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***Araştırma Makalesi*** ***Research Article***

# Oxidative Status and Acute Phase Response in Post-transition Early- and Mid-lactation Holstein Cows and Their Correlations with Some Performance Records

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**Abstract**

Oxidative stress and acute phase response were assessed in post-transition early-lactation and mid-lactation Holstein cows regards to the possible effects on milk production and reproductive performance. In two farms, 113 cows, 2-6 years old within 21-150 days in milk (DIM) were sampled for whole blood three times with 30-day intervals. The concentrations of glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), haptoglobin (Hp) and serum amyloid A (SAA) were measured in plasma. Three DIM stages were defined for further comparisons: DIM=21-70 as a post-transition early-lactation; 71-110 as the late weeks of early-lactation and the early weeks of mid-lactation; and 111-150 as the major portion of mid-lactation. Blood parameters (DIM=21-150) of the cows that conceived before (n=29) and after (n=56) DIM=110 and the cows that remained open (n=27) were also compared with the same test. The relationships between blood parameters (DIM=21-150) and performance indices were studied by Spearman's rho correlation test. The studied performance records were not statistically different (P>0.05) between farms and between the cows that conceived (n=85) and the cows that remained open (n=27). GPx was not different (P>0.05) among DIM stages in the sum of both farms, though it increased significantly in Farm 2 at DIM=111-150. The levels of SOD and MDA were not different between farms and among DIM stages (P>0.05). The levels of Hp and SAA through DIM stages were close to or higher than those reported in fresh or diseased cows and their changes were mostly not significant. Performance records and blood parameters were not different (P>0.05) between the cows that conceived before DIM=110 or later and those that remained open. Negative correlations were observed between Hp and the maximum milk yield of the first 90 days (r=-0.20, P=0.034) and average milk yield during 150 DIM (r=-0.17, P=0.065). No relationship was observed between the plasma parameters and the studied reproductive records. The activity of GPx showed negative correlations with the level of Hp (r=-0.14, P=0.036) and the activity of SOD (r=-0.19, P=0.004). Oxidative stress was not a problem of the studied farms during DIM=21-150. However, high levels of Hp and SAA could reveal continuous mild stressful or inflammatory conditions during DIM=21-150, contributing to the less efficient milk production.

## Introduction

Intensive dairy farming may have problems in achieving desired performance indices irrespective of opportunities such as more hygiene and prophylaxis, protection against diverse climate and good nutritional management (Bertoni et al., 2009). In such systems the cows are ideally expected to conceive during the early weeks of mid-lactation around d 100 of lactation. Many cows, however, conceive later in lactation, impairing the performance indices of the farms, although their insemination programs may be started as soon as day 60 of lactation or earlier (De Jarnette et al., 2007); still some cows may fail to conceive for long periods of time and may be culled for reproductive failure. Ansari-Lari et

al. (2010) studied 8,204 lactations on five large Iranian Holstein farms over 4 years (2004-2007) and reported that the mean (±SD) calving to first service interval and days open were 67 (±38) days and 134 (±89) days, respectively. Atashi et al. (2012) studied 528,034 lactations of 246,132 Iranian Holstein cows over 1994 to 2008 and found that calving interval increased from 394.1 (±65) days in 1994 to 413.2 (±81) days in 2008, concurrent with an increase in herd size and milk production.

Acute phase proteins are mainly synthesized in the liver, mediated by pro-inflammatory cytokines, and their concentration can increase (positive APPs) or decrease (negative APPs) as a consequence of inflammatory stimuli (Murata et al., 2004; Murata, 2007). In bovines, serum proteins that have been recognized as APPs include two major APPs: serum amyloid A (SAA) (Horadagoda et al., 1993) and haptoglobin (Hp). Haptoglobin (Hp) consists of two α and two β chains, connected by disulfide bridges (Morimatsu et al., 1991). In the circulation, Hp is highly polymerised with a molecular weight of approximately 1000–2000 kDa, and exists also as a polymer associated with albumin (Godson et al., 1996). The primary function of Hp is to bind free hemoglobin released from erythrocytes and to thereby inhibit its oxidative activity (Yang et al., 2003). Many studies have indicated the significance of Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle with various diseases (Eckersall, 2000). Serum amyloid A (SAA) belongs to the family of apolipoproteins associated with high density lipoprotein (Uhlar et al., 1994). Different isoforms of SAA are expressed constitutively at different levels in response to inflammatory stimuli (Jensen and Whitehead, 1998). During inflammation, SAA1 and SAA2 are expressed principally in the liver, whereas SAA3 is induced in many distinct tissues, including the mammary gland (Weber et al., 2006).

Acute phase response and oxidative stress have been the focus of numerous dairy cow studies, particularly in a transition period and early lactation (Bertoni and Trevisi, 2013; Bossaert et al., 2012). With progress in lactation, high levels of dietary starch at 80 days lactation has been shown to induce oxidative stress in dairy cows, possibly due to cellular changes related to oxidative phosphorylation (Gabai et al., 2004). These situations may leave no clinical signs, but may consume energy and protein for production of immune responses and scavenging of free radicals, and decrease the nutrient availability for production purposes, impairing the performance of the animal for the whole lactation period. The present study quantified and compared the acute phase response and oxidative stress during post-transition early-lactation and mid-lactation periods. The hypothesis was that acute phase response and oxidative stress may be features of early-to mid-lactation period, affecting the re-productive performance of the cows for extended periods of time.

## Materials and Methods

### Animals, Samplings and Farm Data

This study was conducted in two dairy farms (120 milking cows each) with constant, preset management routines, being almost similar in both farms. Both farms complained of unsatisfactory reproduction performance, low peak lactations and undesired milk persistency. In both farms the multiparous cows were kept in the high string for at least 150 days in milk (DIM) and the primiparous had a separate group. The rations of both groups (Table 1) were constant and almost similar in chemical composition for at least 5 months (about 2 months before to 3 months after samplings). The cows were milked 3 times per day and their milk production was recorded monthly. The cows were stabled in free stalls with mixtures of sand and soil beds in the cubicles and concrete floors in the waking allies. The farms were under veterinary supervision biweekly. All cows received the routine postpartum examinations and treatments and were inseminated based on the veterinary's advises. The voluntary waiting period in both farms was 40 days.

All cows, from 2 to 6 years old, 21-150 days in milk (DIM) were enrolled in the study and were sampled for whole blood three times with 30-day intervals. Thus, each cow could be sampled 1 to 3 times. For example, if a cow had 30 DIM at the first sampling, she was also enrolled in the 2$^{nd}$ and the 3$^{rd}$ samplings. But if the cow had 145 DIM she was not included in the further samplings. A total number of 69 cows in Farm 1 and 56 cows in Farm 2 were sampled 1 to 3 times between DIM=21 to 150. Six cows in each farm were excluded from further statistical studies due to their affection by diseases (dystocia, retained placenta, metritis, peracute mastitis, respiratory diseases, nymphomania, foot abscess and lameness) peripartum and later up to 150 DIM. Sampling was performed during the winter from late December to late February, between 09:00 to 11:00. Whole blood samples were collected from coccygeal vessels in EDTA tubes by a skilled veterinarian under excellent restraint conditions with minimal stress.

**Table 1.** Rations of the lactating cows in the studied farms.

| Feed weight (kg) | Farm 1 | Farm 2 |
|---|---|---|
| As fed | | |
| Multiparous cows | 51.5 | 51.7 |
| Primiparous cows | 46 | 46 |
| Dry matter | | |
| Multiparous cows | 24.9 | 25.1 |
| Primiparous cows | 22.24 | 22.33 |
| **Feed ingredients (Percent as fed)** | | |
| Alfalfa hay | 9.7 | 9.7 |
| Corn silage | 62.14 | 61.9 |
| Beet pulp (dry, with molasses) | 1.94 | 1.93 |
| Barley grain (ground) | 6.6 | 6.77 |
| Corn grain (cracked) | 6.6 | 6.77 |
| Wheat bran | 4.19 | 3.87 |
| Soybean meal | 4.19 | 7.58 |
| Canola meal | 3.15 | - |
| Supplemental fat | 0.39 | 0.39 |
| Salt (white) | 0.14 | 0.14 |
| Sodium bicarbonate | 0.39 | 0.39 |
| Calcium carbonate | 0.17 | 0.17 |
| Mineral-vitamin supplement | 0.39 | 0.39 |
| Vitamin E+Se supplement | 13 grams | 13 grams |
| **Chemical composition** | | |
| Dry matter (%) | 48.4 | 48.6 |
| Dry matter (kg) | 24.9 | 25.1 |
| NDF (%DM) | 35.4 | 34.3 |
| fNDF (%DM) | 23.9 | 23.7 |
| NEl (mcal/kg DM) | 1.56 | 1.58 |
| CP (%DM) | 16.05 | 16.60 |
| RUP (%CP) | 30.5 | 30.7 |
| MP (%DM) | 9.87 | 10.27 |
| NFC (%DM) | 40.8 | 41.3 |
| EE (%DM) | 3.50 | 3.26 |
| Ca (%DM) | 0.75 | 0.73 |
| P (%DM) | 0.50 | 0.47 |
| Ca:P ratio | 1.50 | 1.55 |
| Forage ratio (DM) | 47.8 | 47.5 |

NDF: neutral detergent fiber; fNDF: forage NDF; NEl: net energy for lactation; CP: crude protein; RUP: rumen undegradable protein; MP: metabolizable protein; NFC: non-fibrous carbohydrates; EE: ether extract; Ca: calcium; P: phosphorus.

**Cow Appearance and Farm Data**

Each cow was watched for a while after getting out of the sampling box for detection of its body condition score (BCS) and locomotion score (LS), both on 1 to 5 scales as described by Edmonson et al. (1989) and Bicalho et al. (2007), respectively. The following items were extracted for the studied cows by inspection of the farms' data during one year of the corresponding lactation: diseases of the cows during the peripartum period up to 150 DIM, maximum recorded milk during 90 DIM (not to be mistaken with peak milk), the average recorded milk during the first 150 days of lactation, changes of milk production at d 150 compared to the maximum recorded milk, voluntary waiting period before breeding, service per conception ratios; open days of the conceived cows, and the number of cows that were not conceived for one year after their calving.

**Sample Preparation**

Whole blood samples were transferred to the laboratory within three hours of sampling. Plasma was separated by centrifugation at 3000*g* for 15 minutes.

The erythrocyte sediment was washed with 3 ml of 0.9% NaCl solution four times, was centrifuged each time at 700*g* for 5 minutes at room temperature and the supernatant was separated. The washed erythrocytes were then diluted with 2 ml of cold redistilled water. The plasma and the hemolysates were stored at -80°C for measurement of serum amyloid A (SAA), haptoglobin (Hp), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) 2-4 months later.

**Measurement of Acute Phase Proteins (APP)**

Plasma concentration of SAA was determined by a solid phase sandwich ELISA and that of Hp was measured based on prevention of the peroxidase activity of hemoglobin, which is proportional to the amount of Hp. The analytical sensitivities of the tests were 0.30 µg $mL^{-1}$ for SAA and 0.01 mg $mL^{-1}$ for Hp as determined by the manufacturer (Tridelta Development Plc, Wicklow, Ireland).

**Measurement of Superoxide Dismutase (SOD)**

The activity of SOD was quantified in the hemolysates using a commercial kit (Ransod kit, Randox Com, UK). According to the manufacturer's instructions the hemolysates were diluted to 1: 200 with 10 mM phosphate buffer (pH=7). Xanthine and xanthine oxidase are used to generate superoxide radicals, forming a red formazan dye after reacting with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT). One unit of SOD corresponded to 50% inhibition of INT reduction under assay condition. The enzyme activity was expressed as units per g of hemoglobin, measured by the cyanmethemoglobin method (Jain, 1986).

**Measurement of Glutathione Peroxidase (GPx)**

GPx activity was evaluated in the hemolysate using a commercial GPx detection kit (Ransel kit, Randox Co. UK) according to the manufacturer's instructions. The kit works based on the method of Paglia and Valentine (1967). GPx catalyze the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance against blank was measured at 340 nm. One unit (U) of GPx activity was defined as the amount of enzyme that converts 1 µmol of NADPH to $NADP^+$ per minute. The GPx activity was expressed as unit per mg of hemoglobin (U/g Hb).

**Measurement of MDA**

Lipid peroxidation in erythrocytes was evaluated using an HPLC method based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), to form a colored MDA–TBA adduct (Lykkesfeldt, 2001). Forty µL of the sample was diluted with 100 µL of $H_2O$ and then was mixed with a solution of 20 µL of 2.8 mmol/L butylatedhydroxytoluene (BHT) in ethanol, 40 µL of 81 g/L sodium dodecyl sulfate and 600 µL of TBA reagent (8 g/L TBA diluted 1:1 with 200 ml/L acetic acid adjusted to pH 3.5 with NaOH). The mixture was immediately heated to 95°C for 60 minutes and was cooled with running water. Then, 200 µL of $H_2O$ and 1000 µL of butanol–pyridine (15:1, v/v) were added to the mixture. After vigorous mixing, the solution was centrifuged for 3 minutes at 16,000*g* and the organic layer was separated. The absorbance of the supernatant was measured by a UV-visible spectrophotometer fitted with an 80 µL flow cell at 532 nm (the mobile phase was consisted of 300 mL/L methanol in 50 mM $KH_2PO4$, pH:7.0). 1,1,3,3-tetraethoxypropane was used as a standard, and the values of MDA-TBA reactive substance were expressed as MDA mili-mole per liter (mmol/L). The HPLC system consisted of a solvent delivery pump (JASCO 980-PU, Tokyo, Japan), a reversed-phase column (Luna C18, 250 mm × 4.6 mm, Phenomenex, CA, USA), and a UV–Visible detector (Jasco, UV-975, Tokyo, Japan) operated at 532 nm.

**Statistics**

Three DIM stages were defined within each farm: DIM 21-70, corresponding to early lactation after the end of the transition period; DIM 71-110, the late weeks of early lactation and the early weeks of mid-lactation; and DIM 111-150, the major portion of mid-lactation. The final number of cows after exclusion of the diseased cows and the number of samples in various DIM stages are depicted in Table 2. The SPSS statistical software (version 15.0, SPSS, Inc., Chicago, IL, USA) was used for data analysis. The data for the measured parameters were not distributed normally as shown by Kolmogorov–Smirnov test. The comparisons of the performance records of the farms and the blood parameters in separate DIM stages between the two farms were done using Mann–Whitney U test. Blood parameters in DIM stages within each farm were compared with the Kruskal Wallis test. Blood parameters of DIM=21-150 of the cows that conceived before (n=29) and after (n=56) d 110 of lactation and the cows that remained open (n=27) were also compared with the same test. The relationships between blood parameters (d 21 to 150) and performance indices were studied by Spearman's rho correlation test. Probability values of $P<0.05$ were considered significant.

**Table 2.** Number of cows studied in each farm and number of samples at various study periods.

| | Number of Studied Cows | Total Number of Samples | DIM 21-70 (Early-lactation) | DIM 71-110 (Early-to-mid-lactation) | DIM 111-150 (Mid-to-late-lactation) |
|---|---|---|---|---|---|
| **Farm 1** | 63 | 134 | 46 | 43 | 45 |
| **Farm 2** | 50 | 102 | 36 | 31 | 35 |

## Results

### Performance Data

As shown in Table 3 none of the studied performance indices was statistically different between farms (P>0.05). The number of cows that did not conceive during one year was 8 (12.7%) in farm 1 and 19 (38%) in Farm 2 (Table 3). The studied performance indices also were not statistically different between the cows that conceived (n=85) and the cows that remained open (n=27) (Table 4). Compared to conceived cows, the cows that remained open in the sum of both farms had numerically greater drops in milk production at month 5 of lactation.

### Oxidative Status and Acute Phase Response

Table 5 compares the measured parameters between the farms and among the various DIM stages within farms. There was no noticeable difference between farms except a significantly lower level of GPX (P=0. 011) in farm 2 at DIM=71-110. GPX was not different (P>0.05) among DIM stages in the sum of both farms, though it increased significantly in Farm 2 at DIM=111-150. The levels of SOD and MDA were not different between farms and among DIM stages (P>0.05). SAA was higher (P=0.054) in Farm 1 at DIM=21-70, declining through the succeeding stages (P<0.05). In sum of both farms the changes of SAA through DIM stages were not significant. Similarly, Hp did not show significant changes through DIM stages and between farms. The levels of both SAA and Hp were close to or higher than the levels reported previously in fresh or diseased cows.

Comparison of the indices of oxidative status and acute phase response between the cows that conceived within one year of their calving before d 110 (n=29) or after that (n=56) and the cows that remained open (n=27) revealed no significant difference (P>0.05) between groups (Table 6).

### Correlations

Negative correlations were observed between Hp and the maximum milk yield of the first 90 days (r=-0.20, P=0.034) and average milk yield during 150 DIM (r=-0.17, P=0. 065). No relationship was observed between the studied plasma parameters and the studied indices of reproductive performance. The activity of GPx showed negative correlations with the level of Hp (r=-0.14, P=0. 036) and the activity of SOD (r=-0.19, P=0.004).

**Table 3.** Performance indices of the studied cows (mean±SD).

| | Farm 1 (63 cows) | Farm 2 (50 cows) |
|---|---|---|
| BCS | 2.51±0.25 | 2.57±0.33 |
| Maximum recorded milk (kg) during the first 90 days | 39.53±7.48 | 38.32±4.96 |
| Average milk production (kg) during the first 150 days | 35.74±6.28 | 34.13±3.72 |
| Milk drop (kg) at month 5 of lactation compared to the maximum | 3.79±3.26 | 4.19±2.90 |
| Open days in the cows that conceived during one year | 160.66±69.30 | 172.23±91.96 |
| Service per conception ratio of the cows that conceived during one year | 2.18±1.12 | 2.32±1.28 |
| Number of cows that did not conceive during one year | 8 (12.7%) | 19 (38%) |

**Table 4.** Performance data of cows that remained open for one year compared with cows that conceived during one year after parturition (pooled data of DIM=21-150).

| | Conceived (n=85) | Remained open (n=27) |
|---|---|---|
| BCS | 2.54±0.27 | 2.55±0.34 |
| Maximum recorded milk (kg) during the first 90 days | 38.92±6.71 | 39.59±6.11 |
| Average milk production (kg) during the first 150 days | 35.11±5.65 | 35.02±4.45 |
| Milk drop (kg) at month 5 of lactation compared to the maximum | 3.81±3.05 | 4.57±3.38 |

**Table 5.** Comparison of blood parameters between and within farms in various DIM periods.

| | Farm | DIM 21-70 (Early-lactation) | DIM 71-110 (Early-to-mid-lactation) | DIM 111-150 (Mid-to-late-lactation) | DIM 21-150 (All samples) |
|---|---|---|---|---|---|
| SOD (U/g Hb) | 1 | 767.45±374.66 | 694.71±310.68 | 733.20±373.88 | 731.34±353.83 |
| | 2 | 916.47±367.09 | 806.69±294.30 | 812.95±369.43 | 845.50±343.41 |
| | Both | 837.93±376.09 | 748.57±306.19 | 763.72±371.92 | 782.08±353.10 |
| GPx (U/g Hb) | 1 | 277.97±95.50 | 281.09±85.80* | 280.22±96.67 | 279.82±92.33 |
| | 2 | 245.43±84.69 [a] | 236.77±66.99 [a,*] | 299.85±104.16 [b] | 258.28±88.64 |
| | Both | 262.98±91.56 | 260.01±80.11 | 287.55±99.35 | 270.43±91.18 |
| MDA (mmol/L) | 1 | 5.05±0.68 | 5.14±0.62 | 5.08±0.61 | 5.09±0.63 |
| | 2 | 5.11±0.78 | 5.16±0.65 | 4.95±0.62 | 5.08±0.68 |
| | Both | 5.07±0.72 | 5.15±0.63 | 5.03±0.61 | 5.09±0.65 |
| Haptoglobin (g/L) | 1 | 0.166±0.104 | 0.167±0.104 | 0.150±0.070 | 0.160±0.093 |
| | 2 | 0.141±0.061 | 0.153±0.079 | 0.165±0.073 | 0.153±0.072 |
| | Both | 0.155±0.88 | 0.160±0.093 | 0.156±0.071 | 0.157±0.084 |
| SAA (µg/mL) | 1 | 28.38±34.89[a] | 19.91±22.54 [ab] | 13.70±17.24 [b] | 20.09±25.84 |
| | 2 | 15.37±19.64 | 23.90±27.89 | 19.73±26.95 | 19.83±25.15 |
| | Both | 22.39±29.43 | 21.81±25.15 | 15.95±21.43 | 19.98±25.49 |

*: Significant difference between farms at each DIM stage,
[a,b]: Different letters in rows indicate to significant differences.

**Table 6.** Comparison of cows that remained open for one year with cows that conceived during one year after parturition (pooled data of DIM=21-150). [NS]

| | Conceived (d 60-110; n=29) | Conceived (d>111; n=56) | Remained Open (n=27) |
|---|---|---|---|
| SOD (U/g Hb) | 784.12±347.59 | 761.40±348.31 | 832.79±373.57 |
| GPX (U/g Hb) | 256.54±78.45 | 273.09±92.69 | 280.29±100.98 |
| MDA (mmol/L) | 5.06±0.58 | 5.10±0.69 | 5.09±0.64 |
| Haptoglobin (g/L) | 0.161±0.092 | 0.158±0.083 | 0.150±0.079 |
| SAA (µg/mL) | 22.28±29.32 | 18.89±23.01 | 20.07±27.12 |

[NS]: No significant difference within rows (P>0.05).

## Discussion

Mild and continued stressful or inflammatory conditions could be concluded in the present study as the levels of Hp and SAA were high during the period of the study (DIM=21-150) compared to the levels reported in healthy animals. These conditions appeared to affect the level of production, but not the speed of milk drop and the studied reproductive indices.

Both Hp and SAA increase in blood during acute phase response (APR) (Cray et al., 2009; Humblet et al., 2006), which is a nonspecific reaction to different external or internal challenges such as stress, infection, inflammation or trauma (Bertoni and Trevisi, 2013; Cray et al., 2009; Mohebbi-Fani et al., 2010). In the present study, the average levels of Hp and SAA during DIM=21-150 were close to the levels reported by others in fresh or diseased cows. Hp concentrations in healthy cows are normally as low as 0.1 to 0.15 g/L. After calving a peak of 0.4 to 0.5 g/L occurs around 7 days in milk, followed by a reduction to normal values in 3 to 5 weeks (Bertoni and Trevisi, 2013). In the study of Chan et al. (2010) Hp increased in healthy cows within 3 days after calving to values greater than 0.13 g/L and decreased to levels below that by the third week after parturition. Nazifi et al. (2009) reported averages of 0.09±0.01 g/L for Hp in healthy cows. The level of Hp in the present study was 0.157±0.084 g/L during DIM=21-150 (Table 5). The levels of SAA have been reported to be 13.8 µg/ml and 29.9µg/ml in cows with mild and moderate mastitis, respectively (Eckersall et al., 2001), whereas SAA levels as low as 5.10±0.508 µg/ml have been reported in healthy cows (Nazifi et al., 2009). In the present study, the average concentration of SAA during d 21 to 150 of lactation was 19.98±25.49 µg/ml (Table 5). These acute

phase proteins often increase for a short period of time within 48 hours after the start of the trigger, when the liver is activated by proinflammatory cytokines, and often decline rapidly due to their very short half-life (Bertoni and Trevisi, 2013; Johnson et al., 1999). After week 1 post calving, Hp and SAA have poor sensitivity in detecting specific causes of inflammations (Humblet et al., 2006), while they show large inter-individual variations even in healthy animals (Bertoni and Trevisi, 2013; Humblet et al., 2006; Mohebbi Fani et al., 2010). However, both Hp and SAA have been recognized to be useful as indicators of stress (Mohebbi Fani et al., 2010; Murata, 2007) and their persistent high levels in the present study during 21 to 150 days of lactation could suggest that the cows in the production systems of the farms were prone to long lasting stressful conditions. Stiff bedding (soil instead of sand in the stalls), slippery floors and high accumulation of animals were stressful conditions recognizable in both farms during the study. Many of the reasons that may cause changes in serum APP concentrations remain subclinical (Bertoni et al., 2008; Bertoni and Trevisi, 2013). However, prolonged APR may consume energy and proteins for the production of immune responses and decrease the nutrient availability for production purposes. In the present study negative correlations were observed between the level of Hp and the maximum milk yield of the cows of the first 90 days (r=-0.20, P=0.034) and average milk yield of 150 days (r=-0.17, P=0.065). The speed of milk drop after maximum production, however, appeared not to be affected by this condition. The levels of production at month 5 of lactation of the studied cows were 90.4% and 89% of the maximum recorded milk in Farms 1 and 2, respectively. Assuming that the maximum milk was recorded at day 60, the amount of the drop was less than the recommended levels of 6-9% per month for adult cows after the peak lactation. The studied reproductive indices also were not correlated with the levels of APPs in the present study. Conceived and non-conceived cows were not different in the levels of Hp and SAA (Table 6). Whether these latter findings were attributed to the customization of the animals to such conditions should be assessed.

Oxidative reactions, which are essential for general health of the animal as parts of homeostatic functions, may damage the living tissues if happened in excess of antioxidant capacity of the body (Al-Gubory et al., 2010) and affect the performance of the animals. In ewes, oxidative stress was associated with delayed cyclicity at the beginning of the breeding period (Mohebbi-Fani et al., 2014a). Ewes with different body conditions and ages may be affected similarly during breeding and pregnancy; breeding time being a more challenging period than pregnancy (Mohebbi-Fani et al., 2012a; 2012b; 2014b). Feed quality (Mohebbi-Fani et al., 2012b) and rapid dietary modification may affect oxidative homeostasis in lactating sheep (Sgorlon et al., 2008). Dairy cows can experience oxidative stress during the peri-parturient period (Bernabucci et al., 2005; Castillo et al., 2005; Gaál et al., 2006) influenced by nutrition and season (Bernabucci et al., 2002; Di Trana et al., 2006). High dietary starch at 80 days lactation increased oxidative stress in dairy cows that could be due to cellular changes related to oxidative phosphorylation (Gabai et al., 2004). The diet and the season in the present study, however, were constant. Irrespective of the increasing pattern of GPx in Farm 2 and its lower level at DIM=71-110 in the same farm, the concentrations of SOD were not different among DIM stages and between farms (Table 5). Besides, the levels of MDA as a marker of oxidative stress (Bernabucci et al., 2002; Mohebbi-Fani et al., 2012 a; 2012b; 2014a; 2014b) were within the normal values reported previously (Khoshvaghti et al., 2014) for dairy cows. There was not any relationship between SOD, GPx and MDA with milk production and reproduction in the present study. It could be concluded that oxidative stress was not a problem of the studied farms during DIM=21-150, revealing satisfactory action of the mechanisms involved in the controlling oxidative stress. However, the activity of GPx had a negative correlation with the level of Hp (r=-0.14, P=0.036), revealing that better anti-oxidative activities could be expected with lowering the stressful conditions. The negative correlation between GPx and SOD (r=-0.19, P=0.004), could be explained this way: SOD neutralizes the reactive oxygen species at the initial stages of their formation (Al-Gubory et al., 2010) and when it works well there would not be a need for more activity of GPx.

## Conclusion

Prolonged moderate acute phase response could be a feature of early- to mid-lactation period. More efficient milk production necessities close attention to the conditions such as prolonged stress that may trigger APR. More detailed investigations are required for assessing the effect of such prolonged APR on reproductive performance of the cows. In the conditions of the present study oxidative stress was not a problem of the early-to mid-lactation period.

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