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Correlation between *in vitro* activity of milk leukocytes with milk constituents in high yielding crossbred cows

TRIPTI SHARMA BURAGOHAIN[1], SARATH CHANDRA REDDY[2], PRADIP KUMAR DAS[3], PROBAL RANJAN GHOSH[4], DIPAK BANERJEE[5] and JOYDIP MUKHERJEE[6]

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West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700 037 India

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Milk somatic cells play a pivotal role in defense of the mammary gland against mastitis-causing pathogens (Sordillo *et al.* 1997) and a significant interactions are also described between milk SCC and milk constituents (Ma *et al*. 2000) suggesting that global assessment of milk composition may be needed for evaluation at animal and herd level. *In vitro* activity of milk leukocytes could be used to evaluate the innate immunity of udder (Mukherjee *et al.* 2013) and the relationship between *in vitro* activities of milk leukocytes with milk constituents may be used for global assessment of udder immunity at animal and herd level. Several micronutrients such as copper (Cu) and zinc (Zn) have been reported to influence different aspects of the immune system (Paterson and MacPherson 1990). Therefore, the present study was undertaken to correlate the *in vitro* milk leukocyte (viz. neutrophils, macrophages and lymphocytes) activity with milk composition (viz. fat, protein, lactose, SNF and total solids) and micronutrient (copper and zinc) content in high yielding crossbred cows.

Forty-eight high yielding crossbred cows (second to fourth parity) were selected from the herd of Eastern Regional Station of ICAR-National Dairy Research Institute, Kalyani, Nadia, West Bengal, India. All the cows selected were kept in loose housing system with brick flooring and managed as per the practices followed in the institute's herd. They were offered *ad lib.* green fodder and calculated amount of concentrate mixture based on milk production only at the time of milking. Fresh tap water was available *ad lib.* at all the time of the day. All the animals were screened weekly for clinical mastitis by assessing macroscopical examination (visualization and palpation of the udder, visualization of the milk, electrical conductivity of milk, California mastitis test) and microscopical (individual cow somatic cell counts).

Present address: [1]Ph.D. Scholar (drtriptibsharma@gmail.com), [2]M.V.Sc. Scholar (sarathchandrareddy1987@gmail.com), [3]Associate Professor (pkdaskol@rediffmail.com), [4,5,6] Assistant Professor (drprghosh@gmail.com, dipakndri@gmail.com, joyphy@gmail.com).

Samples of composite milk (representing all 4 quarters) were collected into sterile tubes (250 ml/cow) at the time of milking. Before collection, teat dipping was done with an effective teat dip (0.5% iodine or 4% hypochlorite) leaving the predip on the teat for at least 20–30 sec before removal. Then the teats were carefully and vigorously scrubbed with a cotton or cloth gauze pad moistened (but not dripping wet) with 70% to 80% ethyl or isopropyl alcohol. Immediately after collection the milk samples were transported to the laboratory. SCC and differential leukocyte counts (DLC) of milk samples were measured microscopically (Mukherjee *et al.* 2013). *In vitro* activity of milk leukocytes, viz. neutrophils, lymphocytes, and macrophages, was performed as per Mukherjee *et al.* (2013).

Estimation of milk constituents was done by standard procedure. Estimation of milk copper and zinc was estimated by the methods of Fernandez and Kahn (1971) using AAS (atomic absorption spectrophotometer). All analysis was done using SYSTAT software package. The correlation between and udder immunological parameters (milk SCC, DLC and *in vitro* activity of milk leukocytes) were analyzed by Spearman Rank Order Correlation.

The correlation between udder immunological parameters (milk SCC, DLC and *in vitro* activity of milk leukocytes) and milk constituents is presented in Table 1. Milk SCC was found to be positively correlated with milk fat, protein, Cu and Zn content though the correlation is nonsignificant. But compared to this there was a significant ($P<0.01$) negative correlation between milk SCC and milk lactose, total soid and SNF content. Milk neutrophils were positively correlated with protein ($P<0.01$) and Cu content and negatively ($P<0.01$) correlated with fat content in milk. However milk lymphocytes were positively ($P<0.05$) correlated with milk fat content and negatively ($P<0.01$) correlated with protein content. Milk macrophages were positively correlated with milk SNF ($P<0.05$) and total solid content ($P<0.01$). SI of milk lymphocytes was significantly ($P<0.05$) positively correlated with milk protein, fat percentage and copper content and negatively ($P<0.01$) correlated with milk lactose, milk total solids, solid not fat

Table 1. Correlation between milk constituents and *in vitro* milk leukocyte activity in high yielding crossbred cows

	SI	PAN	PAM	SCC	LYM	NEU	MAC	Fat	Protein	Total soild	SNF	Lactose	Cu	ZN
SI	1	-0.252	0.137	0.605**	-0.169	0.141	0.1	0.204	0.440*	-0.04	-0.239	-0.555**	0.440*	-0.107
PAN	-0.252	1	0.550**	0.239	0.376	-0.227	-0.696**	0.21	-0.277	-0.666**	-0.432*	-0.038	-0.246	0.515*
PAM	0.137	0.550**	1	0.291	0.382	-0.351	-0.212	0.455*	0.099	-0.254	-0.15	-0.033	0.28	0.203
SCC	0.605**	0.239	0.291	1	-0.082	0.141	-0.31	0.463*	0.405	-0.651**	-0.751**	-0.977**	0.363	0.301
LYM	-0.169	0.376	0.382	-0.082	1	-0.976**	-0.196	0.501*	-0.705**	0.166	-0.049	0.073	-0.386	0.415
NEU	0.141	-0.227	-0.351	0.141	-0.976**	1	-0.02	-0.485*	0.650**	-0.328	-0.057	-0.12	0.435*	-0.364
MAC	0.100	-0.696**	-0.212	-0.31	-0.196	-0.02	1	-0.15	0.27	0.723**	0.503*	0.246	-0.229	-0.275
Fat	0.204	0.21	0.455*	0.463*	0.501*	0-.485*	-0.15	1	-0.145	0.197	0.021	-0.507*	-0.022	0.574**
Protein	0.440*	-0.277	0.099	0.405	-0.705**	0.650**	0.27	-0.145	1	-0.167	-0.098	-0.282	.519**	-0.242
Total soild	-0.04	-0.666**	-0.254	-0.651**	0.166	-0.328	0.723**	0.197	-0.167	1	0.853**	0.327	-0.013	-0.313
SNF	-0.239	-0.432*	-0.15	-0.751**	-0.049	-0.057	0.503*	0.021	-0.098	0.853**	1	0.629**	-0.064	-0.253
Lactose	-0.555**	-0.038	-0.033	-0.977**	0.073	-0.12	0.246	-0.507*	-0.282	0.327	0.629**	1	-0.23	-0.317
Cu	0.440*	-0.246	0.28	0.363	-0.386	0.435*	-0.229	-0.022	0.519**	-0.013	-0.064	-0.23	1	-0.601**
ZN	-0.107	0.515*	0.203	0.301	0.415	-0.364	-0.275	0.574**	-0.242	-0.313	-0.253	-0.317	-.601**	1

*Significance at 5% level (P<0.05),** significance at 1% level (P<0.01).

and zinc content. Phagocytic index (PI) of milk neutrophils was positively (P<0.05) correlated with zinc content and milk fat percentage and negatively (P<0.01) correlated with milk total solid and SNF percentage content. PI of milk macrophages was a significantly (P<0.05) positively correlated between milk fat content. It was also found that milk protein, copper, zinc content were also positively correlated with PI of the milk macrophages though correlations were nonsignificant. Similarly there was a nonsignificant negative correlation between PI of milk macrophages with milk total solids, solid not fat and lactose content.

Milk SCC was found to be positively (P<0.05) correlated with milk fat content in the present investigation which was in agreement with Paura *et al*. (2002). However, Gajdusek (1996) did not find a significant influence of SCC on the content of fat in mastitis affected cows. Here in this study, milk SCC was negatively (P<0.01) correlated with lactose content which confirmed the earlier reports of Paura *et al*. (2002). This could be due to the fact that biosynthesis of lactose is diminished due to infection of intramammary infections (Gajdusek 1996).

Milk lymphocyte proliferation response was found to be positively correlated (P<0.05) with protein and copper content of milk where as a strong negative correlation (P<0.01) was found with lactose content of milk. I*n vitro* investigations confirmed the potential for milk proteins to affect lymphocyte function (Otani *et al*. 1992). Our findings confirmed that milk proteins have the potential to affect local as well as systemic lymphocyte function as reported by earlier researchers. In the present investigation it was found that PI of milk neutrophils was negatively correlated with milk protein content which was in contradiction with Miyauchi *et al*. (1998) has confirmed the ability of bovine milk derived proteins to enhance *in vitro* neutrophil function in heterologous species. In the present investigation, PI of milk neutrophils were found to be positively correlated with milk fat content which was in accordance with Listenberger *et al*. (2001) who indicated that the increased concentration fatty acid enhanced phagocytosis-induced oxidative burst.

Studies have shown that phagocytic promoting effects of Cu and Zn (De *et al*. 2015) either by stabilizing the cells against superoxide radicals through the action of superoxide dismutase, which requires zinc for activation or by activating cytochrome-c-oxidase in the mitochondrial electron-transport chain. Our result on the correlation between milk Cu and zinc content on with Con A induced milk lymphocyte blastogenic response was in agreement with the observations of De *et al*. (2015) who showed that phagocytic activity of nutrophils was increased by *in-vitro* supplementation of Cu and Zn in the culture. Zinc is also essential for lymphocyte replication (Tomlinson *et al*. 2008) as indicated by higher lymphocyte proliferation observed with *in vitro* Zn supplementation in the lymphocyte culture of dairy cows without extra Zn supplementation.

In the present investigation, immunopotentiative role of various milk constituents such as milk protein, copper and zinc content on *in vitro* leukocyte activity was established. Therefore, the routine evaluation of milk constituents together with milk SCC may be used to monitor the udder health at animal and herd level.

SUMMARY

The relationship between the *in vitro* activity of milk leukocytes and milk constituents were studied in 48 high yielding clinically healthy crossbred cows. Milk somatic cell counts (SCC) was negatively correlated with milk lactose, total soid and solid not fat (SNF) content. Milk neutrophils were positively correlated with protein and Cu content and negatively correlated with fat content in milk. However milk lymphocytes were positively correlated with milk fat content and negatively correlated with protein content. Milk macrophages were positively correlated with milk SNF and total solid content. Milk lymphocyte blastogenic response (SI) was significantly positively correlated with milk protein, fat percentage and copper

content and negatively correlated with milk lactose, milk total solids, SNF and zinc content. PI of milk neutrophils was positively correlated with zinc content and milk fat percentage and negatively correlated with milk total solid and SNF content. PI of milk macrophages was positively correlated with milk fat content. In the present investigation, immuno-potentiative role of various milk constituents such as milk protein, copper and zinc content on *in vitro* leukocyte activity was established.

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