 1.35, respectively). The lactic acid to acetic acid ratio was highly correlated with lactic acid concentration in corn silage (r^2 = 0.88). Daily doses of over 50 to 100 g of butyric acid can cause ketosis, and doses over 200 g may induce severe ketosis. Similarly, it has been reported that doses of butyric acid ranging between 450 to 950 g constantly induce severe ketosis in nearly any early lactation cows (Oetzel, 2007). In the current study, butyric acid concentration was more than 0.1% DM in the corn silages obtained from four farms. However, the butyric silage had no significant effect on the incidence of SCK in the dairy farms studied.

The preferred BCS of cows at calving is from 3.25 to 3.75

(NRC, 2001). The average BCS of cows at calving in this study was higher than 3.75 in 3 farms while in one farm was less at 3.25. It was reported that excess body condition at calving resulted in increased losses in BW and body condition during lactation and decreased DMI and milk production (Treacher et al., 1986). Cows that are obese at calving have greater adipose tissue reserves that result in increased mobilization of NEFA (Treacher et al., 1986). Therefore, these cows would develop more severe fatty liver during early lactation. Furthermore, obese cows would be more susceptible to the induction of ketosis than would cows of normal body condition. It has been recently reported that high BCS at calving were associated with greater concentrations of BHBA and upregulation of genes involved in ketogenesis in the liver during postpartum period compared to cows with low BCS at calving (Akbar et al., 2015). In the present study, ketotic cows had higher BCS at calving and during the first weeks postpartum than healthy cows, and they lost more body condition compared to the non-diseased cows. The BCS loss during first month after calving was more than 0.5 unit in all the farms studied.

Generally, most of the correlations between BCS and metabolites occur between 20 to 60 days in milk (Ling et al., 2003). In the present study, the BCS of the fresh cows at calving had a correlation with BCS during 3, 14 and 28 d after calving. The BCS at calving did not affect the incidence of SCK in the farms studied. Cows identified as suffering from SCK in this study had a lower average milk production (30 kg/cow per d) over the first 60 d milking as compared to the healthy cows (34 kg/cow/d), however, the average milk yield was not significantly affected by the incidence of SCK.

CONCLUSIONS

Results of this study showed that the prevalence of SCK in the farms was high during the first month after calving. Intake of NEL was below the recommended requirement and had a significant effect on the incidence of SCK in dairy cows. Butyric acid concentration in the corn silage was high in four farms but the statistical analysis showed that butyric acid had no significant effect on the occurrence of SCK. The BCS at calving had no significant effect on the incidence of SCK, however, cows showed more than 0.5 unit loss of BCS during first month after calving because of the low energy content of the TMR used in the farms. Non-fiber carbohydrates and CP contents in TMR were found to have no significant effect on the occurrence of SCK. Therefore, the most effective way to control or minimize the incidence of SCK in dairy farms of similar conditions as that of this study would be to provide sufficient dietary energy to meet the requirements of the cows, especially during the first month after calving when the SCK prevalence is normally high.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Pejman Ajilchi, General Manager of Sana Dam Pars Company (Tehran, Iran) for financial support of this study.

CONFLICT OF INTEREST STATEMENT

The authors of this article declare that they do not have any conflicting interests.

AUTHOR'S CONTRIBUTION

All authors conceived and coordinated the study, conducted statistical analysis, interpreted the results and drafted the manuscript. All authors read and approved the manuscript.

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