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REGULAR ARTICLES

Effects of parity and days in milk on milk composition in correlation with β-hydroxybutyrate in tropic dairy cows

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Abstract

The current study was conducted to evaluate the effect of parity and days in milk on milk yield and milk production traits and their correlation with β-hydroxybutyrate (BHB) concentrations in milk of Chinese tropic Holstein dairy cows which are adapted to a humid subtropical climate in central China. About 3055 milking records of Holstein cows were obtained from three farms in the hot region in the center of China. The records were classified according to parity to 4 categories: first parity, second parity, third parity, and greater than third parity. According to days in milk, there were 4 groups, first group from (1–100 days), second group from (101–200 days), third group from (201–305 days), and fourth group (>305 days). Milk samples collected between April and November 2019 from the three farms were routinely checked for milk components including BHB using mid-infrared spectroscopy a MilkoScan FT+ (Foss, Hillerød, Denmark). Data were analyzed by multivariate analysis of variance (generalized linear model, GLM). Pearson's correlation coefficients were used to measure the correlation between SCC and BHB with milk yield and milk production traits. Results showed the significant effect of parity and days in milk on milk yield and milk production traits. There was a negative effect of parity and days in milk on milk quality, with increasing parity and days in milk being associated with higher somatic cell count (SCC) (P <0.001). Days in milk significantly affected (P=0.001) BHB. It was concluded that with increasing parity and prolonged days in milk, there was a negative effect on milk quality and udder health of the tropic dairy cows in central China. Based on the results of the current study, sampling milk for specific metabolites, somatic cell count, and quality are sufficient to asses herd health.

Keywords Holstein cows · β-Hydroxybutyrate · Days in milk · Milk production · Parity · Somatic cells

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Introduction

Milk production is one of the most important industries all over the world. According to Euromonitor Passport Database, China is one of the largest markets for dairy products and its milk production is expected to increase by 10–25% between 2016 and 2021. Holstein is considered to be one of the most popular dairy breeds in China, characterized by high milk production (Zi et al. 2003). Milk is a crucial product which contains many vital nutrients such as protein, fat, lactose, vitamins, and minerals that affect animal and human health. Worldwide, regular milk analysis is used as a routine measure to check the health and hygienic conditions of dairy farms (Auldist and Hubble 1998). There are numerous factors affecting milk yield and composition such as breed, herd, managerial methods, environmental conditions, and nutrition (Lindmark-Månsson et al. 2000). Furthermore, some physiological factors such as parity, days in milk, and calving interval affect milking performance in Holstein cows

(Zhao et al. 2012). Proper milking hygiene and trained workers also affect dairy production (Yalçın et al. 2010). Somatic cell count is a measurement of milk quality as elevation of somatic cell count (SCC) indicates poor milk quality and high incidence of mastitis. SCC over a threshold value of 200,000 cell/ml is an indication of mastitis (Bradley and Green 2005; Schukken et al. 2003). High SCC in cows is associated with reduction in milk yield, the magnitude of which is influenced by the parity and lactation stage of the animals (Hagnestam-Nielsen et al. 2009; Hand et al. 2012). The SCC was negatively correlated to milk yield (Cerón-Muñoz et al. 2002). Main milk ingredients such as protein, fatty acids, minerals, lactose, enzymatic activity, and PH changed with SCC elevation (Coulona et al. 2002; Fernandes et al. 2004; Lindmark-Månsson et al. 2006).

β-Hydroxybutyrate (BHB) is one of the ketone bodies which is increased in cow's blood during early lactation due to negative energy balance (NEB). NEB results in decreased blood glucose and excessive fat mobilization, leading to the releasing of ketone bodies such as BHB (Benedet et al. 2019). Elevation of ketone bodies in blood indicates metabolic disorders which have an inverse effect on health, welfare, and milk production of cows (McArt et al. 2013). Cows with high BHB concentration in blood or milk are exhibited by poor health conditions and welfare due to loss of appetite, decrease in milk production, and poor body condition score (Seifi et al. 2011; Suthar et al. 2013; Berge and Vertenten 2014). The effect of BHB on milk yield and milk production traits is not clear as it has been shown that elevated BHB levels are associated with decrease of milk production (Santschi et al. 2016) or increase of milk production (Rathbun et al. 2017) or milk production was not affected (Van der Drift et al. 2012; Chandler et al. 2018). There has been much research into the effect of milk quality as affected by the physiological status of the animals while few studies have demonstrated the correlation between the physiological condition of cows, milk production, and health. Therefore, the current study was conducted to estimate the effect of parity and days in milk on milk yield and milk production traits in correlation with BHB concentrations in milk of the tropic dairy cows' farms in the center of China.

Materials and methods

This study was approved by the Scientific Ethic Committee of Huazhong Agricultural University and was conducted according to the guidelines of care and animal use in research (permit number HZAUCA-2019-005).

Data, Animals, and Management

The data of the current study were obtained from Holstein dairy cows in 3 farms located in the center of China from April to November 2019. The selected farms had the same housing and feeding systems as all cows were housed indoor in a tie-stall barn with ad libitum and continuous access to water. Cows were fed a TMR two times daily. TMR was formulated according to NRC (2001) and Mohammad et al. (2017) (Table 1); the TMR was not offered according to the physiological status of cows (parturition and lactation). The mean environmental temperature varied between 10 and 29 °C and the relative humidity between 45 and 78%. This variation was attributed to the long period of sample collection. Cows were adapted to humid subtropical conditions that prevail in central China. Cows were milked two times per day. Approximately, 3055 milking records were obtained from Holstein dairy cows in the 3 farms. The records included milk yield, milk production traits, date of birth, parity, calving date, days in milk, and calving interval of cows.

Table 1 Ingredient and chemical composition of total mixed ration (TMR) (%)

Item	%
Corn grain	18.44
Barley grain	5.00
Beet molasses	1.20
Sunflower meal	11.09
Full fat soybean	7.50
Corn gluten meal	2.50
Wheat bran	9.20
Soy oil	0.60
Alfalfa hay	16.40
Corn silage	26.20
Limestone	0.90
Dicalcium phosphate	0.60
Sodium bicarbonate	0.16
Salt	0.20
Vitamin-mineral premix	0.09
Dry matter (%)	61.83
Net energy (Nel, Mcal/kg DM)	1.58
Crude protein (CP, % DM)	16.89
MP (% DM) *	10.75
Rumen undegradable protein (RUP, % DM) *	5.93
Rumen degradable protein (RDP, % HP) *	65.25
Fat (% DM)	4.60
Ash (% DM)	6.98
Neutral detergent fiber (NDF, % DM)	35.40
Acid detergent fiber (ADF, % DM)	21.95
Non-fiber carbohydrate (% DM)	38.57
Lysine (% MP) *	5.96
Methionine (% MP) *	1.93
Lysine/methionine *	3.10

DM, dry matter; *MP*, metabolize protein (NRC 2001; Mohammad et al.2017)

Classification of the records

To check the effect of parity and days in milk on milk yield and milk production traits and to detect the correlation between somatic cell count (SCC) and milk production traits, records were subjected to further classification. According to parity, records were divided to 4 categories: first parity, second parity, third parity, and greater than third parity (>3 parity). Regarding days in milk, there were 4 groups: the first group from 1 to 100 days, the second group from 101 to 200 days, the third group from 201 to 305 days, and the fourth group (>305 days).

Milk samples analysis

Milk samples were tested monthly, and sample of 50 ml from each cow was collected in clean bottles containing bronopol as preservative material. Milk samples were sent to DHI (Dairy Herd Improvement) center for milk fat, protein, lactose, total solids, solids not fat, urea nitrogen, SCC, and BHB analysis using mid-infrared spectroscopy a MilkoScan FT+ (Foss, Hillerød, Denmark). All ingredients were measured according to specific calibration formulae as instructed by the manufacturer.

Statistical analysis

The results from the current study were expressed as means ± SEM and were analyzed by multivariate analysis of variance (generalized linear model, GLM) and means were compared by Duncan's multiple range test using SPSS Statistics for Windows, version 23.0 (IBM Corp; Armonk, NY, U.S.A.).The normality of the data distribution was evaluated by a Shapiro-Wilk test; Pearson's correlation coefficients were used to measure the correlation between SCC and BHB with milk yield and milk production traits. Pearson's correlation coefficient (r, 1 or −1) depends on the positive or negative relation between the variables; the correlation coefficient values were interpreted as described by Prion and Haerling (2014): very strong correlation (±0.91 to ±1.00); strong correlation (±0.68 to ±0.90); moderate correlation (±0.36 to ±0.67); weak correlation (±0.21 to ±0.35); and negligible correlation (0 to ±0.20). Differences were declared significant when $P<0.05$.

Results

Parity

Parity showed a significant effect ($P<0.001$) on somatic cell count: SCC was (169.00±7.84, 173.45±11.89, 190.30±6.95, and 215.50±6.41) ×10^3 cell/ml for the first, second, third, and over the third parity, respectively. The results showed that first parity cows recorded the lowest milk yield, which tended to increase in the second and third parity and then decreased in the subsequent parity (P=0.01). The milk fat and protein were lower in the second parity and then tended to increase in the third parity and above ($P<0.001$); lactose was high in the first parity and started to decrease with the subsequent parity. The same effect of parity was recorded in total solids (TS) and solids not fat (SNF) ($P<0.001$). Milk urea nitrogen (MUN) was higher in the first and second parity than in the subsequent parity (P=0.008). The results revealed that there was no significant effect of parity on BHB levels in milk (P=0.5) (Table 2).

The increase in SCC was positively correlated with milk fat (r = 0.01), protein (r = 0.11), and total solids (r = 0.01) as affected by parity; however, this correlation was negligible. There was a negligible negative correlation between SCC and milk yield (r =−0.05), lactose (r =−0.20), SNF (r =−0.10), and MUN (r =−0.12). Negligible negative correlation (r =−0.08) was reported between SCC and BHB. The correlation between BHB and milk production traits was negative except lactose which had a negligible positive correlation with BHB (r = 0.2); also, milk yield had a positive correlation with BHB concentration (r = 0.10), although this positive correlation was negligible (Table 3).

Days in milk

A significant difference was observed in SCC trends as affected by days in milk ($P<0.001$), as SCC increased with days in milk. The SCC was (157.72±9.78, 183.22±7.26, 216.12 ±9.46, and 248.42±20.33) ×10^3 cell/ml for the first, second, third, and fourth group, respectively. The results revealed that milk yield decreased with days in milk ($P<0.001$). Milk fat, protein, TS, and SNF were increased with days in milk ($P<0.001$). Lactose was high in the first group then tended to decrease in the second and third group and increased again in fourth group. MUN was lower in the first group than other groups ($P<0.001$) (Table 4). Also shown in Table 4, the effect of days in milk in BHB concentration in milk was significant (P=0.001) as the BHB concentration was 0.75±0.08, 0.94 ±0.03, 0.77±0.02, and 0.74±0.04 mmol/L for the first, second, third, and fourth group, respectively.

A negligible positive correlation between SCC and milk fat (r = 0.01), protein (r = 0.11), and total solids (r = 0.003) was observed as affected by days in milk, while the negligible negative correlation was recorded between SCC and milk yield (r =−0.03), lactose (r =−0.18), SNF (r =−0.10), and MUN (r =−0.15). Negligible negative correlation (r =−0.084) was observed between SCC and BHB. The correlation between BHB and milk production traits was negative except lactose which had a weak positive correlation with

Table 2 Milk yield and milk production traits as affected by parity

Item	Parity				
	First parity	Second parity	Third parity	>3 parity	*P* value
Milk kg/day	26.30±0.56[b]	28.35±0.82[ab]	31.25±1.68[a]	27.30±1.35[b]	0.01
Fat %	4.21±0.02[b]	4.17±0.03[b]	4.45±0.04[a]	4.39±0.06[a]	<0.001
Protein %	3.47±0.01[b]	3.40±0.01[c]	3.49±0.01[b]	3.56±0.02[a]	<0.001
Lactose %	5.03±0. 007[a]	4.93±0.009[b]	4.89±0.011[c]	4.94±0.017[b]	<0.001
Total solids %	13.69±0.04[a]	13.36±0.04[c]	13.52±0.05[ab]	13.63±0.07[a]	<0.001
SCC×10^3 cell/ml	169.00±7.84[c]	173.45±11.89[bc]	190.30±6.95[b]	215.50±6.41[a]	<0.001
Solids not fat %	10.49±0.06 [a]	10.10±0.10 [b]	9.51±0.14[c]	9.66±0.09[c]	<0.001
Milk urea nitrogen %	16.79±0.07[a]	16.81±0.09[a]	16.56±0.16[ab]	16.35±0.04[b]	0.008
BHB mmol\l	0.81±0.02	0.82±0.03	0.74±0.07	0.90±0.06	0.5

Means (± SE) with different superscript letters in the same row are significantly different at $P<0.05$

SCC somatic cell count, *BHB* β -hydroxybutyrate

BHB (r = 0.34); also, BHB concentration had a negligible positive correlation with milk yield (r = 0.11) (Table 5).

Discussion

Parity

The SCC increased with parity; as with age, cows lose their body condition and the udder tissues become more susceptible to invasion of microorganisms with high incidence of mastitis. The findings of the current study agree with Guo et al. (2010) and Zhao et al. (2012) who recorded the same effect of parity on Holstein dairy cows in the east and northwest of China. In contrast, Yoon et al. (2004) stated that the SCC was higher in first parity than the second and third in Holstein dairy cows.

Parity not only affected SCC but also had a significant effect on milk yield and milk production traits as milk yield increased with parity as mentioned by Yoon et al. (2004). Milk fat and protein were higher in the third and subsequent parity; TS were higher in the first parity and tended to decrease in the second parity with marked increase again in the third and subsequent parity. Lactose and SNF decreased with parity. MUN showed the highest levels in the first and second parity. Milk fat of Holstein cows was 3.68 ± 0.83, 3.74 ± 0.76, and 3.75 ± 0.80 for the first, second, and third parity, respectively (Guo et al. 2010). In contrast to the current results, protein percent was higher in the second parity comparing to the first one in Holstein-Friesian Cows (ÇARDAK 2016).

In line with the present study, Du et al. (2020) reported a reduction in milk lactose from first to second to third parity. Lactose decreased with increasing parity as indicated by Węglarz et al. (2008), who also found a drop in total solids in third parity compared to the second, unlike the findings of the current work. Similar to our findings, Jílek et al. (2006) evaluated the effect of parity on urea concentration in cow milk and found a reduction in urea concentration with parity. The milk urea concentration was 5.63±0.090, 5.62±0.092, and 5.47±0.095 mmol/L for the first, second, and third parity, respectively. The same results were obtained in Holstein cows where the milk urea nitrogen concentration of the third parity was significantly lower ($P<0.001$) than the concentration of the first and second parity (Zhijun et al. 2010). The current result does not agree with Yoon et al. (2004) who compared the milk urea nitrogen in first, second, and third parity and recorded the lowest level of milk urea nitrogen in the first parity.

The current result revealed that parity had no significant effect on BHB concentration in milk; however, the highest concentration of BHB was observed in parity >3; as with age, the cows become more susceptible to metabolic disorders which are characterized by high BHB concentrations in milk and blood. The frequency of hyperketonemia was higher in multiparous than primiparous cows (Santschi et al. 2016;

Table 3 Correlation coefficients between BHB and SCC in milk with milk yield and milk production traits as affected by parity

Item	Milk yield and milk production traits								
	Milk yield	Fat	Protein	Lactose	TS	SNF	MUN	SCC	BHB
BHB	0.10*	−0.18*	−0.53*	0.217*	−0.32*	−0.44*	−0.24*	−0.08	
SCC	−0.05	0.01	0.11*	−0.20*	0.01	−0.10*	−0.12*		−0.08

BHB β -hydroxybutyrate, *SCC* somatic cell count, *TS* total solids, *SNF* solids not fat, *MUN* milk urea nitrogen

*$P<0.05$

Table 4 Milk yield and milk production traits as affected by days in milk

Item	Days in milk				
	1–100 days	101–200 days	201–305 days	>305 days	*P* value
Milk kg/day	31.53±2.18[a]	30.38±0.72[a]	27.88±0.65[b]	21.55±0.72[c]	<0.001
Fat %	4.00±0.05[b]	4.06±0.03[b]	4.40±0.04[a]	4.29±0.07[a]	<0.001
Protein %	3.18±0.01[d]	3.35±0.01[c]	3.61±0.01[b]	3.87±0.04[a]	<0.001
Lactose %	5.00±0. 012[b]	4.98±0.008[b]	4.92±0.014[c]	5.18±0.0267[a]	<0.001
Total solids %	12.90±0.06[d]	13.30±0.04[c]	14.27±0.07[b]	14.90±0.19[a]	<0.001
SCC×10^3 cell/ml	157.72±9.78[c]	183.22±7.26[bc]	216.12±9.46[ab]	248.42±20.33[a]	<0.001
Solids not fat %	9.38±0.09 [c]	10.21±0.10 [b]	10.56±0.07[a]	10.22±0.10[b]	<0.001
Milk urea nitrogen %	16.37±0.11[c]	17.01±0.07[a]	16.95±0.12[a]	16.90±0.10[b]	<0.001
BHB mmol\l	0.75±0.08[b]	0.94±0.03[a]	0.77±0.02[b]	0.74±0.04[b]	0.001

Means (± SE) with different superscript letters in the same row are significantly different at $P<0.05$

SCC somatic cell count, *BHB* β -hydroxybutyrate

Rathbun et al. 2017; Chandler et al. 2018). Berge and Vertenten (2014) confirmed the increase of hyperketonemia with parity. In the same line with the current result, Lee et al. (2016) reported the increase of mean-milk BHB of Holstein cattle with parities.

Days in milk

SCC increased with advanced lactation (increase days in milk); SCC elevation may be attributed to the reduction in milk yield at the mid and late stages of lactation and to weakness in the udder cells. SCC increased with lactation days as the number of polymorphs and other cells increased according to Blackburn (1966) and Kennedy et al. (1982). In cow that remain healthy and do not become infected, SCC does not increase with advanced days in milk (Natzke et al. 1972). In this study, milk yield showed linear decrease with increase days in milk and this is in accordance with Yoon et al. (2004) and ÇARDAK (2016). In Holstein-Friesian cows, the fat and protein percent increased with increase days in milk as the percent of fat was 3.82±0.04, 3.89±0.04, and 4.21±0.04 while the percent of protein was 3.17±0.02, 3.27±0.02, and 3.43±0.02 for lactation stages from 6 to 105 days, 106 to 205 days, and more than 205 days, respectively, while there was a reduction in lactose percent with advanced days in milk as the percent of lactose was 4.47±0.00, 4.47±0.00, and 4.46±0.00, although this reduction was not significant (ÇARDAK 2016).

Milk solids-not-fat were increased with increasing days in milk. This result agrees with Jóźwik et al. (2012) who recorded higher SNF in 200 days in milk than 60 days in milk. The lowest milk urea concentration was observed in first month of lactation then increased in the following months (Jílek et al. 2006). In accordance with the current result, the milk urea nitrogen concentration was lower in the first days of milk in Holstein cows as described by Zhijun et al. (2010); furthermore, the result agrees with Yoon et al. (2004) who confirmed the increase of milk urea nitrogen concentration with lactation period as the milk urea nitrogen concentration was 15.7±0.26, 16.9±0.26, and 17.2±0.24 mg/dl for the lactation periods <100 day, 100–200 days, and >200 days, respectively. Previous studies (Ng-Kwai-Hang et al. 1985; DePeters and Cant 1992) reported the same effect of lactation period in milk urea nitrogen concentration. The difference and changes in MUN in the current study may be attributed to the physiological status of cows during parity and days in milk and not attributed to the ration, as cows in the 3 farms were fed the same levels of dietary crude protein.

The result showed the significant effect of days in milk on BHB concentration in milk ($P=0.001$), and as the environmental and management conditions in the 3 farms were almost the same, the change in BHB may be attributed to days in milk. Most cases of hyperketonemia occurred at early lactation due to negative energy balance and the increased energy needs of the cow during late pregnancy and early lactation.

Table 5 Correlation coefficients between BHB and SCC in milk with milk yield and milk production traits as affected by days in milk

Item	Milk yield and milk production traits								
	Milk yield	Fat	Protein	Lactose	TS	SNF	MUN	SCC	BHB
BHB	0.11	−0.26*	−0.56*	0.34*	−0.38*	−0.47*	−0.23*	−0.084	
SCC	−0.03	0.01	0.11*	−0.18*	0.003	−0.10*	−0.15*		−0.084

BHB β -hydroxybutyrate, *SCC* somatic cell count, *TS* total solids, *SNF* solids not fat, *MUN* milk urea nitrogen

*$P<0.05$

Unfortunately, in the current study, the data obtained for BHB concentrations in early lactation (1–14 DIM) was not enough, but we obtained the highest concentration of BHB at 101–200 days in milk. Also, the effect of BHB concentration in this study on milk yield and milk production traits was preliminary, as the highest concentration of BHB in the current study was 0.94±0.03. Hence, cows of the current study suffered from hyperketonemia at levels like those found in previous studies which estimated the BHB concentration in milk during the first 2 weeks of lactation (Van der Drift et al. 2012; Berge and Vertenten 2014; Santschi et al. 2016),

Correlation between SCC, BHB with milk yield, and milk production traits

SCC is an indicator for milk quality and BHB is an indicator for cow health and welfare. The correlation between these indicators and milk yield and milk production traits was obtained from the same data set of parity and days in milk obtained from farm records. Whatever the factor, the results demonstrated the negligible positive correlation of milk fat, protein, and total solid with SCC. The negative correlation of SCC with milk yield, lactose, SNF, and MUN was clear even if this correlation was significant or not. Elevation of milk fat, protein, total solids, and reduction of lactose indicates poor quality milk which frequently associated with high SCC; the same results were obtained by Silva et al. (2018). Similar to the current results, the findings of Najafi et al. (2009) and Cinar et al. (2015) revealed the positive correlation between fat, protein, and total solids with SCC, while the correlation between milk yield and lactose with SCC was negative. Unlike the current results, Cinar et al. (2015) revealed that the correlation between SCC and milk urea nitrogen was positive but not significant (P>0.05). Numerous studies have reported the relationship between SCC and milk production traits, but the agreement between the nature of relationship is mixed. EL-tahawy and EL-far (2010) recorded an inverse correlation between SCC and percentage of fat, protein, total solids, and lactose, while there was no effect of SCC on milk content as described by Albenzio et al. (2005). In accordance with the current findings, Guo et al. (2010) reported the positive correlation between milk fat and protein percentage with SCC, as fat and protein percentages increased ($P < 0.05$) with increasing SCC in Holstein population in the northwest of China. In contrast, Sun et al. (2009) stated that the correlation between protein content and SCC was not statistically significant. Similarly, ÇARDAK (2016) reported an increase of fat content with high SCC, while also reporting a reduction in milk yield, lactose, and milk urea nitrogen concentrations with increasing SCC. On the other hand, Ng-Kwai-Hang et al. (1985) reported a positive correlation between SCC and milk urea nitrogen. The current results revealed that SCC of Holstein cows in central China as affected by parity and days in milk was lower than that found in other countries, such as South Korea Yoon et al. (2004) and Poland Węglarz et al. (2008) and Salamończyk and Guliński (2013).

There is controversial relationship between BHB, milk yield, and milk production traits. The current study revealed the positive correlation between BHB and milk yield and milk lactose; however, this correlation was weak or negligible. There was a negative correlation between BHB and milk fat, protein, TS, SNF, MUN, and SCC as affected by parity and days in milk. In this study, BHB concentrations increased from the first parity to the second then decreased at the third parity and tended to increase again in >3 parity. BHB showed the highest level up to 200 days in milk then tended to decrease again with advanced lactation. Elevated BHB negatively affected milk production of multiparous cows more than primiparous cows (Ospina et al. 2010; Chapinal et al. 2012; Kayano and Kataoka 2015). Cows with high BHB concentrations showed reduction in milk yield from 1.0 to 1.9 kg daily (Dohoo and Martin 1984; Duffield et al. 2009). In accordance with the current results, the previous studies of Santschi et al. (2016) and Chandler et al. (2018) observed the negative effect of high BHB in milk protein.

Conclusions

It is concluded that parity and days in milk of the tropic dairy cows' farms in central China have a significant effect on milk yield and milk production traits. Increasing parity with advanced days in milk led to high SCC with elevation of milk fat, protein, total solids, and solids not fat and reduction in lactose and milk urea nitrogen, and this indicates poor quality and unfavorable milk. However, BHB reached the highest level from 101 to 200 days in milk. With advanced parity and within 101–200 days in milk cows become more susceptible to hyperketonemia which has an adverse effect on cow health and welfare. Hence, milk production traits in different parities and different lactation stage can be used as an indicator to evaluate the health condition of cows which may be helpful in identifying low production animals for culling and to improve farm management.

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Declarations

Ethics approval and consent to participate This study was approved by the Scientific Ethic Committee of Huazhong Agricultural University and was conducted according to the guidelines of care and using animals in research work (permit number HZAUCA-2019-005).

All authors agree to participate in the current work.

Consent for publication All authors agree to publish the findings of the current research.

Conflict of interest The authors declare no competing interests.

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