

1 **Evaluation of different acute-phase proteins for herd health diagnostics in early**  
2 **postpartum Holstein Friesian dairy cows**

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7 **SUPPLEMENTARY FILE**

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9 **Supplementary Results**

10 *Descriptive Statistics*

11 Farm sizes ranged from a total number of 1074 to 2638 cows per farm (average: 1506 cows).

12 The average annual milk yield was 10351 kg (ranging from 7432 to 11982 kg). At the time of  
13 sampling, cows were on average 4.08 days in milk (DIM) (ranging from 0 to 8 days, SD: 1.99)  
14 and within their 2.39<sup>th</sup> lactation (ranging from 1<sup>st</sup> to 5<sup>th</sup> lactation, SD: 1.15). Mean sampling day  
15 and lactation number were not different between farms ( $P = 0.56$  and  $P = 0.16$ , respectively).

16 Rectal body temperature ranged from 37.6 °C to 39.8 °C (mean: 38.6, SD: 0.4) and was different  
17 between farms ( $P < 0.001$ ). A mean Metrichcek™ (MC) score of 1.26 (SD: 1.10), rumen fill  
18 (RF) score of 2.04 (SD: 0.51) and vulvovaginal laceration (VL) score of 1.02 (SD: 0.42) was  
19 determined. Farms did not differ in their mean MC, RF and VL score ( $P = 0.14$ ,  $P = 0.30$  and  
20  $P = 0.28$ , respectively) (Table S1).

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22 *Correlations among different APP*

23 Significant positive Pearson's correlations were ascertained between Hp and Cp ( $r = 0.273$ ,  $P$   
24  $= 0.006$ ) (Table S2) and Hp and SAA ( $r = 0.257$ ,  $P = 0.010$ ). Significant negative correlations  
25 were found between Alb and Hp ( $r = -0.428$ ,  $P < 0.001$ ) and Alb and Cp ( $r = -0.260$ ,  $P = 0.009$ ).

26 The negative correlation between Hp and Alb was the strongest relationship among all APP.

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28 **Supplementary Discussion**

29 *Correlations among different APP*

30 Significant positive correlations between Cp and Hp and SAA and Hp were observed. All three  
31 proteins are considered positive bovine APP whose serum concentrations are known to rise  
32 concomitantly during an APR (Ceciliani et al. 2012; Kaya et al. 2016). The moderately strong  
33 and highly significant negative correlations found between Alb and Hp and Alb and Cp  
34 corroborate the results of many previous studies, where a decrease in serum Alb was described  
35 concomitant with rising concentrations of positive APP during inflammatory reactions in dairy  
36 cows (Trevisi et al. 2011; Krause et al. 2014; Montagner et al. 2016).

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38 **Supplementary References**

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67 **Supplementary Tables**

68 **Table S1.** Descriptive statistics of farm sizes (cows (total)), average annual milk yields (MY  
 69 (av.)), lactation numbers (Lact. no.), days in milk (DIM). and clinical parameters (RT = rectal  
 70 temperature, MC = Metrichheck™ score, RF = rumen fill score, VL = vulvovaginal laceration  
 71 score) of N = 100 Holstein Friesian dairy cows from 10 different farms assessed in this study.  
 72 N = 10 cows per farm.

<b>Farm</b>	<b>Cows (total)</b>	<b>MY (av.)</b>	<b>n</b>		<b>Lact. no.</b>	<b>DIM</b>	<b>RT</b>	<b>MC</b>	<b>RF</b>	<b>VL</b>
1	1076	9311	10	$\bar{x}$	2.9	4.1	38.2	1.7	1.9	1.0
				<b>Min</b>	1	2	37.8	1	1	0
				<b>Max</b>	5	7	39.6	3	3	2
2	1187	11982	10	$\bar{x}$	2.4	4.8	38.2	0.4	1.7	0.9
				<b>Min</b>	1	4	37.6	0	1	0
				<b>Max</b>	3	7	38.6	2	2	2
3	1599	10419	10	$\bar{x}$	2.2	4.8	38.6	1.22	1.9	0.7
				<b>Min</b>	1	3	38.2	0	1	0
				<b>Max</b>	5	7	39.0	3	3	2
4	1097	11281	10	$\bar{x}$	2.2	3.5	38.9	0.6	1.8	0.7
				<b>Min</b>	1	1	38.5	0	1	0
				<b>Max</b>	3	7	39.4	2	3	2
5	1074	10381	10	$\bar{x}$	2.5	3.9	38.5	1.2	2.2	1.2
				<b>Min</b>	1	1	38.3	0	2	0
				<b>Max</b>	5	8	38.8	3	3	2
6	1224	10436	10	$\bar{x}$	2.6	4.3	38.8	1.9	2.2	0.8
				<b>Min</b>	1	1	38.2	0	1	0
				<b>Max</b>	5	7	39.8	3	3	2
7	1457	11128	10	$\bar{x}$	2.6	4.9	38.9	0.9	2.2	1.3
				<b>Min</b>	1	1	38.5	0	2	0
				<b>Max</b>	5	8	39.4	2	3	2
8	1659	11210	10	$\bar{x}$	2.1	3.3	38.7	1.4	2.1	1.5
				<b>Min</b>	1	1	38.4	0	2	0
				<b>Max</b>	5	5	38.9	3	3	2
9	2052	7432	10	$\bar{x}$	2.8	3.6	38.9	2.1	2.1	0.8
				<b>Min</b>	2	0	38.5	0	2	0
				<b>Max</b>	5	6	39.3	3	3	2
10	2638	9931	10	$\bar{x}$	1.6	3.7	38.6	1.2	2.3	1.3

			<b>Min</b>	1	1	38.4	0	2	1
			<b>Max</b>	3	7	39.3	3	3	2
$\bar{x}$	1506	10351		2.39	4.08	38.6	1.26	2.04	1.02
<b>SD</b>	509	1276		1.15	1.99	0.4	1.10	0.51	0.74
<b>P</b>				0.16	0.56	< 0.001	0.14	0.30	0.28

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75 **Table S2.** Pearson's correlation matrix among Haptoglobin (Hp), Serum Amyloid A (SAA),  
76 C-reactive protein (CRP), Coeruloplasmin (Cp), Albumin (Alb) and Total protein (TP) in  
77 serum of N = 99 dairy cows within 0-8 d p.p.

	N		<b>Hp</b>	<b>SAA</b>	<b>CRP</b>	<b>Cp</b>	<b>Alb</b>	<b>TP</b>
<b>Hp</b>	99	$r_p$	1	0.257*	0.116	0.273*	-0.428*	-0.136
		$P_p$		0.010	0.253	0.006	<0.001	0.179
<b>SAA</b>	99	$r_p$	0.257*	1	0.134	-0.019	-0.048	-0.134
		$P_p$	0.010		0.185	0.848	0.638	0.187
<b>CRP</b>	99	$r_p$	0.116	0.134	1	-0.049	-0.095	0.121
		$P_p$	0.253	0.185		0.627	0.349	0.234
<b>Cp</b>	99	$r_p$	0.273*	-0.019	-0.049	1	-0.260*	0.012
		$P_p$	0.006	0.848	0.627		0.009	0.908
<b>Alb</b>	99	$r_p$	-0.428*	-0.048	-0.095	-0.260*	1	0.166
		$P_p$	<0.001	0.638	0.349	0.009		0.101
<b>TP</b>	99	$r_p$	-0.136	-0.134	0.121	0.012	0.166	1
		$P_p$	0.179	0.187	0.234	0.908	0.101	

78 \*significant Pearson's correlation ( $P < 0.05$ )

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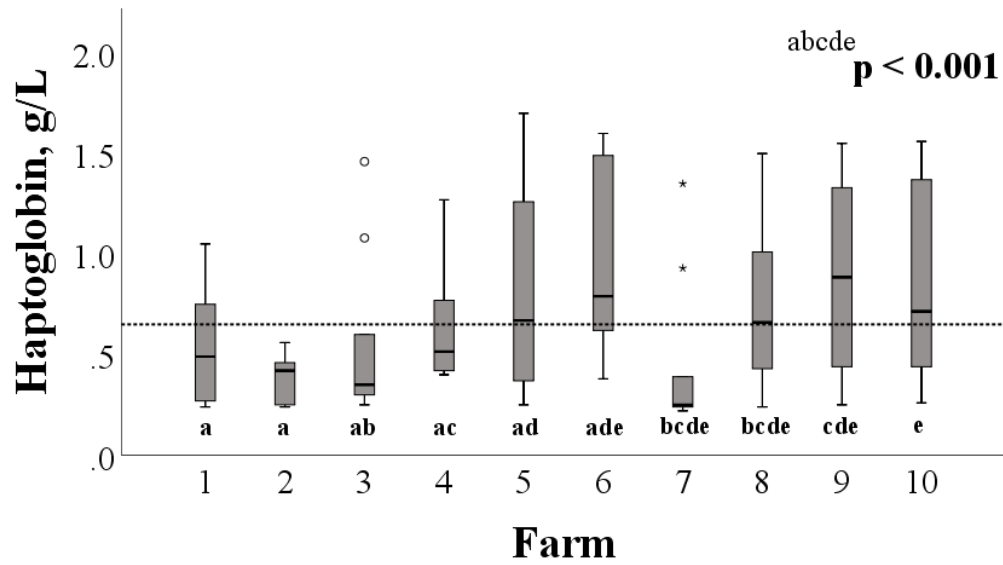
81 **Table S3.** Pearson's and Spearman's rank correlations of Haptoglobin (Hp), Serum-Amyloid-  
 82 A (SAA), C-reactive-Protein (CRP), Ceruloplasmin (Cp), Albumin (Alb) and Total Protein  
 83 (TP) concentrations with rectal body temperature (RT), Metricheck score (MC), Rumen fill  
 84 score (RF) and Vulvovaginal laceration score (VL) respectively, in N = 99 dairy cows within  
 85 0-8 d p.p.

APP	N	RT		MC		RF <sup>1</sup>		VL <sup>2</sup>	
		<i>r<sub>p</sub></i>	<i>P<sub>p</sub></i>	<i>r<sub>s</sub></i>	<i>P<sub>s</sub></i>	<i>r<sub>s</sub></i>	<i>P<sub>s</sub></i>	<i>r<sub>s</sub></i>	<i>P<sub>s</sub></i>
Hp	99	0.45	<0.001	0.48	<0.001	0.07	0.49	-0.12	0.23
SAA	99	0.18	0.08	0.12	0.26	-0.07	0.51	-0.02	0.86
CRP	99	-0.11	0.26	0.11	0.30	0.07	0.48	-0.08	0.41
Cp	99	-0.14	0.16	0.15	0.13	-0.04	0.71	-0.15	0.14
Alb	99	-0.06	0.53	-0.40	<0.001	-0.08	0.46	0.09	0.36
TP	99	-0.26	0.009	0.02	0.84	-0.05	0.61	0.14	0.16

86 <sup>1</sup>Rumen fill (RF) was scored on a 1 – 5 scale according to (Zaaijer & Noordhuizen 2003)

87 <sup>2</sup>Vulvovaginal laceration (VL) was scored on a 0 – 2 scale according to (Vieira-Neto et al.  
 88 2016)

## Supplementary Figures



**Figure S1** Boxplot illustrating the distribution of Haptoglobin (Hp) concentrations in serum of Holstein Friesian dairy cows within 0-8 days p. p. in 10 different farms. (N = 99; --- = threshold value for clinically healthy cows p. p. (0.65 g/L) (Huzzey et al. 2009; Chan et al. 2010))