

Variations in the levels of acute phase proteins and lactoferrin in serum and milk during bovine subclinical mastitis

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SUPPLEMENTARY FILE

Supplementary Table. S1: Comparison of acute phase proteins in the serum and milk of 40 cows in Group 2 against Electrical Conductivity scores of milk

Sl. No.	Parameters (Mean±SE)	Electrical Conductivity score			P value
		Group A	Group B	Group C	
1	Hp in milk (Ln)	6.812 ± 0.183 ^a	8.807 ± 0.464 ^b	5.516 ± 0.048 ^a	0.001
2	Hp in serum (Ln)	10.147 ± 0.104 ^{ns}	10.076 ± 0.293 ^{ns}	9.787 ± 0.003 ^{ns}	0.827
3	CRP in milk (Ln)	6.550 ± 0.023 ^a	6.736 ± 0.045 ^b	6.62 ± 0.125 ^{a,b}	0.023
4	CRP in serum (Ln)	12.604 ± 0.129 ^{ns}	12.693 ± 0.165 ^{ns}	12.08 ± 0.150 ^{ns}	0.741
5	SAA in serum (Ln)	7.666 ± 0.189 ^{ns}	7.731 ± 0.594 ^{ns}	8.343 ± 0.450 ^{ns}	0.834
6	AGP in milk (Ln)	9.405 ± 0.132 ^a	10.722 ± 0.282 ^b	10.098 ± 0.595 ^{a,b}	0.003

7	Albumin in milk (g/dl)	0.391 ± 0.024 ^{ns}	0.542 ± 0.084 ^{ns}	0.225 ± 0.035 ^{ns}	0.469
8	Albumin in serum (g/dl)	2.538 ± 0.070 ^{ns}	2.336 ± 0.159 ^{ns}	2.810 ± 0.180 ^{ns}	0.053

Means with different superscripts differ significantly, ^{ns}- non significant (p<0.05)

(Hp- Haptoglobin, CRP- C-reactive protein, SAA- serum amyloid A, AGP- α -1 Acid glycoprotein, Ln- natural logarithm)

SupplementaryTable. S2: Comparison of acute phase proteins in the serum and milk of 40 cows in Group 2 against SCCcount of milk

Sl. No.	Parameters (Mean±SE)	Somatic Cell Count				P value
		Group A	Group B	Group C	Group D	
1	Hp in milk (Ln)	6.0513 ± 0.2538 ^a	6.0682 ± 0.2204 ^a	7.6607 ± 0.3163 ^b	8.1790 ± 0.3882 ^b	0.000
2	Hp in serum (Ln)	10.309 ± 0.244 ^{ns}	9.816 ± 0.032 ^{ns}	10.184 ± 0.177 ^{ns}	10.246 ± 0.250 ^{ns}	0.254
3	CRP in milk(Ln)	6.529 ± 0.054 ^{ns}	6.579 ± 0.049 ^{ns}	6.585 ± 0.037 ^{ns}	6.590 ± 0.036 ^{ns}	0.763
4	CRP in serum (Ln)	12.689 ± 0.278 ^{ns}	12.187 ± 0.292 ^{ns}	12.869 ± 0.115 ^{ns}	12.666 ± 0.173 ^{ns}	0.153
5	SAA in serum (Ln)	7.579 ± 0.437 ^{ns}	7.126 ± 0.269 ^{ns}	8.061 ± 0.288 ^{ns}	7.989 ± 0.401 ^{ns}	0.192
6	AGP in milk (Ln)	8.888 ± 0.260 ^a	9.329 ± 0.220 ^{a,b}	10.009 ± 0.242 ^b	9.976 ± 0.228 ^b	0.003

7	Albumin in milk (g/dl)	0.442 ± 0.047 ^{ns}	0.330 ± 0.036 ^{ns}	0.449 ± 0.056 ^{ns}	0.398 ± 0.041 ^{ns}	0.225
8	Albumin in serum (g/dl)	2.498 ± 0.139 ^{ns}	2.605 ± 0.117 ^{ns}	2.435 ± 0.117 ^{ns}	2.554 ± 0.144 ^{ns}	0.823

Means with different superscripts differ significantly, ^{ns}- non significant (p<0.05)

(Hp- Haptoglobin, CRP- C-reactive protein, SAA- serum amyloid A, AGP- α -1 Acid glycoprotein, Ln- natural logarithm)

Supplementary Table. S 3: Comparison of variation of acute phase proteins against the bacterial isolates in the milk of cows

Sl. No.	Parameters (Mean±SE)	Pathogen specific variation			P value
		Group A	Group B	Group C	
1	Hp in milk (Ln)	7.429 ± 1.078 ^{ns}	7.304 ± 0.715 ^{ns}	8.110 ± 0.267 ^{ns}	0.490
2	Hp in serum (Ln)	11.041 ± 0.868 ^{ns}	10.307 ± 0.527 ^{ns}	10.110 ± 0.143 ^{ns}	0.185
3	CRP in milk (Ln)	6.703 ± 0.053 ^{ns}	6.628 ± 0.093 ^{ns}	6.569 ± 0.029 ^{ns}	0.283
4	CRP in serum (Ln)	13.056 ± 0.045 ^{ns}	12.950 ± 0.403 ^{ns}	12.738 ± 0.118 ^{ns}	0.582
5	SAA in serum (Ln)	8.145 ± 0.639 ^{ns}	8.692 ± 0.709 ^{ns}	8.003 ± 0.275 ^{ns}	0.696
6	AGP in milk (Ln)	9.529 ± 0.316 ^{ns}	9.637 ± 0.554 ^{ns}	10.093 ± 0.196 ^{ns}	0.492
7	Albumin in milk (g/dl)	0.548 ± 0.181 ^{ns}	0.375 ± 0.116 ^{ns}	0.416 ± 0.037 ^{ns}	0.507

8	Albumin in serum (g/dl)	2.415 ± 0.332 ^{ns}	2.300 ± 0.288 ^{ns}	2.500 ± 0.103 ^{ns}	0.800
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Means with different superscripts differ significantly, ^{ns}- non significant (p<0.05)

(Hp- Haptoglobin, CRP- C-reactive protein, SAA- serum amyloid A, AGP- α -1 Acid glycoprotein, Ln- natural logarithm)

Supplementary Table S4; Comparison of results of CMT, EC and SCC with variations in the levels of acute phase proteins between the healthy animals and animals affected with subclinical analysis

Name of APP	Diagnostic tests and statistical differences		
	CMT	EC	SCC
Serum Hp	NS	NS	NS
Serum CRP	NS	NS	NS
Serum SAA	NS	NS	NS
Serum albumin	NS	NS	NS
Milk Hp	S	S	S
Milk CRP	NS	NS	NS
Milk lactoferrin	S	S	NS
Milk albumin	NS	NS	NS
Milk AGP	S	S	NS

S- Significant. NS- Non significant

Supplementary Table S5 Primers used for identification of bacterial isolates in milk samples from the 40 cows in Group II, their target location in the genome and references.

Sl. No.	Pathogens	Target	Primers	References
1	<i>Staphylococcus</i> spp.	16S rRNA	AACTCTGTTATTAGGGAAGAACA CCACCTTCCTCCGGTTTGTCACC	Ciftci <i>et al.</i> 2009
2	<i>S. aureus</i>	23S rRNA	GGACGACATTAGACGATCA CGGGCACCTATTTTCTATCT	El-Razik <i>et al.</i> 2010
3	<i>Streptococcus agalactiae</i>	16S rRNA	CGCTGAGGTTTGGTGTTTACA CACTCCTACCAACGTTCTTC	El-Razik <i>et al.</i> 2010
4	<i>Streptococcus dysgalactiae</i>	16S rRNA	AAAGGTGCAACTGCATCACTA GTCACATGGTGGATTTTCCA	El-Razik <i>et al.</i> 2010
5	<i>Streptococcus uberis</i>	16S rRNA	TAAGGAAGACGTTGGTTAAG TCCAGTCCTTAGACCTTCT	Moatamedie <i>et al.</i> 2007