Studies On Concentration of Some Milk Metabolic Enzymes at Different Parities, Stage of Lactation and Their Correlation with Composition and Yield of Milk in Red Sindhi Cows

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ABSTRACT
The study investigated the impact of parity and lactation stage on milk metabolic enzyme concentrations and their correlation with milk composition and yield in Red Sindhi cows. A total of 45 apparently healthy cows were selected from two cattle farms in Pakistan. Milk samples were collected, tested for subclinical mastitis, and analyzed for chemical composition and enzyme activity. Notably, alkaline phosphatase (ALP) activity increased significantly from 1st parity to 3rd, 4th, and beyond 4th parity during early, mid, and late lactation. Aspartate amino transferase (AST) concentration was higher from 1st to 2nd parity across all lactation stages, followed by a decrease with increasing parity. Lactate dehydrogenase (LDH) concentration varied significantly during different lactation stages and parities concentration was significantly higher during early lactation of 4th parity cows, mid lactation of cows at >4th parity, and late lactation of 1st parity cows Additionally, milk fat and solid-not-fat (SNF) content were notably higher at >4th parity and 1st parity, respectively. Density of milk decreased and total solids (TS) increased with parity. Slightly higher pH was recorded in milk at 1stand 2ndparity. Protein, ash and chloride and milk yield increased with parity and lactation stage and milk yield .has positive correlation with AST and negative correlation with LDH and ALP. Ash, Fat Protein, and lactose have positive correlation with LDH, while chloride and pH have negative correlation with AST and LDH These findings provide valuable insights for the dairy industry.

Keywords: Metabolic Enzymes, Red sindhi cows, Parity, Lactatio

INTRODUCTION

Milk is considered a complete diet for young mammals because it contains all the essential nutrients needed for growth and development, including proteins, carbohydrates, fats, minerals, and vitamins. Additionally, it serves as a vital source of water for newborns. Quality of milk is dependent on its chemical composition that effects on milk processing in dairy industry. (Ozrenk and SelcukInci, 2008). Many researchers have reported that milk yield and its composition vary with variation in parity and lactation. Besides seasonal variation and changes in dietary condition and physiological status animal and genetic factors impact on mil production and qaulaity (Slots et al., 2009; Mapekula et al., 2011; Frelich et al., 2012; Myburgh et al., 2012 and Lee et al., 2014). (Gonzalo et al., 1994), (AlSaiady, 2006), (Oravcova et al., 2007) and (El-Tarabany and ElBayoumi, 2015) also mentioned that Productive and reproductive traits of lactating cows and small ruminants are affected by these factors. Udder health plays important role in milk quality. If not cared properly it can adversely effects on production and quality milk which in turn pose great economic loss to dairy industry. Therefore, veterinary health workers and livestock farmers must be aware of the tools for early detection of udder health. Elevated somatic cell count (SCC) and leukocytes in milk indicates the inflammation of udder (Nabih and Rahman, 2015). Increased SCC in milk due to infection and damages in mammary epithelium negatively affect by reducing milk synthesis and altering concentration of fat and lactose in milk. Increased urea inversely influences percentages of milk protein and SCC (Johnson and Young, 2003; Novac and Anderi, 2020). Activity of some metabolic enzymes are activated or inhibited in response to inflammatory conditions in udder. Cytoplasm of animal cells bacteria and yeast contains a non-lysosomal enzyme called LDH that increases in milk when any damage occurs in mammary

epithelial cell and inflammation therefore it is used a biochemical marker for detection subclinical mastitis. It is an indicator of oxidative stress in mammary cells occurring due to increased metabolism of mill constituents (Novac and Anderi, 2020). Activities of liver catabolic enzymes i.eAlanine aminotransferase (ALT), Aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), and alkaline phosphatase (ALP)andn their concentration in plasma and serum of postpartum Holstein cows indicates health of cows (Stojević et al., 2005; and Śamanc et al., 2011) and activities of these enzymes in milkindicates udder function and health (Liu et al., 2012 and 2013). Analysis of these enzymes is used for evaluation of udder health in dairy cows (Fox and Kelly, 2006). Alkaline phosphatase is naturally found in milk, its levels are elevated above normal when tissue mammary tissues are damaged (Patil et al., 2015). Function of liver, skeletal muscle and heart is determined by the concentration of AST in serum of cows (Kaneko et al., 2008). Elevated concentration of AST reflects liver failure (Sattler and Fürll, 2004) and damage in cell structure (Milinković-Tur et al., 2005). Activities of ALP, AST and LDH are used as biochemical markers for detection inflammatory changes or any damage to mammary epithelium of dairy cow. Their concentration is increased in high yielding cow with high somatic cell count and subclinical mastitis.

MATERIAL AND METHODS

Study site

The proposed study was conducted on milk of Red Sindhi Cattles selected from two government farms i.e., Red Sindhi Cattle Farm Tando Muhammad khan and Red Sindhi Cattle Farm Korangi Karachi and Commercial dairy Farms around Hyderabad Division,Pakistan

Animals and experimental design

Table I shows total number of 45 Red Sindhi Cattles were selected and grouped into different parities and stage of lactation.

Table I. Selection of animals according to parity and lactation stage

Parity	Lactation stage			Total
	Early	**Mid**	**Late**	
	1-3 months	**3-6 months**	**>6 months**	
1st	n= 03	n=03	n=03	n=09
2nd	n= 03	n=03	n=03	n=09
3rd	n= 03	n=03	n=03	n=09
4th	n= 03	n=03	n=03	n=09
>4th	n= 03	n=03	n=03	n=09
Total	n= 15	n=15	n=15	n=45

Defatting and Enzyme Assays: Each 10 ml sample was defatted, and the milk whey was stored for enzyme assays. Remaining milk was used for composition analysis.
Subclinical Mastitis Testing: All samples were tested for subclinical mastitis using an EC meter.

Analysis of Milk:

pH Measurement: The pH of the milk was measured using a pH meter.
Total Milk Fat Content: The Gerber Method was used to determine fat content.
Total Protein Content: Formol titration method was employed. The formula for total protein calculation is: total protein = (V2-V1) × 1.94.
Chloride/Salt Content: Determined using the Silver nitrate titration method.
Ash Content: Gravimetrically calculated by heating pre-weighed crucibles with milk in a muffle furnace.
Density of Milk: Measured using a lactometer.
Total Solids: Calculated based on moisture content.
Solid-Not-Fat (SNF): SNF (%) = Fat × 0.22 + LR × 0.25 + 0.72.
Lactose Content: Lactose (%) = Total Solids % - (Fat % + Protein % + Ash).
Enzyme Assay: Activities of ALP, AST, and LDH were measured using Kinetic methods.

STATISTICAL ANALYSIS

Collected data was tabulated in excel sheet and transferred to data sheet of statistical analysis software Statistic 8.1 by using two-way ANOVA test. Descriptive statistics was performed to now means and standard errors. LSD was calculated and statistical difference between the groups and level of significance was be determined at $P<0.05$.

RESULTS

Metabolic enzymes in Milk

Influence of Parities and Lactation stage on concentration of alkaline Phosphatase on milk of Red Sindhi Cows

Table II shows that in the first parity concentration of alkaline phosphate in milk was recorded significantly (P<0.05) higher in the third lactation stage followed by second then first lactation stage. While among all three lactation stages of Red Sindh cow numerical non-significant variation (P<0.05) was noted in Alkaline phosphate concentration in the milk in second and third parity except statistical variation in the first lactations of bothparties (1 and 2nd). Moreover, Statistical analysis (ANOVA) results revealed that in the 4th and 5th parities significantly (P<0.05) higher concentration of alkaline phosphatase was recorded in the milk of Red Sindhi cow in the second lactation of 4th parity, though non-significant variations (P<0.05) were noted in alkaline phosphatase level among all three lactation stages of 4th and 5th parities. Furthermore, it is concluded that among all three parities and lactation stages statistically (P<0.05) higher concentrations of Alkaline phosphatase was noticed in the 2nd lactation of 4th parity compared to all lactation stages and parities.

Table II. Concentration of alkaline phosphatase (IU/L) in milk during different parities and lactation stages of Red Sindhi Cows

Parities	Alkaline phosphatase (IU/L)		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	110.27^{de}	147.03^{c-e}	392.11^{ab}
2nd	28.59^{e}	91.92^{de}	73.37^{de}
3rd	281.83^{a-d}	104.60^{de}	84.53^{de}
4th	55.45d^{e}	434.17^{a}	183.60^{b-e}
>4	188.27^{b-e}	275.70^{a}-^{d}	363.40^{a-c}
LSD (0.05) = 240.66	SE ± = 117.49		

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of Parities and Lactation stage on concentration of Aspartate Amino transferase activity (ASTIU/L) on milk of Red Sindhi Cows.

Table III Shows the influence of different parities and stage of lactation concentration of aspartate amino transferase (AST) in milk of Red Sindhi Cows. Results revealed significant difference in AST concentration between 1st, 2nd, 4th and 5th parities. Significantly (P<0.05) highest concentration was recorded milk of Red Sindhi Cows at second parity followed by 4th and 3rd parity. Lowest concentration was recorded at 5th parity. During 1st and 3rd parity highest concentration was recorded during late lactation. Whereas in 2nd parity significantly (P<0.05) higher concentration was recorded at mid lactation. During 4th and above parity cows' concentration of AST decreased from early to late lactation stage.

Table III. Concentration of Aspartate transaminase (IU/L) in milk during different parities and lactation stages of Red Sindhi Cows

Parities	AST (IU/L)		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	23.33^{d}	20.51^{d}	26.10^{d}
2nd	83.60^{ab}	113.83^{a}	62.23^{bc}
3rd	19.70 ^{d}	37.63^{cd}	39.03^{cd}
4th	75.90^{b}	67.02^{bc}	40.40^{cd}
>4	15.69^{d}	11.03^{d}	12.47^{d}
LSD (0.05) = 32.698	SE ± = 15.962		

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of Parities and Lactation stage on Lactate dehydrogenase (LDH, IU/L) on milk of Red Sindhi Cows

Table IV depicts the effect of different parities and stage of lactation on concentration of lactate dehydrogenase (LDH) in milk of Red Sindhi Cows. Statistical analysis of results revealed significantly (P<0.05) highest LDH concentration in the milk samples of cows at parity above 4th. Highest concentrations were recorded at parity above 4th followed 4th, 2nd, 3rd and lowest at parity 1st. Significantly (<0.05) higher concentration of LDH was recorded in milk of cows above 4th parity during mid lactation. Whereas lowest at during mind lactation of first parity.

Table IV. Lactate dehydrogenase (LDH, IU/L) in milk during different parities and lactation stages of Red Sindhi Cows

Parities	LDH (IU/L)		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	112.88^{cd}	222.28^{b-d}	422.74^{a-c}
2nd	134.93^{cd}	548.68^{ab}	176.00^{cd}
3rd	74.66^{cd}	406.73^{a-c}	115.00^{cd}
4th	411.04^{a-c}	248.88^{b-d}	210.00^{b-d}
>4	182.43^{b-d}	755.53^{a}	313.60^{b-d}

LSD (0.05) = 368.41 SE ± = 179.8

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Milk composition

Influence of parities and lactation stage on the pH of milk of red Sindhi Cow

Table V depicts the influence of different parities and stage of lactation on milk composition of Red Sindhi Cows. It is evident from the data that pH of milk samples from cows at 1st and 2nd parity was slightly higher than those at parity 3rd, 4th and above. Milk of early lactating cows during 1st parity had slightly lower pH as compared to late lactation whereas during mid-lactation pH was significantly (P<0.05) higher than early lactation.

Table V pH of milk of different parities and lactation stages of Red Sindhi Cows

Parities	Ph		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	6.6800^{a-c}	6.7300^{a}	6.6967^{a-c}
2nd	6.7067^{ab}	6.6600^{bc}	6.6567^{bc}
3rd	6.6600^{bc}	6.6500^{c}	6.6667^{bc}
4th	6.6633^{bc}	6.6800^{a-c}	6.6533^{bc}
>4	6.6700^{bc}	6.6633^{bc}	6.6667^{bc}

LSD (0.05) = 0.0546 SE ± = 0.0266

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of parities and lactation stage on the density of milk in red Sindhi cows

Data shown in Table VI depicts the influence of different parities and stage of lactation on milk composition of Red Sindhi Cows. It is evident from the data that highest density of milk was recorded during 1st parity at early, mid and late lactation. Cows above 4th parity had significantly (P<0.05) lower density as compared to 1st parity during early, mid and late lactation stages.

Table VI. Density of milk of different parities and lactation stages of Red Sindhi Cows

Parities	Density		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	1.0310[a]	1.0307[a]	1.0290[bc]
2nd	1.0290[bc]	1.0280[d]	1.0280[d]
3rd	1.0290[bc]	1.0287[b-d]	1.0287[b-d]
4th	1.0293[b]	1.0280[d]	1.0283[cd]
>4	1.0283[cd]	1.0280[d]	1.0280[d]

LSD (0.05) = 0.0526 SE ± = 0.0245

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of parities and lactation stage on the fat of milk of red Sindhi Cows

Table VII depicts the influence of different parities and stage of lactation on the fat content of milk of Red Sindhi Cows. Results reveals that milk from cows over 4th parity had significantly (P<0.05) higher fat content as compared to those at 1st parity. Early lactating cows of over 4th parity had lower fat content as compared to mid and late lactation stage.

Table VII. Fat of milk of different parities and lactation stages of Red Sindhi cows

Parities	Fat %		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	2.5833[d]	2.7500[d]	3.5000[c]
2nd	3.5500b[c]	3.9167[ab]	3.9833[a]
3rd	3.5000[c]	3.7833[a-c]	3.9000[ab]
4th	3.5333b[c]	4.0167[a]	4.0500[a]
>4	3.8500[a-c]	4.1167[a]	4.1167[a]

LSD (0.05) = 0.3854 SE ± = 0.1882

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of parities and lactation stage on the SNF of milk Red Sindhi Cow

Table VIII depicts the influence of different parities and stage of lactation on the SNF of milk of Red Sindhi Cows. Parity wise comparison of the results showed significantly (P<0.05) highest SNF in milk of 1st parity cows than those above 4th parity. Milk Early lactating of 1st parity cows showed higher SNF than late lactation stage.

Table VIII. SNF of milk of different parities and lactation stages of Red Sindhi cows

Parities	SNF %		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	9.0967[a]	9.0200[ab]	8.8233[c-e]
2nd	8.8333[cd]	8.8167[de]	8.7633[d-f]
3rd	8.8133[de]	8.8000[de]	8.7800[de]
4th	8.9233[bc]	8.7700[de]	8.7800[de]
>4	8.7733[de]	8.7300[ef]	8.6633[f]

LSD (0.05) = 0.1029 SE ± = 0.0502

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of parities and lactation stage on the total solids (TS) in milk of Red Sindhi cows

Data shown in Table IX depicts the influence of different parities and stage of lactation on TS in milk Red Sindhi Cows. Results showed significantly (P<0.05) higher total solid concentration in milk of cows over 4th parity during early, mid and late lactation stages and lowest in milk of 1st parity cows. It is also evident from the results that total solids increased as lactation progressed. Lowest density was recorded during early lactation than mid and late lactation stages.

Table IX. TS of milk of different parities and lactation stages of Red Sindhi cows

Parities	Total Solids(TS) %		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	11.670[e]	11.770[e]	12.323[d]
2nd	12.383[cd]	12.733[ab]	12.747[ab]
3rd	12.313[d]	12.583[a-d]	12.697[a-c]
4th	12.457[b-d]	12.797[ab]	12.830[a]
>4	12.747[ab]	12.847[a]	12.797[ab]

LSD (0.05) = 0.3470 SE ± = 0.1694

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of parities and lactation stage on the protein in milk of Red Sindhi cow.

Table X depicts the influence of different parities and stage of lactation on protein in milk of Red Sindhi Cows. Significantly (P<0.05) higher protein content was recorded in milk samples from cow over 4th parity and lowest in milk of those at 1st parity. Moreover, it is observed that milk of late lactation stage had higher protein content as compared to early lactation stage.

Table X. Protein of milk of different parities and lactation stages of Red Sindhi Cows

Parities	Protein %		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	3.0667^{e}	3.1333^{de}	3.2333^{cd}
2nd	3.3000^{bc}	3.4000^{ab}	3.4000^{ab}
3rd	3.2000^{c-e}	3.4000^{ab}	3.4667^{a}
4th	3.3000^{bc}	3.4667^{a}	3.5000^{a}
>4	3.4000^{ab}	3.4667^{a}	3.5333^{a}

LSD (0.05) = 0.1573 SE ± = 0.0768

Superscripts with different letters in rows and columns varied significantly (P < 0.05).

Influence of parities and lactation stage on the lactose in milk of Red Sindhi cow.

Table XI shows the influence of different parities and stage of lactation on lactose content in milk of Red Sindhi Cows. It is evident from the data that lactose content increased as parity increases. Significantly (P<0.05) higher lactose content was recorded in milk samples of cows above 4th parity and lowest at 1st parity. No significant difference was observed during early, mid or late lactation stages.

Table XI. Lactose of milk of different parities and lactation stages of Red Sindhi cows

Parities	Lactose %		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	2.5733^{c}	2.7500^{c}	3.5000^{b}
2nd	3.5500^{b}	3.9167^{a}	3.9833^{a}
3rd	3.5000^{b}	3.7833^{ab}	3.9167^{a}
4th	3.5333^{b}	4.0033^{a}	4.0500^{a}
>4	3.9500^{a}	4.1167^{a}	4.1000^{a}

LSD (0.05) = 0.3592 SE ± = 0.1754

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of parities and lactation stage on the milk yield of milk in Red Sindhi cow

Table XII depicts the influence of different parities and stage of lactation on milk yield of Red Sindhi Cows. Result of milk yield shows that milk yield increases as parity increases. The highest milk yield was recorded at parity 4th. Then yield decreased as parity increased over 4th. There was significant (P<0.05) difference between milk yield at 1st, 2nd, 4th and over 4th parity. Increased milk yield was recorded at mid lactation stage of all parity cows except 2nd parity cows whose milk yield was higher during early lactation.

Table XII Milk yield of different parities and lactation stages of Red Sindhi cows

Parities	Milk yield (L/time)		
	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	3.2333^{de}	3.9000^{ab}	2.5000^{f}
2nd	4.2333^{b-e}	3.5000^{a}	3.0667^{e}
3rd	3.5667^{b-d}	4.2333^{a}	3.7000^{bc}
4th	3.7333^{bc}	4.3333^{a}	3.5667^{b-d}
>4	3.3000^{c-e}	3.7000^{bc}	3.1000^{e}

LSD (0.05) = 0.4362 SE ± = 0.2130

Superscripts with different letters in rows and columns varied significantly (P < 0.05).

Influence of parities and lactation stage on the Ash of milk in red Sindhi Cows.

Data shown in Table XIII. depicts the influence of different parities and stage of lactation on ash content in milk of Red Sindhi Cows. It is evident from the data that ash content increased as parity increased. 1st parity cows had lowest ash

content and cows greater than 4th parity had highest ash content. However, there was no significant difference in ash content amongst parities. During 1st and 4th parity ash content in milk was higher at late lactation stage. Whereas in 2nd and 3rd parity ash content was higher in milk at mid lactation stage. Milk of Cows over 4th parity had higher ash dur ing early lactation period.

Table XIII. Ash of milk of different parities and lactation stages of Red Sindhi cows

Parities Ash %

	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	0.7333^{b}	0.8000^{ab}	0.9333^{a}
2nd	0.7333^{b}	0.9000^{ab}	0.8667^{ab}
3rd	0.8667^{ab}	0.9000^{ab}	0.7667^{ab}
4th	0.8667^{ab}	0.8000^{ab}	0.9000^{ab}
>4	0.9333^{a}	0.8333^{ab}	0.8333^{ab}

LSD (0.05) = 0.1961 SE ± = 0.0957

Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Influence of parities and lactation stage on the Chloride concentration of milk in red Sindhi cow.

Data shown in Table XIV. depicts the influence of different parities and stage of lactation on chloride concentration in milk of Red Sindhi Cows. The results show increase in chloride content as parity increases. Statistical analysis of data determined the significantly higher (P<0.05) chloride content at 4th parity. Moreover, chloride content increased as lactation stage increase. Milk of early lactating cows had lower chloride as compared late lactating cows.

Table XIV. Chloride concentration of milk of different parities and lactation stages of Red Sindhi cows

Parities Chloride concentration %

	Lactation stage		
	Early (1-3 months)	**Mid (3-6 months)**	**Late (more than 6 months)**
1st	0.0233^{c-f}	0.0253^{a-c}	0.0250^{b-d}
2nd	0.0227^{d-f}	0.0223^{ef}	0.0260^{ab}
3rd	0.0233^{c-f}	0.0247^{b-e}	0.0263^{ab}
4th	0.0227^{d-f}	0.0257^{a-c}	0.0277^{a}
>4	0.0220^{f}	0.0217^{f}	0.0260^{ab}

LSD (0.05) = 2.651 SE± = 1.294

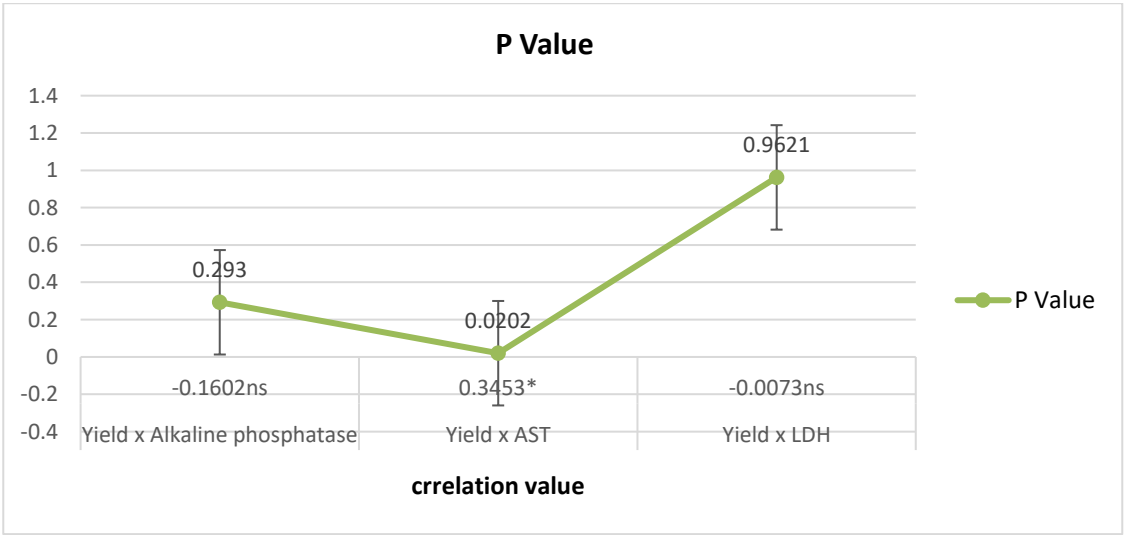
Superscripts with different letters in rows and columns varied significantly (P < 0.05)

Correlation matrix

Correlation between milk yield and metabolic enzymes in milk of Red Sindh Cows

Figurer 1. shows the correlation amongst milk yield and metabolic enzyme of milk i.e. Alkaline phosphatase (ALP), Aspartate transaminase (AST) and Lactate dehydrogenase concentration in milk of Red Sindhi Cows. Milk yield had significantly (P<0.05) positive correlation with AST. Whereas there was negative correlation between milk yield with Alkaline phosphatase and LDH.

Figure 1. Correlation matrix (Pearson) among yield, alkaline phosphatase, AST, and LDH enzymes concentration in milk



Correlation amongstsome metabolic enzymes in milk and its chemical composition.
Alkaline phosphatase had non-significant correlation with ash, fat, protein, lactose, chloride concentration and pH of milk as shown in Figure 2a. whereas data shown in Figure 2b. shows significant (P<0.5) positive correlation of LDH with Ash, Fat, Protein, and Lactose content in milk. Negative correlation was recorded between AST and LDH with chloride concentration, and pH of milk.

Figure 2a. Correlation matrix (pearson) among milk enzymes and chemical characteristics

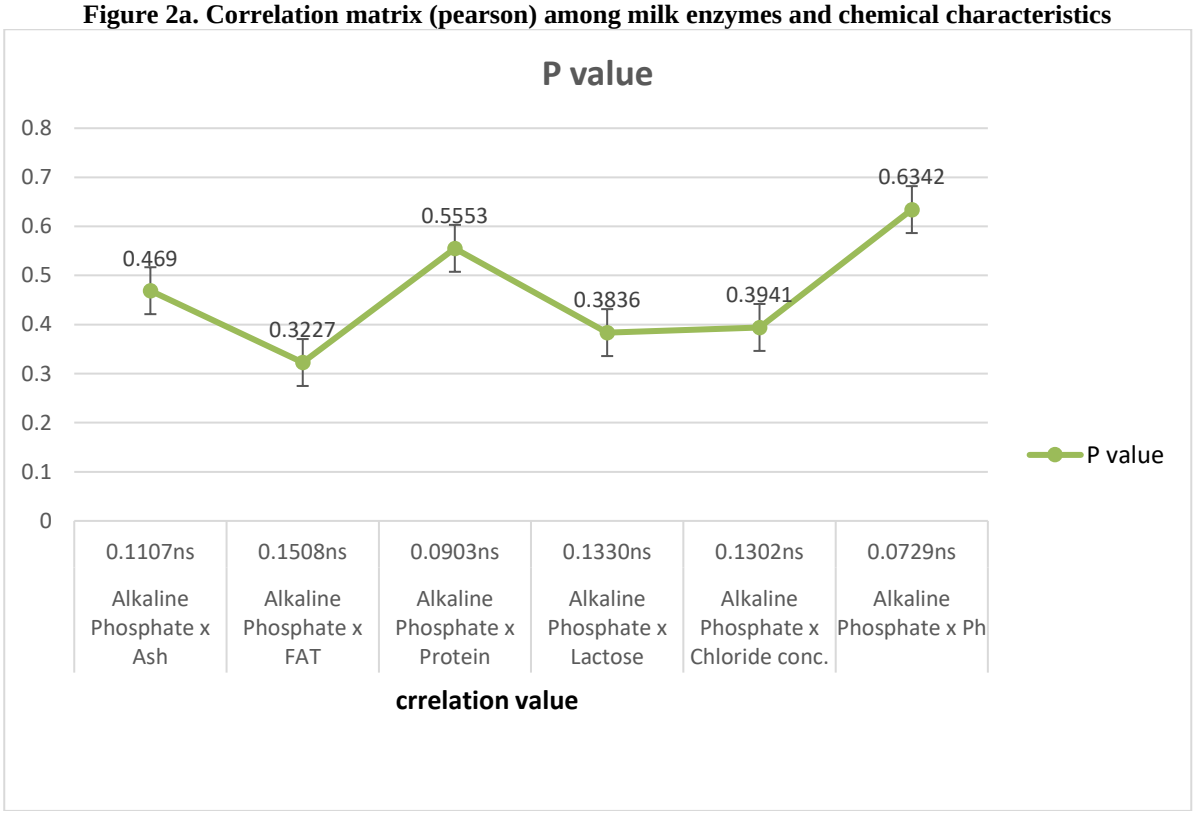
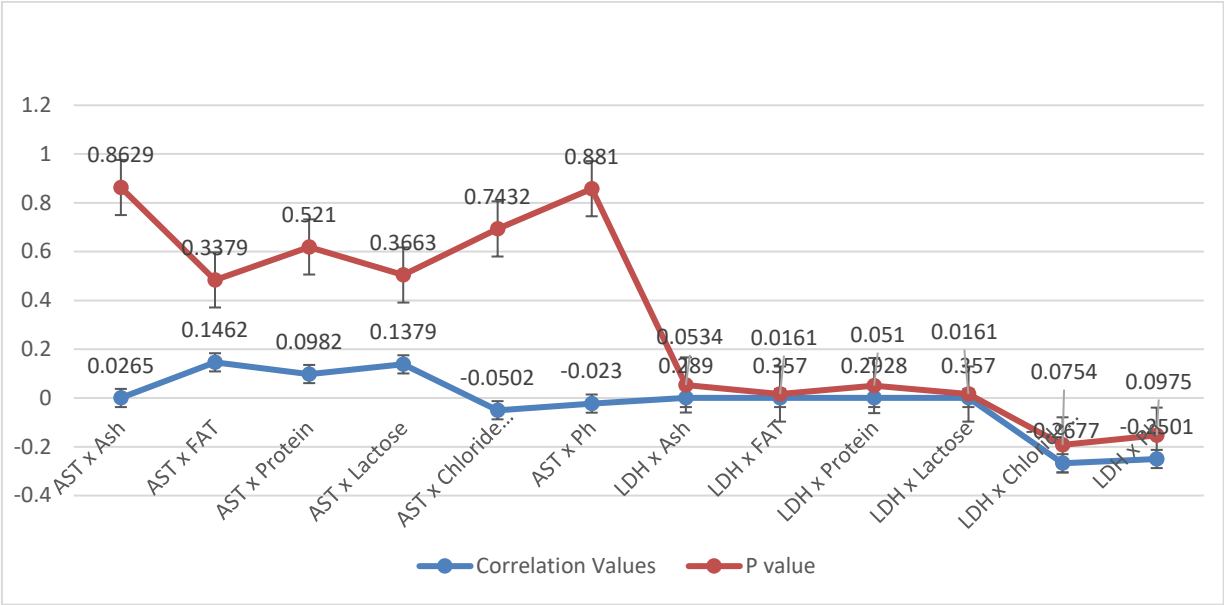


Figure 2b. Correlation matrix (Pearson) among milk enzymes and chemical characteristics 0.0265



DISCUSSION

Alkaline phosphatase is an important metabolic enzyme. It is increased in the milk in response to any infection leading to increased leukocyte infiltration and causes cellular damage in mammary epithelium. Thus used as biochemical marker for early detection of sub-clinical mastitis and udder health condition in lactating dairy cows (Hassan et al., 2014; Qayyum et al., 2018; Hussain et al., 2014; Akbari Aliabad et al., 2022). Studies on milk of cattle (Babaei et al., 2007) and camel (Ali et al., 2016) also indicated that ALP increases in mastitis condition. During present study we recorded that mean alkaline phosphatase was 132.82 IU/L during early lactation, 832.86 IU/L during mid lactation and 219.40 IU during late lactation. These results reflect that alkaline phosphatase increased with stage of lactation which could be due to increase milk yield causing stressful condition on the udder and damages to tissue due to oxidative stress or proinflammatory condition. Alkaline phosphatase activity reduces during late lactation when milk yield reduces. Similar trend is reported in the studies of (Mahmoud, 2014) who found less alkaline activity in early than mid lactation i.e 1771±330.0 and 2253±288.7 IU respectively. Djokovic et al., 2019) also reported increasing trend activity of alkaline phosphatase in milk of Holstein dairy cows at different stage of lactation. They found, 121.64±32.56, 241.11±109.31, 199.23±186.23U/L ALP during, early, mid-and late lactating cows respectively. The results of present studies are this in accordance with findings of these researchers. (Nabih and Rahman, 2015) assessed the influence of intramammary infection on activities of AST. Alkaline phosphatase in non-infected buffalo cows, singly infected and mixed infected animals were 279.60±28.11, 420.07±40.55b and 566.25±60.38 IU/L respectively. In present study values of alkaline phosphatase activity during late lactation were higher than that of non-infected buffaloes which is 279.60±28.11. Higher values of alkaline phosphatase in singly or mixed infections in buffaloes support the increased levels of ALP during mid lactation in present study as udders are more prone to infections when milk yield is increase. However, no apparent mastitis was observed but there may be no-specific subclinical infections that might had contributed in increasing ALP.

Activity of LDH is also considered as an important factor in determining the udder health. Besides the cow factor also influence the LDH activity such as parity, lactation and seasons. The activity of LDH remain higher during first week after calving and then increase as lactation, and parity advances. LDH activity is increase as result of inflammatory condition in the udder resulting in epithelial damage in mammary glands and increase in somatic cell count (Babaei et al., 2007).Who observed significantly higher LDH in mastitis milk as compared to normal milk in Holstein Friesian Cows. He recorded 127.0 ± 29.6, 222.0 ± 34.67, 943.5 ± 105.3 and 1098.5 ± 107.1 IU/L LDH in normal, mastitis grade 1, 2 and 3 respectively. Similarly, (Guha et al., 2012) indicated higher LDH activity in sub-clinical mastitis than in normal milk i.e., LDH (U/L) 96.64±31.64 and 263.10±18.95 in normal and subclinical mastitis milk respectively. Increased LDH activity indicates the inflammatory condition in udder thus may be used as marker for udder health in dairy hers (Guha et al., 2012; Gera and Guha, 2011a). (Nyman et al., 2014) found that parity has significant influence on the LDH levels milk of dairy cows. They recorded 1.27 (1.08-1.48), 1.53 (1.30-1.80), 1.45 (1.22-1.72), 1.66 (1.37-2.01) and 2.10 (1.69-2.60) LDH U/L during first, second, third, 4th and >4th parity. The results of their study showed that LDH activity was less in early parity than in advanced parity or in older cows.

Aspartate aminotransferase (AST) is one of the liver enzymes that indicates the metabolic imbalanced. Various studies have been conducted to investigate activity of AST in blood serum and milk in animals in different parities and stage of lactation. Present study was conducted to investigate the influence of different parities and stage of lactation concentration of aspartate transaminase (AST) in milk of Red Sindhi Cows. Results of our study revealed significant increase (P<0.05) in concentration of AST with increase in parity. The highest concentration was recorded in milk of Red Sindhi Cows at second parity followed by 4th and 3rd parity. Lowest concentration was recorded at >4th parity. During 1st and 3rd parity highest concentration was recorded during late lactation.Whereas in 2nd parity significantly (P<0.05) higher concentration was recorded at mid lactation. During 4th and above parity cows' concentration of AST

decreased from early to late lactation stage (Kuczyńska et al., 2021) reported that aspartate aminotransferase (AST) activity increased until 2nd parity and then decreased as parity increased. Its concentrations were lower during late lactation period. Studies have shown relation of metabolic traits of blood and milk with the age and lactation (Sakowski et al., 2012; Vanholder et al., 2015). Studies of (Vanholder et al., 2015) reported negative correlation between AST and parity. They found decreased AST after the third parity and attributed this change to increased risk of developing ketosis. AST decreased from early to late lactation. Earlier studies by (Doornenbal et al., 1988) describe those levels of AST is increased as age increases. Heifer milk contain 123.8 IU/L whereas older cow at age of 6 to 10 year contain 132.7 U/L AST in their milk. Cow over third parity had decreased AST which increase the risk of ketosis and reduced milk production. In present study higher values of AST in milk may be due to the ketosis in Red Sindhi Cows because that is major cause of low milk production besides presence unnoticed sub-clinical mastitis may not be rule out.

(Stojević et al., 2005) studied the activities of AST, ALT and GGT in clinically healthy cows. They observed that activity of AST was higher during early lactation, and it increase as the lactation progress than decrease during late lactation and dry period.(Muhamod, 2104) also reported higher AST in milk of cows during early lactation i.e. 51.30±9.954 IU/L as compared to mid lactation i.e. 48.45±5.424 IU/L The finding of present study are in contrast to those of Mohammad (014) where we found decreased concentration late lactation than early lactation in 1st and 3rd parity cows and in 2nd parity significantly (P<0.05) higher concentration was recorded at mid lactation. (Kuczyńska et al., 2021) reported that aspartate aminotransferase (AST) activity increased until 2nd parity and then decreased as parity increased. (Djoković et al., 2013) found that AST concentration 69.46±27.54 IU/l in early-lactation cows which is higher than 39.31±18.90 IU/l in mid lactation, they attribute this change to metabolic changes during early lactation that were more intensive as a function of (EB) enrage balance, compared to mid lactation.(Sakowski et al., 2012) found significant negative correlation between average daily milk. They found increased level of AST in milk of high yielding cows as compared to low yielding cows. In low yielding cows' values of AST (unit/l) were 55.92± 0.08 and 53.76±A 0.13 during early 60 day and late 200 day respectively. Whereas in high yielding cows during early 6o day and at 200 day AST U/L were 75.36± 0.15 76.59± 0.17 respectively. (Guha et al., 2012) the results of their studies indicated that AST (U/L) of normal milk was 23.32±1.35 as compared to mastitis milk i.e., 22.87±1.02. (Nabih and Rehman, 2015) investigated relation between T3 milk level and enzymes in milk i.e., GOT, ALP, and LDH. They found 21.30±2.60, 32.01±2.26, 48.20±3.1 AST (IU/L) I normal, single infection and mixed infection milk respectively. The results revealed that the means of AST, ALP & LDH activities in milks from buffalo cows with subclinical mastitis were significantly (P<0.05) higher than those from healthy normal buffalo cows. This indicates that using by determination of enzymes activities in serum milk is a sensitive and reliable method for detection of bovine subclinical mastitis. (Hussain et al., 2014) have also reported that the enzymes including lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase were significantly higher in mastitis than healthy buffaloes.

Milk constituents varies amongst the cow composition of bovine milk is affected by stage of lactation, season, diet, dam age, physiological status, environmental conditions, region of production in addition to the genetic background (Slots et al., 2009; Mapekula et al., 2011; Frelich et al., 2012; Myburgh et al., 2012 and Lee et al., 2014). We conducted studies to evaluate the composition of milk as influenced by age, parity and lactation stage in Red Sindhi Cows. Results of present studies revealed slightly higher milk pH during 1st and 2nd parity cows than that of 3rd, 4th and above 4th parity cows. Moreover, pH increased with stage of lactation being higher in mid lactation. Density of milk also increases with increase in parity being significantly less (P < 0.05) in cows at p4th and higher parities as compared to 1st parity. Significantly higher (P < 0.05) fat content was observed in milk of cows over 4thparity; early lactating cows had had lower fat content. Significantly (P<0.05) highest SNF is recorded in milk of 1st parity cows than those above 4th parity. Milk early lactating of 1st parity cows showed higher SNF than late lactation stage. Total solids increased significantly (P<0.05) as lactation progressed. Protein content was also significantly (P<0.05) higher in milk samples from cow over 4th parity and lowest in milk of those at 1st parity. Late lactation stage had higher protein content as compared to early lactation stage. Similarly, lactose content was found significantly increased with progression in parity. No significant difference was observed during early, mid or late lactation stages. Ash content also increased as parity increased. 1st parity cows had lowest ash content and cows greater than 4th parity had highest ash content. As content was found higher during late lactation of 1st and 4th parity cows, mid lactation in 2nd and 3rd parity cows. Milk of Cows over 4th parity had higher ash during early lactation period. Chloride content also significantly increased (P < 0.05) as parity and stage of lactation increases. Milk yield was positively correlated to the increase in parity and decline in parity over 4th. Mid lactating cows had significantly highest milk yield. Significantly (P<0.05) positive correlation is recorded between milk yield and AST Whereas Alkaline phosphatase and LDH. Alkaline phosphatase was negatively correlated milk yield. With non-significant correlation with ash, fat, protein, lactose, chloride concentration and pH of milk as shown in table 16a. Whereas data shown in Table 16b shows significant (P<0.05) Positive correlation of LDH with Fat and Lactose content in milk. Negative correlation was recorded between AST and LDH with chloride concentration, pH of milk. (Shiue et al., 2016) have reported significant effect of breed n milk constituents i.e., milk fat, SNF (%), protein (%) and lactose (%). In present study significantly higher (P < 0.05) fat content is recorded in milk of Red Sindhi Cows that were over 4th parity and les fat content was recorded in milk of early lactating. Whereas SNF was significantly higher in milk of 1st parity cows than those above 4th parity. Early lactating of 1st parity cows showed higher SNF than late lactation stage. Total solids increased significantly (P < 0.05) as lactation progressed.

Milk composition is significantly influenced by type of cow breed. Local breed has significantly less milk contents compared to cross breed. All milk constituents except lactose is influenced by lactation stage and parity. There was increased fat percent on early and late lactation and significant increased fat in milk of cross bred cows during late lactation (Shiuep et al., 2016). They believed that reduced fat in milk during early and mid-lactation in crossbred cows is due to higher milk yield. Other studies mentioned that fat in milk fat increases with advancement of lactation that may be due to low mill yield in cow. (Auldist et al., 1998). (Bohmanova et al., 2009) found that milk fat was less in the late stage of lactation. Dietary changes such as higher concentrate and low roughage in feed of lactating cows may cause reduction in fat percent in milk (Sutton, 1989). (Nyamushamba et al., 2014) suggested that fat percent in cow milk is inversely proportional to the age of first calving. They also reported that due to increased anabolism of certain milk constituent during late lactation are increased e.g. SNF in milk of local as well as cross bred cows. In present study protein content was significantly ($P<0.05$) higher in milk of early parity cows than late parity cows and during over 4^{th} parity and similarly protein content increased as lactation increased. Our findings are contrast to those of (Shuiep et al., 2016) reported that local cows are significantly ($P \leq 0.05$) higher milk protein content in early and mid-lactation compared to the crossbred cows, but he did not found any significant difference in lactose e content between milk of local and cross bred cows at different parties from first to fourth and stage of lactation in same breed of cows. Similar observations were reported by (Mech et al., 2008). Less influence of lactation stages on lactose and its stability is defined by (Pollott, 2004) who consider the correlation of water secretion and lactose synthesis and its secretion in milk as possible reason for its stability throughout lactation and non-significant effect of lactation on lactose content. They recorded 4.20±0.95 to 5.23±0.50 L and 8.17±0.95 to 8.90±0.53 L milk yield of local and cross bred cows respectively. Showing significantly higher yield ($P \leq 0.05$) in crossbred cows. It was also observed that both stage of lactation and parity significantly ($P \leq 0.01$) effects daily milk yield. (Sahib et al., 2019) recorded average milk yield (kg), fat, SNF, protein and lactose percent irrespective of parity, pregnancy and stage of lactation were 7.9, 3.6, 8.1, 3.2 and 4.2, respectively. They reported that milk constituents and milk yield are not affected by the order of parity. Similar findings are reported by (Gurmessa and Melaku, 2012) and (Pratap et al., 2014) who observed contrast to their results (Yoon et al., 2004) reported that milk yield, milk fat and protein varied significantly that milk yield and major milk components (fat, SNF, protein and lactose) are not influenced by parity. Whereas in with parity. (Sahib et al., 2019) found that milk yield was significantly ($P<0.05$) lower and fat percent was significantly higher in the lactation stage above 200 days when compared to lactation stage below 100 days. Similar results were reported by (Gurmessa and Melaku, 2012) and (Shuiep et al., 2016). They attributed increase in milk fat percent in late lactation may be due to reduced milk yield during this period as reported by (Auldist et al., 1998). However, (Sudhakar et al., 2013) reported that milk constituents and milk yield were not influenced by the stage of lactation.

CONCLUSION

It is concluded from the results of present study that milk yield, composition of milk Concentration of ALP, LDH and AST is significantly influenced by parity and lactation stage of normal cows. Therefore, it is suggested that the stage of parity and lactation should be considered for evaluating milk for mastitis.

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Statement of conflict of interest

The mentioned authors have declared no conflict of interest.

CONTRIBUTION

JK, SAS, ABK and TA conceived and designed the experiments .JK performed the experiments.SH, MUF, SN and NM analyzed the data. ZK, BI, AU and AK revised the manuscript. AK, JK wrote the manuscript.

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