



Metabolic and Endocrine Attributes of Culled Dairy Cows Raised under Hot Climate

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ABSTRACT

Culling is a critical step that excludes the herd's unhealthy, low-than-average milk yield and sub-fertile cows. Heat load in the Arabian Gulf area imposes more burdens on cows' performance. Thus, the main goal of the present study was to explore the leading causes of culling in a private dairy farm located in the Arabian Peninsula and their inherent metabolic and hormonal attributes. This study encompasses 45 Holstein cows that were sorted to be culled from the farm due to repeated abortion (n=9; 20%), general health illnesses (n=9; 20%), low milk yield (n=10, 22%), repeat breeder (n=9, 20%), and udder diseases (n=8; 18%). Results revealed an increase in the activity of aspartate aminotransferase (AST) in cows with udder illnesses; however, alanine aminotransferase (ALT) exhibited the highest activity in cows with low milk yield and general health issues and the lowest activity in cows that had abortions. Glucose concentration was higher ($\approx 105\text{mg/dL}$, $P<0.05$) in low milk-producing cows, cows with general health problems, and those with udder diseases than in repeated abortion and repeat breeder cows ($\approx 75\text{mg/dL}$). Plasma total protein concentration was higher in the case of the diseased udder (85.01g/L) than in aborted (64.35g/L) cows. Low milk yield- cows revealed the highest low-density lipoproteins (LDL; 151.16mg/dL) and the lowest high-density lipoproteins (HDL; 45.13mg/dL). Progesterone concentration was lower (0.54ng/mL ; $P<0.01$) in low-milk cows compared with other categories. Luteinizing hormone (LH) concentration was highest in cows suffering from general health issues; however, other culling categories revealed low LH levels. Insulin and insulin-like growth factor-1 (IGF-1) concentrations were lowest in cases of low-milk yield and udder illnesses; however, other categories revealed higher levels of both hormones. In conclusion, cows suffering from reproductive inefficiency possesses lower pituitary functions, as shown by low LH. In contrast, cows suffering from udder issues and low milk yield exhibited less metabolic hormone concentrations, i.e., IGF1.

Key words: Dairy cows, Culling reasons, Metabolic parameters, Hormones, Heat stress

INTRODUCTION

Culling in dairies is defined as excluding individual cows who do not meet the standard parameters of productive ones raised in the herd under certain circumstances. Reasons for culling have been categorized as voluntary and involuntary (Rilanto et al. 2020). The culling decision represents cost loss in the herd, leading to improved productivity and reproductive efficiency. Several reasons for culling predominate in most dairies, including lameness, reproductive problems, udder malformation (i.e., deformity of the teats, mastitis, blind teat), low milk yield, major health disorders, and mortality (Compton et al. 2017; Kulkarni et al. 2023). Dutch cows' average culling rate was 25.4% due to slaughter and mortalities during 2007-2010 (Nor et al. 2014). Dairy cows, especially high-milking cows, are susceptible to

external high temperatures (Lucy 2019; Abdul Sammad et al. 2020). Lately, Chen and his colleagues have studied the effect of environmental heat stress on heat-tolerant and heat-sensitive dairy cows and concluded that the thermoregulatory mechanism in heat tolerant was more active, as it promptly activates the hypothalamic-pituitary-adrenal axis to secrete more types of neurotransmitters (i.e., epinephrine, norepinephrine, oxytocin, dopamine) (Chen et al. 2023). The high temperature and drought in the Arabian Gulf area might negatively affect the productivity and sustainability of dairy cows. These environmental cues might shorten the productive life of dairy cows and deteriorate the reproductive organs, leading to an excess culling rate. Therefore, the main goal of the present study was to explore the most common reasons for culling in large dairies and the interrelationships with metabolic and hormonal attributes.

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MATERIALS AND METHODS

Animals and management

A private dairy encompassing 1500 milking Holstein Friesian cows allocated in six yards has collaborated with Qassim University in this study. Forty-five cows (aged 2-11 years) were selected to be culled from the milking herd in the eastern region of the capital, Riyadh. The lowest daily milk yield considered the border for culling is <25kg milk/head/day. Culling is a critical practice that replaces the cows that do not comply with the set standards. During the summer of years 2021 and 2022, cows located in one of the yards were sorted, and 45 individuals were culled due to recurrent abortion (n=9), failure to conceive (n=9), general health issues (n=9), lower-than-average milk yield (n=10), and udder malformation and/or mastitis (n=8). Cows were offered the TMR (16.5% crude protein and 73.5% total digestible nutrients) diet as maintenance (1% of body weight) and production (1kg concentrate/2.5L milk), in addition to alfalfa hay, fresh water, and integrated salt licks were offered as free choices. Animals were vaccinated regularly and monitored for any health problems. The rotary milking machine accommodates 80-120 cows. Cows are manipulated for milking twice daily with an average milk yield of 25 liters/cow.

Culling reasons

Several categories of culling were mainly centered on low milk production, failure to conceive, recurrent abortions, udder malformation and mastitis, and general health problems (i.e., lameness, acidosis, mortality).

Blood sampling

Whole blood samples were collected from each cow twice at 30-day intervals via jugular venipuncture. Samples were collected in EDTA tubes and spun at 3000rpm/3min in a cold (5°C) centrifuge. Plasma samples were harvested and stored deep frozen (-80°C) until assayed.

Metabolic parameters determinations

Plasma AST levels were determined by a commercial ELISA kit (MyBioSource, San Diego, CA, USA), according to Babson et al. (1962), with a sensitivity of 0.05IU/mL. Plasma ALT levels were determined by a commercial ELISA kit (MyBioSource, San Diego, CA USA), according to Matsuzawa and Katunuma (1966), with a sensitivity of 0.6IU/mL. We monitored glucose levels according to the colorimetric method of Barham and Trinder (1972). We quantified total proteins in plasma according to the colorimetric method reported by Gyllensward and Josephson (1957). Low-density lipoproteins (LDL) levels were quantified in plasma using the Okada et al. (1998) method. High-density lipoproteins (HDL) levels were determined in plasma according to the method of Gordon et al. (1977).

Hormonal determinations

Plasma progesterone was determined using a simple solid-phase ELISA commercial kit (HUMAN, Gesellschaft für Biochemica und Diagnostica mbH, Germany). The determination followed the method reported by Joyce et al. (1981). All samples were determined in one assay with an intra-assay CV of 5.6% and sensitivity of 0.03-0.07 ng/mL.

Bovine LH determination in plasma was performed using a commercial sandwich ELISA kit (MyBioSource, Cat. No. MBS030927, California, USA). The standard curve range was set at 1.56-50mIU, the sensitivity was 0.4mIU, and the intra- and inter-assay CVs were <10 and <15%, respectively. Bovine insulin was determined by a commercial sandwich ELISA kit (MyBioSource, Cat. No. MBS9309584, California, USA). The standard curve range was set at 3.12-100mIU/mL, the sensitivity was one-mIU/mL, and the intra- and inter-assay CVs were <10% and <15%, respectively. Bovine IGF-I was determined by a commercial sandwich ELISA kit (MyBioSource, Cat. No. MBS165384, California, USA). The standard curve range was set at 1-40ng/mL, the sensitivity was 0.53ng/mL, and the intra- and inter-assay CVs were <8 and <10%, respectively.

Statistical analysis

Descriptive analyses were performed to evaluate variables: AST, ALT, glucose, total protein, LDL, HDL, progesterone, LH, insulin, and IGF-1. A one-way analysis of variance (ANOVA) was performed to make statistical comparisons among groups (i.e., culling categories) (IBM 2012). Analysis of the normal distribution of data was examined with the Kolmogorov-Smirnov test; if the data were not normally distributed, then a Kruskal-Wallis one-way ANOVA (non-parametric statistical test) was used to test for the presence of significant differences among all groups (SPSS, version 22). The data were considered statistically different if $P < 0.05$. Data were expressed as the mean \pm SEM. The Pearson correlation method is used to evaluate the association between variables. If the P value is <5%, then the correlation between the two variables is significant.

RESULTS

Culling reasons

In the whole herd, the percentage of culling due to lameness is 4.5%, low milk yield is 12%, reproductive failure is 6.5%, general illnesses are 2%, abortions are 2%, and mastitis/udder malformation is 3.5%. However, in the sample taken for the present study, the percentage of culled cows was recurrent abortions (n=9; 20%), general health illnesses (n=9; 20%), low milk yield (n=10, 22%), repeat breeder (n=9, 20%), and udder diseases (n=8; 18%).

Metabolic attributes

As illustrated in Table 1, non-significant differences in AST were found among culling groups. Even though a mathematical elevation ($P > 0.05$) was observed in the cows culled due to their udder abnormalities and/or mastitis compared to the other culled cow's categories whose values were statistically similar. On the contrary, significant ($P < 0.05$) elevations in ALT activity were obtained in cows suffering from general health issues and those producing low milk yield. Cows that suffered from repeated abortions revealed the lowest ALT activity. Concerning blood glucose, there exist significant ($P < 0.05$) differences among culling groups, showing high concentrations in cows culled for udder disorders, low milk production, and general health illnesses. However, cows culled for reproductive failures (i.e., repeated abortions and conception failures) exhibited lower glucose concentrations by about 23-41% of their counterparts.

Additionally, there exists a significant ($P<0.05$) elevation in total protein (Table 1) in cows culled due to udder disorders and/or mastitis, exceeding that in cows culled for frequent abortions by about 24%. There were non-significant differences in total protein among other culled groups.

The range of plasma LDL among culled cows was 98.89–151.16mg/dL (Fig. 1). The highest level ($P<0.05$) of LDL was found in the cows culled for low milk yield compared with that level in cows culled for general health illnesses. Other categories of culled cows have shown similar levels of LDL with non-significant ($P>0.05$) differences. HDL level was significantly higher ($P<0.05$) in cows that suffered from recurrent abortions and conception failure (i.e., cows having reproductive failures) than other culled cows. Meanwhile, cows culled for low milk yield and the unhealthy cows showed significantly lower ($P<0.05$) levels of HDL. Moreover, cows with diseased udders revealed intermediate HDL levels with non-significant differences from other groups.

Hormonal parameters

Cows that had recurrence abortions, conception failures, general health problems, and udder malformation and/or mastitis revealed higher ($P<0.05$) levels of progesterone compared with cows that produced low milk yield. The increase of progesterone in all culled cows over those culled for low milk productivity was 4.4 folds. As shown in Table 1, the highest ($P<0.05$) concentration of estradiol-17 β was present in the cows that were culled for various health disorders. However, other culled group cows revealed low estradiol concentrations with non-significant differences. Cows that suffered from general health problems revealed the highest ($P<0.05$) level of LH

compared with other culled cows who exhibited similar ($P>0.05$) LH concentrations. Insulin concentrations were higher ($P<0.05$) in cows that were culled for repeated abortions ($4.6 \pm 0.75 \mu\text{IU/mL}$), conception failure, and general health illnesses compared with LH concentrations in cows that were culled for low milk production and those cows that were culled for udder malformation and/or mastitis. The reduction of insulin in culled dairy cows due to the unhealthy conformation of their udders, and consequently due to their low milk output, approached 65% of those levels recorded in culled cows based on their reproductive inefficiency. The concentrations of insulin-like growth factor 1 (IGF1) were higher ($P<0.05$) in cases of recurrent abortions and conception failure cows than in cows yielding low milk. However, no significant differences ($P>0.05$) were detected among cows with udder malformations and/or mastitis and cows with several health illnesses.

Correlations between tested parameters

Table 2 exhibits the possible correlation coefficients between different parameters measured in the study. A significant positive relationship ($r=+0.252$) exists between AST and ALT. Likewise, a positive relationship ($r=+0.236$) was obtained between ALT and LH. A similar finding was found between glucose and plasma total protein ($r=+0.243$). However, significant negative relationships were observed between glucose and HDL ($r=-0.325$) and glucose and insulin ($r=-0.422$). Also, a negative correlation was found between LDL and IGF-I ($r=-0.273$). On the contrary, a positive correlation exists between HDL and insulin ($r=+0.276$). They also found a positive correlation between insulin and IGF-I ($r=+0.362$). Otherwise, there were non-significant correlations between other parameters.

Table 1: Effect of culling reasons of dairy cows on plasma biochemical and hormonal attributes

Parameters	Culling reasons				
	Abortion	Repeat breeder	Low milk yield	Health illnesses	Udder disorders
AST (U/L)	24.58 \pm 4.36	24.03 \pm 2.15	25.73 \pm 1.64	28.44 \pm 3.09	32.31 \pm 3.17
ALT (U/L)	16.35 \pm 1.64c	20.06 \pm 1.71b	21.48 \pm 1.29a	23.17 \pm 2.30a	20.62 \pm 1.47ab
Glucose (mg/dL)	85.24 \pm 6.52b	64.70 \pm 9.07b	105.34 \pm 5.11a	100.50 \pm 6.76a	110.00 \pm 11.21a
Total protein (g/L)	64.35 \pm 7.10b	72.43 \pm 6.98ab	79.30 \pm 4.39ab	79.06 \pm 7.37ab	85.01 \pm 6.42a
Progesterone (ng/mL)	2.05 \pm 0.56a	2.57 \pm 0.60a	0.57 \pm 0.36b	2.56 \pm 0.75a	2.98 \pm 0.94a
Estradiol (pg/mL)	32.59 \pm 13.78b	33.96 \pm 17.23b	46.09 \pm 15.96b	101.59 \pm 45.49a	16.05 \pm 10.38c
LH (Miu/mL)	4.24 \pm 0.63b	4.84 \pm 0.39b	3.27 \pm 0.28b	8.29 \pm 1.83a	4.48 \pm 0.70b
Insulin ($\mu\text{IU/mL}$)	4.60 \pm 0.75a	6.34 \pm 1.49a	1.73 \pm 0.11b	5.19 \pm 0.87a	2.00 \pm 0.37b
IGF-1 (ng/mL)	45.14 \pm 4.12a	49.63 \pm 3.96a	35.95 \pm 3.78b	41.79 \pm 3.05ab	40.62 \pm 2.78ab

Values (Mean \pm SE) in the same row with different alphabets differ significantly ($P<0.05$).

Table 2: Correlation coefficients between parameters measured in culled cows.

	AST	ALT	Glucose	Protein	LDL	HDL	Insulin	IGF-I	LH	Progest. [§]
AST	-									
ALT	+0.252*	-								
Glucose	0.163	0.039	-							
Protein	-0.201	0.155	+0.243*	-						
LDL	-0.022	-0.115	0.044	0.093	-					
HDL	-0.118	-0.148	0.325**	-0.089	-0.096	-				
Insulin	-0.069	0.038	-0.422**	-0.146	-0.148	+0.276*	-			
IGF-I	-0.117	0.039	-0.155	-0.049	-0.273*	0.200	0.362*	-		
LH	0.112	+0.236*	-0.008	-0.079	-0.175	0.092	0.105	-0.021	-	
Progest. [§]	0.053	-0.083	-0.109	0.083	-0.052	0.179	0.208	0.167	0.139	-

[§]Progesterone; * Significant at $P<0.05$, ** Significant at $P<0.01$. AST=Aspartate amino transferase; ALT=Alanine amino transferase; LDL=Low-density lipoproteins; HDL=High-density lipoproteins; IGF-I=Insulin-like growth factor 1.

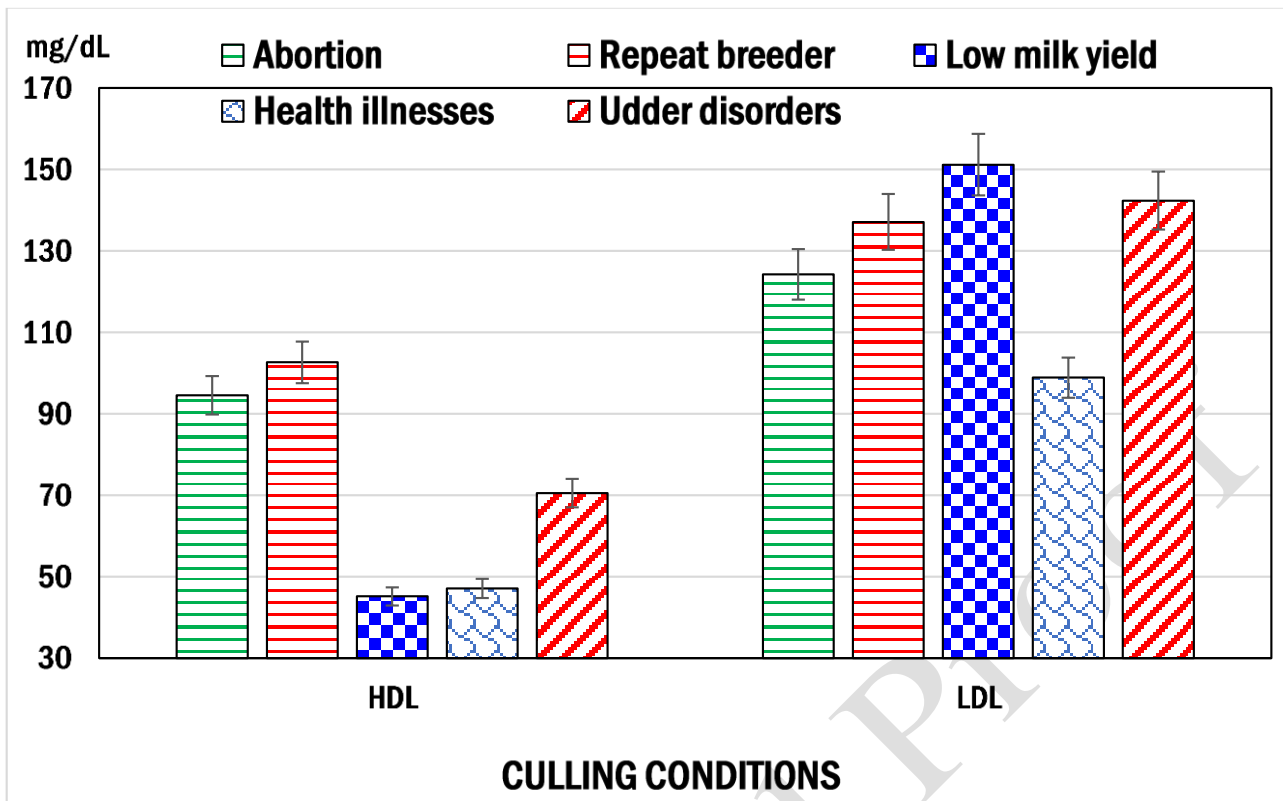


Fig. 1: Plasma (Mean±SE) high-density lipoproteins (HDL) and low-density lipoproteins (LDL) as affected by the culling reasons of dairy cows.

DISCUSSION

Culling is a critical process in any dairy enterprise. Several culling reasons have been investigated regardless of looking at the environmental situation. In the current study, the researchers investigated the reasons for culling in a sizeable private dairy farm in the Kingdom of Saudi Arabia. Several causes for cow's elimination from the herd have been recognized. Primary reasons for the culling decision were recurrent miscarriage, low milk yield, low conception rates, general health illnesses, and udder malformation and/or mastitis. The extremely high temperature during summer negatively impacts the milking cows' production and reproduction ability (Gantner et al. 2011). The climate temperature during the hot summer months (June-September) ranges between 38 (minimum) - 42°C (maximum) in the shade. Lin et al. (2010) stated that an ambient temperature of 18-23°C and relative humidity of 35-70% are suitable for mammalian comfort. Thus, it has been suggested that the temperature/humidity index is the best indicator for measuring heat stress in Saudi Arabia (Al-Bouwarthan et al. 2019). Extreme heat stress with relative humidity ranging from 20-70% probably imposes physiological changes that lead to productive and reproductive inefficiency.

General health issues and mastitis are significant reflections of liver diseases resulting in low milk yield in dairy cows. Recently, Kerwin et al. (2022) reported that the liver health index (LHI) directly correlates with milk production, general health, and reproduction in multiparous but not primiparous dairy cows. Moreover, elevated liver enzymic activities have been used to indicate fatty liver (fat

cow syndrome), which led to decreased milk yield in dairy cows (Bombik et al. 2020). ALT and AST are positively correlated in the current study. Average blood glucose in milking dairy cows was reported to be 78-92mg/dL, depending on the cow breed and body condition score (BCS) (Jaackson et al. 2013). Endocrine and metabolic profiles affect many nutrients: insulin, insulin-like growth factor 1, glucocorticoids and growth hormone, which significantly impact the provision of nutrients in ruminants' bloodstream (Baumgard et al. 2017). Pawliński and his coworkers recently reported a significantly higher blood glucose level in high- than in low milk yield-dairy cows (Pawliński et al. 2023).

Moreover, glucose exhibited a positive correlation with plasma protein ($r=0.243$), a negative correlation with insulin ($r=-0.422$), and a negative correlation with HDL ($r=-0.325$). Andjelić et al. (2022) found a positive correlation between glucose and protein in the plasma of dairy cows during lactation. Furthermore, it has been found that heat stress resulted in a high blood glucose concomitant with a high insulin concentration in milking dairy cows (Abbas et al. 2020), as it has been known that HDL is a good indicator of general health and milk production. In the current study, the cows culled for general health issues, low milk yield, and udder malformation and/or mastitis revealed lower-than-average HDL levels, which were in line with previous findings (Sehati et al. 2022). Additionally, there were negative correlations between plasma glucose and insulin in the cows culled for low milk production and cows with unhealthy udders. Leiva and his colleagues reported glucose intolerance in milking dairy cows with low milk yield or excessive energy provision (Leiva et al. 2015).

The only category of culled cows in the current study that had shown a significantly reduced level of progesterone (P4) is those eliminated for the low milk yield. However, the other categories of culled cows revealed similarly high progesterone levels. Nonetheless, all categories of culled cows had lower-than-normal levels of progesterone. In this context, Widayati and coworkers indicated a negative correlation between circulating progesterone level and milk yield in dairy cows since the progesterone level was 3.07ng/mL in ordinary but 1.17ng/mL in repeat breeder cows (Widayati et al. 2019). The primary source for progesterone production is HDL, which has shown a concomitant reduction in low milk-producing cows with P4 circulating levels (Wiltbank et al. 2014). Also, the metabolic regulator, HDL, as a source of cholesterol for P4 build-up, and the circulating level of LH have shown a positive relationship. These distinct relationships were evident in the low milk yield cows. It has also been stated that, in high-producing milk cows, elevated liver blood flow is accompanied by high P4 metabolism. Luteinizing hormone concentration revealed low figures in cows culled for reproductive subfertility issues (i.e., miscarriage, repeat breeding without conception, low milk yield, and unhealthy udders). It is well known that LH is responsible for ovulation, luteinization, and maintenance of the corpus luteum (CL) (Stevenson and Britt 1979). The low levels of circulating LH in cases of abortions, failure to conceive, low milk yield, and udder illnesses might explain the insufficient amount of this hormone to trigger its main actions in such cows.

Metabolic hormones are excellent indicators of the health status of mammals. In ordinary dairy cows, the circulating insulin level is 7.16 μ IU/mL. However, the insulin levels in culled cows due to low milk production and udder illnesses were as low as 24 and 28% of their counterparts in the regular cows (Azarbayejani and Mohammadsadeh 2021), even though the levels of insulin in other categories of culled cows were reduced than the average level by 35.7, 11.5, and 27.5% in cases of recurrent abortions, conception failure, and general health issues, respectively. Simultaneously, IGF-1 levels showed the same trend as insulin levels. The lowest IGF-1 levels were found in the low-milk yield cows and cows with udder diseases. Moreover, a significant negative correlation was found between circulating IGF-1 and milk yield (Falkenberg et al. 2008). In conclusion, in a hot climate, the harsh environment might burden dairy cows' productivity and reproductive performance. Culling the less-than-normal cows from dairy farms indeed elevates the profit of the enterprise and keeps up the high production and better reproduction of the whole herd.

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Competing interests

The authors declare that they have no competing interests.

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