

# Effects of ketosis in dairy cows on blood biochemical parameters, milk yield and composition, and digestive capacity

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## Abstract

**Introduction:** This study aimed to characterise the effects of ketosis on milk yield and composition and digestive capacity in transition dairy cows. **Material and Methods:** Seven ketotic and seven healthy cows were housed in individual stalls for six days. Samples of plasma, milk, refused total mixed ration, and faeces were collected, and the blood biochemical parameters, milk yield and composition, dry matter intake, and faecal dry matter (FDM) production were determined. **Results:** Compared with healthy cows, the ketotic cows had significantly higher concentrations of milk fat and citrate, but lower levels of milk protein and lactose. The cows exhibited a need for acid detergent fibre in forage and better digestion of neutral detergent fibre, starch, crude protein, and phosphorus than healthy cows, but more fat and gross energy were excreted in their faeces. Ketotic cows had higher energy-corrected milk yields and lower FDM than healthy cows. **Conclusion:** Lower feed intake coinciding with the requirement to maintain high milk production is considered to be the cause of ketosis in dairy cows. Ketotic cows exhibited lower dry matter fat digestion.

**Keywords:** dairy cow, ketosis, milk yield, digestive capacity, dry matter intake.

## Introduction

Ketosis is a significant metabolic disease in dairy cows during transition periods. The incidence of ketosis can range from 10% to 60% for subclinical cases and 2% to 15% for clinical cases in the first month of lactation (10). Essentially, all dairy cows experience a negative energy balance (NEB) during early lactation due to the energy intake failing to meet the requirements of milk production (3, 29). An excessive NEB usually leads to ketosis, which can result in decreased milk production, rapid weight loss, dry faeces, less rumination (11), reduced reproductive performance (28), and increased risk of other illnesses, such as fatty liver, displaced abomasum, and metritis (23). These characteristics are usually indicative of an abnormal digestive capacity. The present study explored whether ketosis modifies milk yield, milk composition, and digestive capacity.

## Material and Methods

**Treatments and management.** Fourteen Holstein cows with 2–3 parities were selected at 7–14 days postpartum. Cows with body weight of  $606.4 \pm 80.2$  kg, body condition score of  $2.86 \pm 0.08$ , and previous lactation milk yield of  $8,721 \pm 1,084$  kg were the seven animals assigned to the ketosis group based on a plasma  $\beta$ -hydroxybutyric acid (BHBA) level of  $\geq 1.40$  mmol/L, and the other seven cows with body weight of  $616.2 \pm 42.3$  kg, body condition score of  $2.88 \pm 0.04$ , and previous lactation milk yield of  $8,500 \pm 1,025$  kg were assigned to the healthy group as dictated by a BHBA level of  $< 1.00$  mmol/L and absence of other clinical diseases. The selected cows were housed in individual stalls, bedded on rubber mattresses, had free access to water throughout the trial, and were fed a total mixed ration (TMR) (Table 1) *ad libitum* (more than 5% refusals) at 05:00 and 13:00 daily. Cows were milked three times daily, at 04:00, 12:00, and 20:00.

Each experimental period lasted six days, with two days of adaptation and four days of observation and sample collection.

**Table 1.** Components of the feed

Ingredient, g/kg	Diet
Corn silage	380.40
Alfalfa	50.71
Oat grass	25.35
Chinese hay	22.82
Cotton seed	32.96
Soybean	21.30
Tableting corn	32.45
Concentrated feed	234.63
Water	199.38

**Sample collection and analysis.** Blood samples of each cow were collected daily before morning feeding by venepuncture of the caudal vein, and heparin sodium was used as an anticoagulant. The samples were immediately centrifuged at 1,400 g for 10 min. The plasma was subsequently stored at  $-80^{\circ}\text{C}$ . Blood parameters, including BHBA, nonesterified fatty acids (NEFA), glucose (Glu), calcium (Ca), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglycerides (TG), total bilirubin (TBIL), and  $\gamma$ -glutamyl transpeptidase (GGT) were measured with a biochemical kit (Beijing Jiuqiang Biotechnology Co., China) and an automatic clinical analyser (Synchron DXC800; Beckman Coulter, USA). Each cow was milked three times daily, and the milk was sampled (25 mL) at each milking time, treated with preservative (1 mg/mL of potassium dichromate), and stored at  $4^{\circ}\text{C}$ . Daily milk samples from each cow were mixed and analysed to determine the fat, protein, lactose, nonfat milk solids (NFS), dry matter (DM), urea, and citrate contents using a CombiFoss 4000 combined milk composition analyser and somatic cell counter (Foss, Denmark). The daily milk yield for each cow was recorded and converted into the energy-corrected milk (ECM) yield based on the fat and protein contents of the milk, as follows:  $\text{ECM (kg/cow per day)} = 0.25 \times \text{mass of milk yield in kg} + 12.2 \times \text{mass of fat yield in kg} + 7.7 \times \text{mass of protein yield in kg}$ . The ECM formula was given by Bell *et al.* (1) and cited by Sjaunja *et al.* (22).

The TMR refused by each cow was collected and weighed daily before the morning feed. Refused feed was sampled on a daily basis and subjected to immediate DM analysis ( $105^{\circ}\text{C}$  for 23 h) to estimate the DM intake (DMI). Each aliquot of refused TMR was added to a sealed plastic bag and stored at  $-20^{\circ}\text{C}$  to create a bulked sample. At the end of the period, the bulked samples were thawed and four daily samples from each cow were mixed to analyse the acid detergent fibre (ADF), neutral detergent fibre (NDF) (26), starch, crude protein (Kjeldahl determination),

crude fat (Soemmering's extract), gross energy (combustion method), calcium (by inductively coupled plasma mass spectrometry ICP-MS), phosphorus (by ICP-MS), and total carbon contents (elemental analyser).

Faeces of each cow were collected from the ground in individual stalls every 4 h (at 24:00, 04:00, 08:00, 12:00, 16:00, and 20:00), and then mixed and weighed. Daily mixed faeces in a 500g mass was used to analyse the DM ( $105^{\circ}\text{C}$  and  $65^{\circ}\text{C}$  for 23 h) for calculating the daily faecal dry matter (FDM) of each cow. Fresh 200 g faeces samples from each cow were collected rectally daily at 04:00, 12:00, and 20:00 and frozen immediately at  $-20^{\circ}\text{C}$ . After thawing, equal weight aliquots of four daily faeces samples from each cow were mixed and used to analyse the DM ( $105^{\circ}\text{C}$  and  $65^{\circ}\text{C}$  for 23 h), NDF, ADF, starch, crude protein, crude fat, gross energy, calcium, phosphorus, and total carbon contents. Daily DMI and FDM of each cow were calculated for the DM digestive capacity analysis as  $\text{DM digestion (\%)} = ((\text{DMI} - \text{FDM})/\text{DMI}) \times 100$  (8).

**Statistical analysis.** All of the data were analysed using SPSS (version 19.0; IBM-SPSS, USA) and the results were expressed as the means  $\pm$  SD. Daily plasma and milk parameters and the ECM yield, DMI, FDM, and DM digestibility were used as the experimental variables. Four days' samples of faeces and refused TMR from each cow were mixed as experimental units. The data were analysed using independent-sample *t*-tests to determine the effects of ketosis in dairy cows.  $P < 0.05$  was considered statistically significant.

## Results

**Plasma and milk variables.** As shown in Table 2, the plasma concentrations of BHBA, NEFA, AST, TBIL, and GGT were significantly higher in ketotic cows compared with healthy cows, whereas the plasma Glu and Ca concentrations were significantly lower. Compared with healthy cows, ketotic cows had significantly lower levels of milk protein, lactose, and NFS, but higher levels of milk fat and citrate.

**Nutritional content of faeces and refused TMR.** As shown in Table 3, the TMR refused by ketotic dairy cows had a lower level of ADF, and therefore it is theorised that ketotic cows had a significantly higher ADF intake than healthy cows. In addition, dairy cows with ketosis had significantly higher levels of crude fat and gross energy in their faeces.

**Digestive capacity characteristics.** As shown in Table 4, ketotic cows had a higher milk yield than healthy cows, but there was no difference between the control and ketosis groups. Ketotic cows had significantly lower DMI and FDM levels. Based on 1 kg of DMI, ketotic cows had a significantly higher ECM yield, but less FDM was excreted. Thus, ketotic cows exhibited better DM digestion than healthy cows.

**Table 2.** Plasma and milk variables

Item	Healthy	Ketosis
Plasma samples		
BHBA (mmol/L)	0.68 ± 0.15	3.41 ± 1.52**
NEFA (mmol/L)	0.41 ± 0.18	1.18 ± 0.37**
Glu (mmol/L)	4.23 ± 0.58	2.74 ± 0.51**
Ca (mmol/L)	2.18 ± 0.14	2.08 ± 0.15 *
AST (U/L)	82.88 ± 13.15	139.03 ± 52.6**
ALT (U/L)	20.56 ± 1.41	22.22 ± 5.52
TC (mmol/L)	3.21 ± 0.77	3.12 ± 1.09
TG (mmol/L)	0.17 ± 0.03	0.15 ± 0.03
TBIL (mmol/L)	1.79 ± 0.15	4.98 ± 1.52**
GGT (U/L)	21.0 ± 2.4	42.09 ± 18.14**
Milk samples		
Milk fat (%)	3.35 ± 0.86	4.43 ± 0.88**
Milk protein (%)	3.32 ± 0.38	2.78 ± 0.35**
Fat/ Protein	1.04 ± 0.31	1.55 ± 0.34**
Milk lactose (%)	5.12 ± 0.3	4.91 ± 0.21**
Nonfat milk solids (%)	9.2 ± 0.44	8.79 ± 0.53**
Dry matter (%)	12.79 ± 2.99	13.35 ± 1.26
Milk urea (mg of MUN/dL)	15.05 ± 4.97	17.01 ± 2.88
Milk citrate (mmol/L)	8.63 ± 1.43	11.45 ± 1.93**

BHBA –  $\beta$ -hydroxybutyric acid; NEFA – nonesterified fatty acids; Glu – glucose; AST – aspartate aminotransferase; ALT – alanine aminotransferase; TC – total cholesterol; TG – triglycerides; TBIL – total bilirubin; GGT –  $\gamma$ -glutamyl transpeptidase; MUN – milk urea nitrogen; \* – ketosis has statistical differences compared with healthy cows ( $P < 0.05$ ), \*\* – ketosis has statistical differences compared with healthy cows ( $P < 0.01$ )

**Table 3.** Nutritional levels in manure and refused TMR

Item	Refused TMR		Faeces	
	Healthy	Ketosis	Healthy	Ketosis
DM (%)	50.97 ± 1.98	49.95 ± 1.03	16.81 ± 1.46	16.37 ± 1.39
Composition, % DM				
NDF	39.66 ± 3.77	40.52 ± 3.71	50.67 ± 3.51	49.08 ± 2.76
ADF	21.05 ± 0.87	19.06 ± 0.84*	33.77 ± 1.85	32.79 ± 2.02
Starch	16.43 ± 0.39	15.73 ± 0.37	2.45 ± 0.20	2.44 ± 0.26
Crude protein	10.99 ± 0.08	10.75 ± 1.09	2.48 ± 0.33	2.32 ± 0.22
Crude fat	5.81 ± 1.69	5.18 ± 0.46	0.53 ± 0.42	1.73 ± 0.78**
Total carbon	42.53 ± 0.36	43.10 ± 1.73	41.77 ± 0.57	42.30 ± 2.05
Ca (g/kg)	10.57 ± 1.56	10.25 ± 1.23	5.7 ± 0.97	5.8 ± 0.63
P (g/kg)	4.33 ± 0.34	4.47 ± 0.55	1.63 ± 0.34	1.48 ± 0.27
Gross energy (Mcal/kg)	2.19 ± 0.13	2.12 ± 0.08	0.60 ± 0.07	0.71 ± 0.05*

TMR – total mixed ration; DM – dry matter; NDF – neutral detergent fibre; ADF – acid detergent fibre; \* – ketosis has statistical differences compared with healthy cows ( $P < 0.05$ ); \*\* – ketosis has statistical differences compared with healthy cows ( $P < 0.01$ )

**Table 4.** Milk production characteristics and digestive capacity

Item	Healthy	Ketosis
Milk yield (kg/cow/day)	27.56 ± 9.75	27.83 ± 8.11
Fat yield(kg/cow/day)	0.93 ± 0.41	1.18 ± 0.41*
Protein yield (kg/cow/day)	0.86 ± 0.24	0.77 ± 0.22
Lactose yield(kg/cow/day)	1.42 ± 0.54	1.36 ± 0.39
ECM (kg/cow/day)	25.03 ± 8.65	27.31 ± 8.08
DMI (kg/day)	14.41 ± 2.54	11.30 ± 3.01**
FDM (kg/day)	5.26 ± 1.19	3.24 ± 0.98**
ECM/DMI	1.44 ± 0.38	2.15 ± 0.76**
FDM/DMI	0.37 ± 0.08	0.29 ± 0.05**
DM digestibility (%)	63.14 ± 7.8	71.41 ± 4.81**

ECM – energy-corrected milk; DMI – dry matter intake; FDM – faecal dry matter; DM – dry matter. \* – ketosis has statistical differences compared with healthy cows ( $P < 0.05$ ); \*\* – ketosis has statistical differences compared with healthy cows ( $P < 0.01$ )

## Discussion

Ketosis is understood to occur mainly in high-producing dairy cows. However, in this study, although ketotic cows had higher ECM yield, there was no significant difference compared with healthy cows. The association between milk production and ketosis is ambiguous, as it depends on the postpartum period and parity in cows (14, 17). Indeed, Duffield *et al.* (6) detected negative and positive effects when ketosis was diagnosed during weeks 1 and 2 postpartum, respectively. In the present study, cows were selected between 7 and 14 days postpartum when ketosis was first diagnosed, where a higher ECM yield was considered a factor related to ketosis. However, as ketosis continues, this disease is known to reduce milk production. Essentially, all transition dairy cows experience NEB because their DMI increases more slowly than the energy demand after parturition. In the present study, significantly lower DMI than that required to meet the demand imposed by lactation was considered to be the major factor that caused ketosis. To counteract a NEB, fat from adipose tissue stored in dairy cows is mobilised to form glycerol during gluconeogenesis, while large quantities of NEFA in the blood are taken up by the liver and  $\beta$ -oxidised to ketone bodies, thereby leading to ketosis with high concentrations of BHBA and NEFA in the blood. Several studies have shown that if the blood BHBA threshold is raised to 1.4 mmol/L from the usual 1.2 mmol/L, this can correct for underestimation of ketosis associated with suboptimal test sensitivity and specificity at 1.2 mmol/L (19). In the present study,  $>1.40$  mmol/L plasma BHBA was used to detect ketosis. During monitoring for four days, slight changes in the plasma BHBA concentration were detected in each cow and on only one day did a dairy cow have a plasma BHBA of 1.31 mmol/L. Ketotic cows had high concentrations of NEFA, which ranged from 0.75 to 1.99 mmol/L. Several studies have shown that postpartum NEFA concentrations resolved to a greater risk of ketosis than the postpartum BHBA concentrations (17, 20). The increased plasma NEFA can be used as an energy source *via*  $\beta$ -oxidation processes or as a substrate to produce Glu *via* gluconeogenesis, but large quantities of NEFA are esterified to triglycerides in the liver, which leads to hepatic injury with increased plasma concentrations of AST, TBIL, and GGT. In the present study's results, plasma concentrations of ALT in ketotic cows showed no significant difference from those of healthy animals. The level of ALT is an indicator of the degree of cell membrane damage, whereas that of AST is an indicator of mitochondrial damage, because mitochondria contain 80% of the enzyme (24). High NEFA-mediated mitochondrial oxidative damage (5) was considered to cause high plasma concentrations of AST in ketotic cows. Increased serum GGT may be a marker of oxidative stress (7), and a study stated that serum GGT

was strongly associated with hypertension, dyslipidaemia, and abnormal glucose tolerance, suggesting that when elevated in serum, this transferase accompanies hepatic insulin resistance, regardless of the presence of non-alcoholic fatty liver disease (30). In this study, liver oxidative damage caused by high NEFA and low Glu suggested elevated plasma AST, GGT, and TBIL (3). In addition, a high plasma concentration of NEFA is temporally correlated with an impaired peripheral blood neutrophil function, which affects the response to immune challenges (21).

In this study, high milk fat and citrate and low milk protein and lactose were detected in ketotic dairy cows. Under NEB (meaning blood glucose deficiency), high blood NEFA content mobilised from adipose tissue suggested increased content of milk fat and changed milk fatty acid composition, and also caused decreased milk protein content (6, 25). Several authors reported that ketosis decreases both milk lactose yield and lactose percentage (4), and high availability of blood glucose and a cow's positive metabolic energy status are always translated into high milk lactose yield and lactose percentage (18). A high milk fat:protein ratio is considered to be a signifier of a high risk of ketosis in dairy cattle (2). Research described cut-off points for the energy balance during early lactation using milk variables, *i.e.*, a milk fat:protein ratio  $> 1.4$ , milk protein  $< 2.9\%$ , milk fat  $> 4.8\%$ , and milk lactose  $< 4.5\%$  (16). In the present study, the milk fat:protein ratio was 1.55:1 in ketotic cows. In addition, there was a significantly high milk citrate level in ketotic cows, which ranged from 8.75 to 15.89 mmol/L (11.45 mmol/L mean), whereas the milk citrate level in healthy cows ranged from 6.41 to 11.40 mmol/L (8.63 mmol/L mean). Milk citrate is a marker of mitochondrial metabolism in the mammary gland and it induces the ferric citrate transport system, where it competes with lactoferrin for iron. Recent research has shown that the milk citrate level has a positive correlation with milk BHBA and fat (9). In the present study, it was not clear whether the increased milk citrate level was caused by mitochondrial injury in the mammary gland and further research is needed to clarify this issue, but milk citrate was identified as an early indicator of NEB.

The ingestion characteristics related to ketosis in dairy cows were also evaluated in this study by analysing the nutritional features of refused TMR. A significantly lower level of ADF was detected, which indicates that the forage ADF intake was higher in ketotic cows than healthy ones. ADF is the main component of fibrous and structural carbohydrates; alfalfa and cereal silage have higher ADF concentrations compared with corn silage (12). Vickers *et al.* (27) found that feeding a higher forage diet before calving can reduce rates of subclinical ketosis in transition dairy cows. In the present study, the weight of refused TMR was only 5–10% of the DMI (more than 5% refusals), indicating that the ADF ingestion

characteristics of ketotic cows also require further investigation. However, more crude protein and crude fat were ingested by both healthy and ketotic dairy cows compared with the nutritional contents of the TMR, which suggests that the ingestion characteristics are more concentrated in transition period cows. Interestingly, the nutritional analysis of the faeces produced by ketotic cows indicated lower NDF, ADF, starch, crude protein, and phosphorus contents than healthy cows, although the differences were not significant, and these differences suggest that ketotic cows exhibited better digestion characteristics than healthy cows. The lower DMI of ketotic cows was thought to explain their superior digestion. Kaufman *et al.* (14) found that multiparous cows with ketosis ruminated for  $25 \pm 12.8$  min/day less than healthy cows from week -2 to week +4 of calving. Rumination time is consistently associated with the dietary NDF intake and particle size. Maekawa *et al.* (15) found that rumination time was also positively correlated with a greater DMI, body weight, and higher milk yield. Ketotic cows have a shorter rumination time (13), but significantly higher DM digestion was detected in these animals in the present study. Of note is the detection of significantly higher crude fat and gross energy levels in faeces of ketotic cows in this study. As an energy source, fat is generally used as an additive for transition period cows, but supplemental fat can decrease the acetate level and increase the propionate level in the rumen. The addition of fat can interfere with ruminal fermentation and decrease the digestion of fibre, whereas an increase in the hay content of the basal diet might reduce the fermentation problems that are normally caused by fat. Based on the high DM digestion found in the present study, it seems that the high fat content had no effect in the rumen. More research is needed to determine whether the hepatic injury caused by severe NEB might affect fat absorbed in the intestines. In the present study, we found that ketotic dairy cows had significantly higher ECM/DMI levels compared with healthy cows, but large quantities of fat were mobilised to counteract the lack of feed.

In conclusion, feed intake lower than that required to maintain high milk production in the early postpartum period is assumed to cause ketosis in dairy cows. High plasma concentrations of BHBA and NEFA, high milk fat and citrate levels (the latter here considered an early indicator), and lower milk protein and lactose concentrations are characteristics of ketosis. We found that ketotic cows exhibited better DM digestion than healthy cows. Dairy cows with ketosis had a greater appetite for ADF, but more fat and gross energy were excreted in their faeces. With high NEB levels, dairy cows with ketosis had higher ECM/DMI levels than healthy cows. Thus, more forage is wasted because fat reserves are mobilised. Further research is needed to clarify the effect mechanism of lower fat digestion in ketotic cows.

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