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Survey on Serum Amyloid A and Haptoglobin Concentrations in Normal or Sensitive to Rumen Acidosis Holstein Dairy Cows

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Abstract

BACKGROUND: Diagnosis and prognosis of inflammatory conditions based on acute phase proteins (APPs) level in blood has been exploited for a long time in human medicine and their changes in SARA condition is considered in veterinary medicine.

OBJECTIVES: To evaluate the variations of serum amyloid A (SAA) and haptoglobin (Hp) as main acute phase proteins in cow with normal pH or cows that experienced rumen pH ≤5.8 during first days after parturition.

METHODS: A total of 106 multiparous Holstein dairy cows were randomly selected after parturition in two different seasons of winter and summer 2017. Cows were divided into 2 groups as normal cows with pH >5.8 or rumen pH ≤5.8. Ruminal fluid samples were collected through stomach tube for rumen pH and blood samples were taken from the coccygeal vein of cows concurrently once per day at days 4, 11 and 18 post-partum. SAA and Hp were determined in blood samples. The PROC MIXED of SAS (2003) was used for all determined variables with repeated measures. BCS, parity and milk yield were included as fixed and cows as random effect. The significant level was declared at P≤0.05, and tendency toward significance was considered at 0.05<P≤0.10 by the Tukey test. Correlation between rumen pH and APPs were surveyed using PROC CORRELATION of SAS (2003).

RESULTS: The results of experiment showed that rumen pH was lower (P<0.05) in summer samples than winter (6.33 vs. 6.46). Rumen pH was lower (P<0.0001) in cows composed by subjects with rumen pH ≤5.8 than pH >5.8. For all examined cows, SAA concentration was greater in winter than summer (P<0.001), as well as at day 4 than days 11 and 18 after calving (P<0.05). Also, Hp concentration was greater for winter samples than summer (P<0.05), and at day 18 than days 4 and 11 after calving (333.33 vs. 299.3 and 300.1 respectively) (P<0.05). SAA and Hp concentrations were not affected by rumen pH. There was no significant correlation between rumen pH and APPs concentrations in both groups of pH ≤5.8 and pH >5.8.

CONCLUSIONS: Results showed that rumen pH ≤5.8 seems not to stimulate the APPs production.

KEYWORDS: Acute phase protein, Dairy cows, Haptoglobin, Rumen pH, Serum amyloid A

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Introduction

The acute phase proteins (APPs) are sensitive factors that allow the early and precise detection of inflammation in ruminants. The most frequently investigated APPs in cattle are haptoglobin (Hp), serum amyloid A (SAA), fibrinogen (Fb), ceruloplasmin, alpha 1-antitrypsin and alpha1-acid glycoprotein (α1-AGP) (Pošćić et al., 2017). APPs have been exploited for a long time in human medicine and recently have been paid attention to in veterinary medicine (Abbak and Alkım Ulutaş, 2017). Liver is a major organ responsible for the synthesis of acute phase proteins (Cannizzo et al., 2012). In cattle some AAPs may be synthesized extra-hepatically, e.g. Hp and SAA may also be produced in the mammary glands (Pradeep, 2014). In recent years, it has become more popular to use APPs as predictors or risk factors for infectious diseases. In heifers with retained placenta (RP), Hp and SAA were detectable in the last week before delivery and differences in Hp and SAA levels between heifers with and without RP were statistically significant (Manimaran et al., 2016). Hp concentration was used as an early predictor of metritis in dairy cattle. Cows with Hp concentrations ≥1 g/L at day 3 postpartum were 6.7 times more likely to develop metritis (Manimaran et al., 2016). In milk, amyloid A (AA) seems to have greater potential for the detection of mastitis than Hp since it had higher sensitivity, specificity and efficiency in differentiating between cows with mastitis and healthy cows (Thomas et al., 2015). Based on a polish study which had been carried out in 15 dairy farms during 2009- 2010, increased serum level of Hp was found in 35% of cows in early lactation whereas increased serum Hp concentration at the peak and end of lactation were noticed in 14.6% and 10.5% respectively (Jawor and Stefanika 2011). The metabolic effects of acute systemic inflammation include adipose tissue mobilization, breakdown of liver glycogen, and liver triglyceride accumulation, all of which occur during the transition period. More specifically, cytokines promote the breakdown of fat stores through decreased feed intake (Makki et al., 2013), impaired insulin sensitivity, and direct stimulation of lipolysis (Makki et al., 2013). All of these conditions are associated with ketosis and fatty liver in dairy cattle. Plasma concentrations of a number of inflammatory markers were increased in cows that developed fatty liver (Cannizzo et al., 2012). Newborn cows are particularly vulnerable to acidosis because of the fairly abrupt change in fermentable carbohydrate intake that occurs after parturition. SARA increased dramatically after parturition in dairy cow (Danscher et al., 2015).

Increased serum APPs reported by mentioned experimental and induced rumen acidosis in cattle, therefore, present study was conducted to evaluate the correlation between serum APPs concentrations and rumen pH in normal and sensitive group of cows to rumen acidosis during the first 21 days' lactation.

Materials and Methods

Animals, feeding and housing

The experiment was conducted in a large commercial dairy farm with almost 3000 milking Holstein cows located in Qazvin province. A total of 106 Holstein dairy cows were randomly selected after parturition in two different seasons of winter and summer 2017. Cows were divided into groups as normal cows with pH >5.8 or rumen pH ≤5.8. The cows were housed in free stall barns, with concrete stalls bedded with processed manure. Conditions such as DIM, milk yield and lactation were similar for all cows. Cows were fed twice daily at 08:30 and 16:30 with about 5–10% refusal/d (Table 1). The TMR (total mixed ration) feed was the only feed composition available to cattle in the herd. Daily milking was done at 08:00, 16:00 and 24:00.

Ruminal fluid sampling

The Rumen fluid samples were collected by a stomach tube connected to the vacuum pump at a volume of at least 500 mL. The rumen fluids were taken once per day approximately 5 to 6 hours after morning feeding at days 4, 11 and 18 postpartum. Rumen pH was measured immediately in two consecutive duplicates by a portable pH meter, and the mean of two numbers was recorded as rumen pH.

Blood sampling

Blood samples were taken from healthy cows at 4, 11 and 18 days postpartum 3 to 5 hours after morning meal delivery. Blood samples were collected via the coccygeal vein using a 10-mL plain vacuum tube (Pars Khavar, Qazvin, Iran). After collection, all blood samples were centrifugation at 2000 g for 15 min and separated sera were stored at -20 °C until analysis. SAA and Hp concentrations were measured by using an ELISA kit (Cloud-Clone Corp, Fernhust, USA). Samples were added to the appropriate microplate wells with a biotin-conjugated antibody specific to Hp and SAA. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contain haptoglobin, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Hp and SAA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Statistical analysis

The PROC MIXED of SAS (2003) was used for all determined variables. The Kolmogorov-Smirnov test was used to determine the distribution of the data, whether the data were normally distributed or not and it was found that data for rumen pH, SAA and Hp had normal distributions. BCS, parity and lactation were included as a fixed and cows as a random effect. The significant level was $p \leq 0.05$, and tendency toward significance was considered at $0.05 < p \leq 0.10$ by the Tukey test. PROC CORRELATION of SAS (2003) was used for determination of correlation between rumen pH and APPs.

Table 1. Ingredient and nutrient compositions of diets administered during the experimental period.

	Summer	Winter
Ingredients [a], % as DM fed		
Alfalfa hay	15.00	12.12
Corn silage	16.03	16.66

	Summer	Winter
Beet Pulp	15.00	15.00
barley grain, ground	10.20	7.30
Corn grain, ground	20.22	20.22
Soybean meal	10.78	5.78
Canola meal	0.00	4.50
Cotton seed	0.00	5.20
Fat powder	0.75	0.00
Extruded soybean	4.78	4.78
Calcium carbonate	0.75	0.75
Magnesium oxide	0.17	0.17
Sodium bicarbonate	0.75	0.75
Salt	0.37	0.37
Dicalcium phosphate	0.36	0.36
Mineral premix	0.77	0.77
Vitamin premix	0.77	0.77
Mycotoxin binder	1.00	1.00
Meat Meal	1.90	1.90
Urea	0.40	0.00
Corn gluten meal	0.00	1.60
Nutrient composition on DM basis		
DM, % as fed	(0.05)48.5	(0.06)48.5
% ,CP	(0.05) 18.5	(0.05) 18.6
%,ASH	(0.05)9.82	(0.05)9.82
% ,EE	(0.04) 4.32	(0.02) 4.12
% ,NDF	(0.7) 35.4	(0.7) 35.4
% ,ADF	(0.3)17.5	(0.3)17.5
%,NFC	31.96	32.06
(NE_L (Mcal/kg	1.64	1.62

[a] The diet was formulated according to the NRC (2001) nutrient requirements for Holstein dairy cows weighing 650 kg and producing, 39 kg of 3.5% fat-corrected milk.
[b] Contains 500,000IUof vitamin E., 15,000,000 IU of vitamin A.,400,000IUofvitaminD3, and6000IUofvitaminEperkilogram.

Results

The results of rumen pH, SAA and Hp concentrations in all examined cows are shown in Table 2 and Fig 1-3. The results of experiment showed that ruminal pH was lower (P<0.05) in summer than winter (6.33 vs. 6.46). In all cows, rumen pH did not differ between days 4, 11 and 18 of experiment. However, rumen pH at day 11 of summer was significantly lower than same day in the winter samples (6.32 vs. 6.53) (P<0.05) (Fig 1). Also, ruminal pH was significantly lower (P<0.0001) in cows grouped as pH≤5.8 than pH>5.8 (5.66 vs. 6.47). As shown in Table 2, SAA concentration was greater in winter samples than summer (P<0.001), specially at day 4 for winter than summer samples (P<0.001) (Fig. 2).For all examined cows, SAA concentration was greater at day 4 than days 11 and 18 after calving (P<0.05). Similarly, in winter, SAA value was greater at day 4 than days 11 (P<0.001) and 18 (P<0.05) postpartum. Hp concentration was greater in winter samples than summer cases (P<0.05), specially at day 18 postpartum. Also, Hp concentration at day 18 after calving was greater (P<0.05) than day 4 and day 11 (333.33 vs. 299.3 and 300.1 respectively). As shown in Table 3, SAA (P> 0.05) and Hp (P=0.08) concentrations did not differ between cows grouped as pH≤5.8 or pH>5.8. There were no significant correlations between rumen pH and APPs for all examined cows as well as cows grouped as pH≤5.8 or pH>5.8, however, concentration of SAA was significantly correlated to Hp in all examined cows and cows grouped as pH>5.8 (Table 4).

Table 2. Rumen pH and Acute phase proteins (Hp and SAA) levels in all early lactation Holstein dairy cows

Items	Season		Days			S		
	S	W	4	11	18	4	11	18
pH	$6.33^{a}\pm0.03$	$6.46^{b}\pm0.03$	6.39±0.04	6.43±0.04	6.38±0.04	6.39±0.06	6.33±0.06	6.28±0.06
SAA (mg/L)	$17.42^{b}\pm0.26$	$18.59^{a}\pm0.23$	$18.66^{a}\pm0.3$	$17.71^{b}\pm0.3$	$17.64^{b}\pm0.3$	17.44±0.4	17.63±0.4	17.18±0.4
HP (mg/L)	$281.32^{a}\pm5.9$	$310.89^{b}\pm5.87$	287.33±7.2	297.75±7.2	303.22±7.3	275.41±10.1	295.40±10.3	273.14±10.4

Items	W			P- Value		
	4	11	18	season	day	season× day
pH	6.40±0.06	6.52±0.06	6.46±0.06	0.05>	NS	NS
SAA (mg/L)	$19.88^{a}\pm0.45$	$17.77^{b}\pm0.29$	$18.10^{b}\pm0.45$	0.001>	0.05>	0.05>
HP (mg/L)	$299.26^{b}\pm10.3$	$300.10^{a}\pm10.0$	333.31a±10.2	0.001>	NS	0.05>

[a,b] Means with different superscript letters are differ based on P value described in table

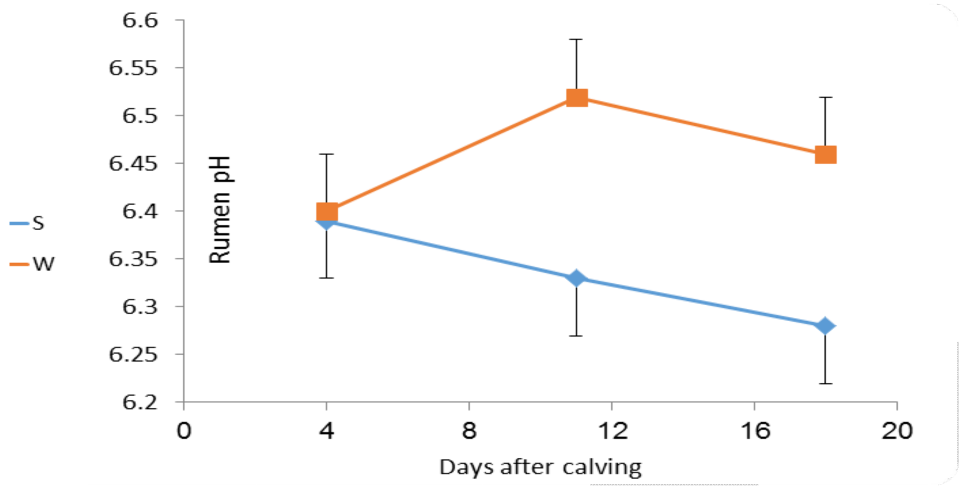

Figure 1. Mean ± SEM of changes in rumen pH on days 4, 11 and 18 after calving in summer (S) and winter (W). * Indicates a significant difference at the level described in above table

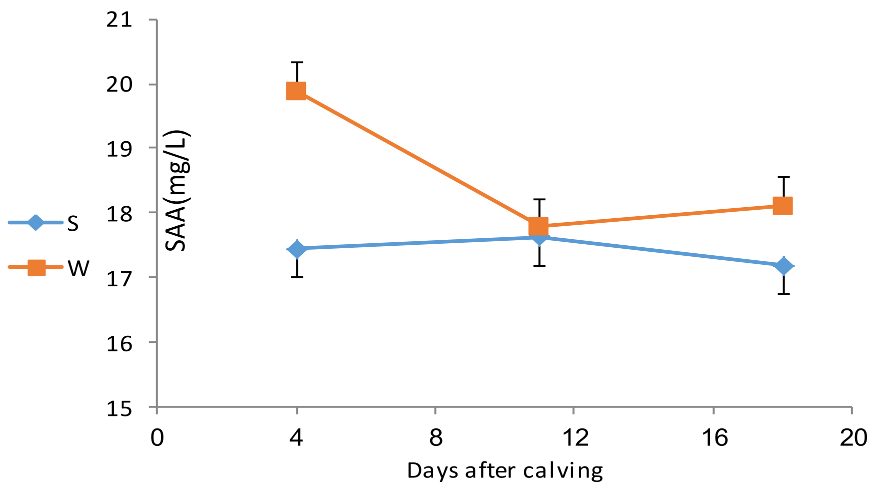

Figure 2. Mean ± SEM of changes in serum SAA on days 4, 11 and 18 after calving in summer (S) and winter (W). * Indicates a significant difference at level described in above table

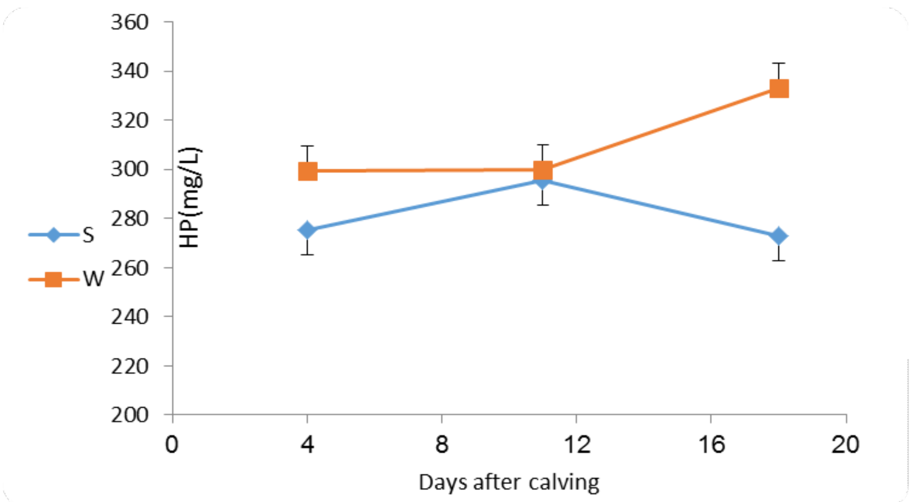

Figure 3. Mean ± SEM of changes in serum Hp on days 4, 11 and 18 after calving in summer (S) and winter (W). * Indicates a significant difference at the level described in above table

Table 3. Rumen pH and acute phase proteins (Hp and SAA) levels in the early lactation Holstein dairy cows with rumen pH ≤5.8 or pH >5.8

Items	cows		P- Value
	pH ≤5.8	pH >5.8	
pH	5.66[b]±0.06	6.47[a]±0.02	<0.0001
SAA (mg/L)	17.12±0.26	18.05±0.13	NS
HP (mg/L)	273.86±5.9	298.56±5.1	=0.08

[a,b] Means with different superscript letters are differ based on P value descripted in table

Table 4. Correlation between Rumen pH and acute phase proteins (Hp and SAA) levels in the early lactation Holstein dairy cows

Items	pH			SAA (mg/L)			HP (mg/L)		
	All cows	pH ≤5.8	pH >5.8	All cows	pH ≤5.8	pH >5.8	All cows	pH ≤5.8	pH >5.8
pH	1.00 NS	1.00 NS	1.00 NS	0.10 =0.07	0.19 NS	0.06 NS	0.06 NS	-0.04 NS	0.03 NS
SAA (mg/L)	0.10 =0.07	0.19 NS	0.06 NS	1.00 NS	1.00 NS	1.00 NS	0.14* <0.05	0.04 NS	0.14* <0.05
HP (mg/L)	0.06 NS	-0.04 NS	0.03 NS	0.14* <0.05	0.04 NS	0.14* <0.05	1.00 NS	1.00 NS	1.00 NS

Correlation with *superscript letters is significant based on P value descripted in table

Discussion

Ruminal acidosis is the consequence of feeding high grain diets to dairy cows, which are adapted to digest and metabolize a predominantly forage based diet. This condition occurs especially in high producing dairy cows after calving (Garret and Oztel., 2007). Ruminal pH is largely a function of the balance between the production of volatile fatty acids (VFA) in the rumen, their neutralization by salivary and dietary buffers, and their absorption across the ruminal wall (Gomez et al., 2014). In the present study, rumen pH of all examined cows was lower in the summer than winter samples (6.33 vs. 6.46) (P<0.05), as well as cows grouped as pH ≤ 5.8 than cows with rumen pH >5.8 (5.66 vs. 6.47) (P<0.0001). According to Gozho et al., (2005) ruminal pH below 5.6 for an average of 187 and 174 min/d can activate a systemic inflammatory response in the steers. In this experiment, cows experienced pH ≤ 5.8 were observed as sensitive cows to SARA. Different prevalence of SARA during transition period of dairy cattle has been reported by researchers, so that 14% SARA among 197 dairy cows was reported by Kleen et al., (2009) in the Netherlands. Cannizzo et al., (2012) reported 18% SARA in 108 samples in Italy, in Ireland the prevalence of disease in early lactation dairy cows was 11% which was reported by O'Grady et al., (2008). In this experiment, a total number of 36 cases were observed with pH ≤5.8, therefore the overall prevalence of SARA was 11.18%. Previously cows that experienced ruminal pH<5.5 using ruminocentesis method reported more than 14% in Danish and 20% in German and US dairy cows after parturition time (Oetzel, 2017). Also, in 213 cows from nine Polish high-yielding dairy herds (≥20 cows per herd) almost 14% (30/213) of cows were found as cows (pH<5.6) that experienced SARA (Stefanska, et al., 2016). Dairy practitioners need new tools and diagnostic aids to monitor and manage this period with the lowest damage. APPs are sensitive factors that allow precise detection of inflammation in ruminants, so evaluation of APPs in fresh dairy cows may be a useful aid for dairy practitioners to monitor some disorders after parturition. Chan et al., (2010) reported that SAA concentration in healthy cows reached the greatest values within 3 days after delivery (Mean value of 66 mg/l). In this experiment, for all examined cows, SAA was greater in winter than summer, as well as at day 4 than days 11 and 18 after calving (P<0.05). In agreement with this, Chan et al., (2010) reported that the serum level of SAA reached its maximum concentration (15± 16 mg/L) the third day after parturition, which might be due to injuries to birth canal tissues and expulsion of fetal membrane. In contrast to this, Nazifi et al., (2009) reported SAA in healthy Iranian Holstein cows was about 72.71±5.9, which was higher that the values obtained for all examined cows (range of 17.18 to 19.88) or cows grouped as pH ≤5.8 (15.70 to 18.60). In all examined cows, Hp concentration was in a range of 275 to 333 mg/L for days 4 to 18 after calving, which was greater in winter samples than summer (P<0.05). In opposition, Chan et al., (2010) reported the highest Hp concentration in the period of three days after parturition. Hp is one of the most important acute phase proteins, which increases in the inflammatory condition (Emmanuel et al., 2007). Although the concentration of Hp and SAA was obtained in ranges reported by previous studies (Fathi 2014, Aziz 2016), Hp value was greater than the value (200±30 mg/L) reported by Nazifi et al., (2009) in healthy cows. Our results showed there was no significant correlation between changes in rumen pH and APPs concentration, however the earlier studies argued that decrease in rumen pH led to immune reaction and increasing in acute phase proteins subsequently (Plaizier et al., 2009, Cannizzo et al., 2012, Danscher et al., 2015, Gozho et al., 2005 & 2007). In line with our results, Cannizzo et al., (2012) did not report an association between rumen pH with SAA and Hp levels by examining three groups of cows with ruminal pH < 5.5 to more than 5.8. The maximum level of serum SAA (300 mg/L) and Hp (4000 mg/L) in SARA affected cows was reported by Danscher et al.,(2015). Cannizo et al., (2012) found that with decrease in rumen pH from 6.2 to 5.77 and 5.55 the serum Hp concentrations were changed from 0.24 (μg/L) to 0.11 (μg/L) and 016 (μg/L) respectively. Based on the Table 3, rumen pH was significantly lower in cows grouped as rumen pH ≤5.8, while the concentrations of SAA and Hp did not differ between cows pH ≤5.8 and pH >5.8. In line with present observation, Cannizzo et al., (2012) demonstrated that, the groups at higher risk for sub-acute acidosis (rumen pH < 5.5) unexpectedly had a lower Hp level than baseline.

Sato (2016) defined that reduction in the ruminal pH lead to destruction of gram-negative bacteria in the rumen and a cascade of inflammatory events will occur which is accompanied by increased serum SAA and

Hp concentrations. Therefore, increased level of SAA and Hp was accompanied as an index in SARA in dairy cows. Khafipour et al., (2009) reported that there is no comprehensive agreement on the pH threshold indicative of sub-acute acidosis; however, it is likely to increase by rumen pH less than 5.5-6.0. The feeding diets containing 60% concentrate: 40% forage in Murcia dairy goats resulted in decrease in rumen pH to less than 5.5 and causes diarrhea in half of dairy goats. However, SAA was not affected by pH, while Hp level increased. These results indicated that in the goat, measuring Hp may be a better indicator of the sub-acute acidosis (González et al., 2010). Similarly Jia et al., (2014) showed SARA goats that experienced ruminal pH below 5.8 for more than 3 h per day, increase in plasma Hp and cortisol but not SAA concentration. In this regard, Kafipour et al., (2009) and Rodríguez-Lecompte et al., (2014) demonstrated that sub-acute acidosis is not exclusively a rumen-dependent disorder and does not necessarily trigger immune responses, so that diagnosis of sub-acute acidosis, in addition to measuring rumen pH, should be done based on feed intake and milk yield. Management conditions such as dairy cow burns, proper transitions and daily monitoring, especially during the transition period (21 days before and after calving time) may be effective on decreasing stress and inflammation factors and maintain metabolic parameters in health conditions.

Conclusion: The results of the present experiment showed that sensitive group of cows to SARA had lower rumen pH, so that SARA cows experienced rumen pH less than 5.6, which accompanied as trigger factor of many metabolic disorders. However, there was no significant association between rumen pH and serum APPs concentrations. It is concluded that rumen pH ≤5.8 seems not to be a stimulating factor for APPs production in fresh dairy cows, and other inflammatory conditions related to calving may be more effective.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۸، دوره ۱۴، شماره ۱، ۵۹-۴۸

تغییرات غلظت سرم آمیلوئید آ و هاپتوگلوبین در گاوهای تازه زا حساس به اسیدوزیس

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چکیده

زمینه مطالعه: تشخیص شرایط التهابی مبتنی بر پروتئین های فاز حاد (APPs) در خون برای مدت طولانی در پزشکی انسانی مورد استفاده قرار گرفته است و اخیرا به عنوان یک ابزار تشخیصی در دامپزشکی مورد توجه می باشد.

هدف: هدف از این مطالعه بررسی تغییرات مقدار برخی پروتئین های فاز حاد و ارتباط آن ها در گاوهای شیرده سالم تازه زا با اسیدیته مایع شکمبه طبیعی و یا دارای اسیدیته ۵/۸ یا کمتر بود.

روش کار: ۱۰۶ راس گاو شیری هلشتاین چند شکم زایش بلافاصله پس از زایش به صورت تصادفی انتخاب شدند. گاوهای سالم تازه زا به دو گروه با pH مایع شکمبه طبیعی و pH معادل ۵/۸ یا کمتر تقسیم شدند. مایع شکمبه با استفاده از لوله شکمبه ای جمع آوری شد و نمونه های خون پس از وعده غذایی صبح در روزهای ۴، ۱۱ و ۱۸ از ورید دمی گرفته شد. پروتئین های فاز حاد SAA و Hp و pH مایع شکمبه اندازه گیری شدند. تجزیه و تحلیل اطلاعات با استفاده از روش مختلط نرم افزار آماری SAS و همبستگی بین فراسنجه ها بررسی شد. نمره وضعیت بدنی، شکم زایش و تولید شیر شکم قبل به عنوان اثرات ثابت و گاو به عنوان اثر متغییر در مدل قرار گرفتند. اختلاف میانگین ها با آزمون چند دامنه ای توکی و سطح معنی داری ۵٪≥ تعیین شد و بیش از ۵ تا ۱۰٪ به عنوان تمایل به معنی داری در نظر گرفته شد.

نتایج: نتایج آزمایش نشان داد در کل گاوهای مورد بررسی، مقدار pH مایع شکمبه در نمونه های تابستان (۶/۳۳) در مقایسه با زمستان (۶/۴۶) کمتر بود (P<۰/۰۵). pH مایع شکمبه به طور معنی داری در گاوهای گروه pH ≤ ۵/۸ (۵/۶۶) در مقایسه با گاوهای دارای pH طبیعی (۶/۴۷) کمتر بود (P<۰/۰۰۰۱). همچنین در کل گاوهای مورد بررسی سطح SAA و Hp در نمونه های زمستان بیشتر از فصل تابستان بود(P<۰/۰۵). سطح SAA در روز ۴ در مقایسه با روزهای ۱۱ و ۱۸ و سطح Hp در روز ۱۸ در مقایسه با روزهای ۴ و ۱۱ بیشتر بود(P<۰/۰۵). با این وجود، سطح SAA (P>۰/۰۵) و Hp (P=۰/۰۸) بین گاوهای با pH شکمبه ۵/۸ و کمتر در مقایسه با گاوهای دارای pH شکمبه طبیعی تفاوت معنی دار نداشت. نتایج آزمایش نشان داد بین pH شکمبه و پروتئین های سرم آمیلوئید آ و هاپتوگلوبین همبستگی معنی داری وجود نداشت (P>۰/۰۵).

نتیجه گیری نهایی: نتایج آزمایش نشان دادpH شکمبه ۵/ ۸ و کمتر در گاوهای حساس به اسیدوز سبب تحریک تولید پروتئین های فاز حاد نشد.

واژه‌های کلیدی:

پروتئین فاز حاد، هاپتوگلوبین،سرم آمیلوئیدآ، گاو شیری،pH شکمبه.

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