ARAŞTIRMA

F.Ü.Sağ.Bil.Vet.Derg.
2015; 29 (3): 151 - 155
http://www.fusabil.org

Effect of Acarbose on Ruminal Function and Variations of Serum Acute Phase Proteins in Dairy Cows with Subacute Ruminal Acidosis

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The aim of this study was to investigate the effects of acarbose addition to the ration of dairy cows on changes in ruminal fermentation characteristics and variations of acute phase proteins (APPs) in dairy cows with subacute ruminal acidosis (SARA). A total of 24 SARA positive Holstein cows that were divided into 4 groups containin 6 cows in each were used in the study. Treatments were 1) 1 mg of acarbose/kg of BW, 2) 2 mg of acarbose/kg of BW, 3) 3 mg of acarbose/kg of BW and 4) control, no additive. Rumen fluid samples were taken starting from immediately after morning feding and at 4-h intervals until 12 h period. Acarbose increased ruminal pH in all groups immediately after feeding compared to control group (P<0.05). Total volatile fatty acid (VFA) and molar percentages of propionate and butyrate increased (P<0.05), whereas percentage of acetate decreased (P<0.05) in acarbose administrated groups when compared with control group. Ruminal levels of lactate were extremely low and below the level of detection (<1 nM) after acarbose administration. Decreases in haptoglobin (Hp) and Serum Amyloid A (SAA) levels were found to be statistically significant (P<0.05) in acarbose administrated groups when compared with control group. Thus, it was concluded that acarbose may improve the ruminal function and accelerate the repair process of the organism.

Key Words: *Subacute ruminal acidosis, acarbose, volatile fatty acids, acute phase proteins, dairy cow*

Subakut Ruminal Asidoz Teşhis Edilen Süt İneklerinde Serum Akut Faz Proteinlerdeki Değişiklikler ve Akarbozun Ruminal Fonksiyon Üzerine Olan Etkisi

Bu çalışmada, subakut rumen asidozlu (SARA) süt ineklerinin rasyonuna tedavi amaçlı ilave edilen akarbozun, rumenin fermentasyon özellikleri ve akut faz proteinlerinin (APP) düzeylerine olan etkileri araştırılmıştır. Çalışmada her birinde 6 hayvan olmak üzere, 4 grupta toplam 24 SARA pozitif Holstein inek kullanılmıştır. Tedavi olarak 1) 1 mg akarboz / kg CA, 2) 2 mg akarboz / kg CA, 3) 3 mg akarboz / kg CA ve 4) kontrol (herhangi bir katkı maddesi yok) kullanılmıştır. Rumen sıvısı örnekleri, sabah yemlemesi sonrasından 12 saate kadar 4'er saatlik aralıklarla alınmıştır. Kontrol grubu ile karşılaştırıldığında, akarbozun yemlemeden sonra tüm gruplarda rumen pH değerini arttırdığı görülmüştür (P<0.05). Kontrol grubu ile karşılaştırıldığında, rasyonuna akarboz ilave edilen gruplarda toplam uçucu yağ asidi (UYA) ile propiyonat ve bütiratın molar yüzdeleri artarken (P<0.05), asetat oranları azalmıştır (P<0.05). Akarboz uygulamasından sonra, rumen laktat düzeylerinin oldukça düşük olduğu (<1 nM) tespit edilmiştir. Kontrol grubu ile karşılaştırıldığında, akarboz verilen grupların serum Haptoglobin (Hp) ve serum amiloid A (SAA) düzeylerinde istatistiksel olarak anlamlı (P<0.05) azalmalar saptanmıştır. Sonuç olarak, akarbozun rumen fonksiyonlarını iyileştirici ve organizmanın onarım sürecini hızlandırıcı etkilere sahip olabileceği kanaatine varılmıştır.

Anahtar Kelimeler: *Subakut rumen asidozu, akarboz, uçucu yağ asitleri, akut faz proteinleri, süt ineği*

Geliş Tarihi : 09.12.2014
Kabul Tarihi : 27.03.2015

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Introduction

Rumen acidosis is the most frequently seen and important nutritional problem that negatively affects health and productivity of both beef and dairy cattle (1-3). It occurs as 2 distinct syndromes: acute acidosis and subacute acidosis (4). Some acidosis should be normally expected when feeding high levels of concentrates to increase the energy intake for high levels of productivity. However, changing diet too rapidly without a proper transition management (5) or feding excessive quantities of concentrate and insufficient forage result in a fiber-deficient ration that is likely to cause a rapid lactate production called subacute ruminal acidosis (SARA). SARA is defined as a ruminal pH of approximately 5.2 to 5.6 (6, 7). The bouts of low ruminal pH mostly occur between calving and reach to peak at about three to four months post-calving. Although, decrease of dry matter intake (DMI) as a consistent clinical sign is a sensitive indicator (5, 8), measuring the rumen fluid pH is the only reliable and accurate tool to diagnose SARA (9). Acetate and ethanol are produced above a pH of 5.7, while lactate levels do not increase markedly until the pH drops below 5.2 (10). If the diet is not balanced correctly or mixed properly, this problem may arise in the most of total mixed ration

(TMR). Several strategies have been used to improve ruminal pH and milk production during SARA. Supplementing the diet with direct-fed microbials, ionophores or α-amylase and glucosidase inhibitors may reduce the risk of SARA. Acarbose is an α-amylase and glucosidase inhibitor that slows the rate of degradation of starch to glucose, thereby reducing the rate of volatile fatty acids (VFA) production and maintaining rumen pH at a more stable level when large amounts of highly fermentable carbohydrate are fed (4). Acarbose is extracted from cultures of Actinomyces bacteria, which acts as a potent competitive inhibitor of many intestinal alpha-glucosidases (1). Some studies have shown that acarbose improves the ruminal function (11, 12).

The acidic rumen environment, changes in osmotic pressure, and ruminal lipopolysaccharide (LPS) may render the rumen epithelium susceptible to injury, resulting in the translocation of rumen endotoxin into the bloodstream (5, 13, 14). The presence of LPS in the bloodstream results in the production of multiple proinflammatory cytokines, reactive oxygen and nitrogen intermediates, and bioactive lipids, which affect the host's metabolic response to inflammation (13). An elevation of haptoglobin (Hp) and serum amyloid A (SAA) was previously suggested as a useful parameter for controlling SARA (13, 15, 16).

However, little information is available on the effects of acarbose on the rate of VFA production. Thus, the aim of this study was to investigate the effects of acarbose addition on changes in ruminal fermentation characteristics and variations of acute phase proteins (APPs) and to test the ability of acarbose on treatment of SARA in dairy cows.

Materials and Methods

Animals and Nutrition: SARA was suspected to be present in a commercial dairy farm in Elazığ province, Turkey. All the animals in the herd were adapted to a 68:32 concentrate:roughage (high-concentrate feeding program) TMR fed twice daily for ad libitum intake for 10 days before treatment. TMR on a DM basis on the dairy farm was formulated as corn silage (32%) and concentrate mix (68%). Table 1 shows the chemical composition analysis of TMR administered before the treatment. The study was performed on 67 Holstein cows at the dairy farm. In all cows included in the study, samples of rumen fluid were taken by a customized stomach pump and tube for the diagnose of SARA. The stomach tube was inserted to a minimum length of 2 m. In order to prevent mixing of rumen content with saliva it was collected from 1 1/2 to 2 litters of rumen fluid. Samples were collected 5 hours after morning feeding. In samples of rumen fluid, immediately after obtaining, pH value was measured within 3 min using a pH meter (WTW 330 i). It was found that pH of rumen content were between 5.5 and 6.8 in 15 cows. Fourty three cows had values between 5.2 and 5.5 and 9 cows had pH value below 5.2. At pH ≤ 5.2 to ≤ 5.6, it was considered as SARA-positive. Results of rumen pH values, of cows included in the study, shown that 64% of the animals were SARA positive. In total, 24 lactating Holstein cows (612±24.5 kg BW) were selected from SARA-positive cows and were divided into four different treatment groups of 6 animals each. Treatments wereas follow, Group 1) 1 mg of acarbose/kg of BW, n= 6; Group 2) 2 mg of acarbose/kg of BW, n= 6; Group 3) 3 mg of acarbose/kg of BW, n= 6 and Group C) control, no additive, n= 6. All the animals were in the first 60 – 80 days of lactation and were housed in individual tie stalls. The body condition score (BCS) values of the animals in the experiment were between 2.5-3.0, in a 1 (emaciated) to 5 (fat) scale, according to the procedure of Edmonson et al. (17). All the cows had an average milk production (about 6000-8000 kg per year). In acarbose treated groups, acarbose (prepared from Glucobay tablets of Bayer AG) was mixed in the concentrate and then was added to the silage in the TMR mixer, with an estimated 10% excess. Animals were given ad-libitum access to clean water.

Sampling of Rumen Fluid: Rumen fluid samples were taken using 4 customized stomach pumps and tubes using one for each of four groups staring from immediately after morning feeding and at 4-h intervals until 12 h (0h, +4h, +8h and +12h) for measurements of pH, VFA and lactate levels. Approximately 200 mL of fluid were taken in each sample, with the first 100 mL of fluid discarded to minimize the saliva contamination. In samples of rumen fluid, immediately after obtaining, pH value was measured within 3 min using a pH meter (WTW 330i). The samples were then stored at −20°C for later analysis of VFA and lactate.

Chemical Analysis: The analytical DM TMR was analyzed for CP, ether extract, Ca, P, Mg, and K according to AOAC (18) methods. The NDF and ADF contents were determined as described by Van Soest (19). The VFA concentrations in rumen fluid samples were determined with the common method (20) involving gas chromatography (model 439, Packard). The concentration of lactic acid was analysed with Boehringer kits using a spectrophotometer according to the method described by Petit and Flipot (21).

Blood Samples and Biochemical Assays: Blood samples were taken from the jugular vein with 10 mL silicone vacutainer tubes without anticougulant before treatment and 12 h after treatment. Samples were centrifuged at 3.000×g at 4°C for 10 min to separate the serum from the erythrocytes. The serum was frozen at −20°C until the time of analysis.

Haptoglobin measurement was based on prevention of the peroxidase activity of haemoglobin, which is directly proportional to the amount of Hp. The analytical sensitivity of this test in serum has been determined as 0.0156 mg/mL for Hp by the manufacturer (Tridelta Development Plc, Ireland).

Serum amyloid A was measured by a solid phase sandwich-ELISA. The analytical sensitivity of this test in serum has been determined as 0.3 µg/mL for SAA by the manufacturer (Tridelta Development Plc, Ireland).

Statistical Analysis: All results were expressed as mean ± standard deviation (SD). SPSS/PC software one way ANOVA was used to determine statistical differences between mean values of the studied parameters among the groups. Differences were considered as significant at P<0.05.

Results

The nutrient content of TMR used in the experiment is listed in Table 1. The prefeeding (0 h) and postfeeding (4, 8, 12 h) values of pH, concentrations of total VFA and lactate and molar percentages of acetate, propionate, and butyrate are summarized in Table 2. Ruminal pH and molar percentages of propionate, and butyrate increased in acarbose administered groups after h 4 compared to control group (P<0.05). Concentrations of total VFA and molar percentages of acetate decreased in acarbose administrated groups when compared to the control group (P<0.05). Concentrations of total lactate were extremely low and below the level of detection (<1 nM) in acarbose administrated groups when compared with control group.

The prefeeding (0 h) and postfeeding (12 h) mean levels of Hp, and SAA are summarized in Table 3. Serum Hp and SAA levels decreased after feeding (12 h) in acarbose administered groups except for control group when compared with prefeeding (0 h) levels (P<0.05).

Table 1. Nutrient content of TMR used in the experiment

Component	TMR
Dry matter, %	56.60
CP	17.02
Ether extract	2.93
Ash	5.91
NDF	22.82
ADF	13.12
NFC	51.32
Ca	1.00
P	1.25
Mg	0.38
K	1.02

Table 2. Effects of acarbose addition on ruminal fermentation

Parameters	Hours	Group C (n= 6)	Group 1 (n= 6)	Group 2 (n= 6)	Group 3 (n= 6)
pH	BT	5.34 ± 0.38	5.31 ± 0.30^{a}	5.29 ± 0.32^{a}	5.34 ± 0.29^{a}
	4h AT	5.32 ± 0.36^{A}	6.17 ± 0.40^{Bb}	6.30 ± 0.39^{Cb}	6.36 ± 0.41^{Cb}
	8h AT	5.39 ± 0.28^{A}	6.35 ± 0.32^{Bb}	6.44 ± 0.32^{Cb}	6.45 ± 0.44^{Cb}
	12h AT	5.38 ± 0.35^{A}	6.42 ± 0.36^{Bb}	6.53 ± 0.40^{Cb}	6.59 ± 0.30^{Cb}
Total VFA, nM	BT	119.28 ± 6.72	120.01 ± 5.55^{a}	121.85 ± 6.04^{a}	120.66 ± 5.88^{a}
	4h AT	119.57 ± 6.04^{A}	91.02 ± 4.43^{Bb}	86.44 ± 3.87^{Cb}	85.71 ± 3.02^{Cb}
	8h AT	120.01 ± 6.04^{A}	90.52 ± 3.99^{Bb}	85.56 ± 4.44^{Cb}	85.08 ± 4.53^{Cb}
	12h AT	117.36 ± 5.78^{A}	90.02 ± 4.24^{Bb}	85.52 ± 4.55^{Cb}	84.80 ± 3.12^{Cb}
Acetate, %	BT	77.11 ± 4.12	78.12 ± 3.81^{a}	78.33 ± 4.54^{a}	77.78 ± 3.87^{a}
	4h AT	76.76 ± 4.55^{A}	68.61 ± 3.44^{Bb}	67.24 ± 4.22^{Bb}	66.98 ± 3.12^{Bb}
	8h AT	77.05 ± 4.02^{A}	68.07 ± 3.12^{Bb}	67.41 ± 3.55^{Bb}	66.21 ± 3.87^{Bb}
	12h AT	76.22 ± 4.39^{A}	68.10 ± 4.07^{Bb}	67.19 ± 3.12^{Bb}	66.10 ± 3.21^{Bb}
Propionate, %	BT	8.88 ± 0.72	8.50 ± 0.84^{a}	8.44 ± 0.51^{a}	8.18 ± 0.44^{a}
	4h AT	8.91 ± 0.82^{A}	13.08 ± 1.12^{Bb}	13.96 ± 1.02^{Bb}	14.09 ± 1.02^{Bb}
	8h AT	8.99 ± 0.52^{A}	13.11 ± 0.92^{Bb}	13.98 ± 0.98^{Bb}	14.06 ± 1.57^{Bb}
	12h AT	8.42 ± 0.97^{A}	13.15 ± 0.86^{Bb}	14.01 ± 0.84^{Bb}	14.12 ± 1.85^{Bb}
Butyrate, %	BT	12.61 ± 1.02	12.90 ± 1.72^{a}	12.21 ± 0.92^{a}	12.50 ± 1.79^{a}
	4h AT	12.60 ± 1.22^{A}	14.29 ± 2.26^{Bb}	14.86 ± 1.54^{Bb}	15.02 ± 1.05^{Bb}
	8h AT	12.76 ± 0.83^{A}	14.33 ± 0.93^{Bb}	14.66 ± 1.11 ^{Bb}	14.95 ± 1.02^{Bb}
	12h AT	12.44 ± 0.54^{A}	14.34 ± 0.68^{Bb}	14.54 ± 0.87^{Bb}	14.90 ± 0.83^{Bb}
Total lactate, mM	BT	18.72 ± 1.74	20.04 ± 2.28	18.44 ± 1.91	21.26 ± 2.33
	4h AT	18.12 ± 0.84	N.D	N.D	N.D
	8h AT	18.19 ± 1.58	N.D	N.D	N.D
	12h AT	18.30 ± 1.25	N.D	N.D	N.D

^{a,b} Values with different superscript letters within a row differ significantly at P<0.05.
^{A,B,C} Values with different superscript letters within a column differ significantly at P<0.05.
BT: Pretreatment; AT: Posttreatment.
N.D: Not detectable.

Table 3. The mean levels and standard deviations (±SD) of Hp, and SAA in cows with SARA

Parameters		Group C (n= 6)	Group 1 (n= 6)	Group 2 (n= 6)	Group 3 (n= 6)
SAA (mg/mL)	BT	66.36 ± 6.54	72.26 ± 7.25^{A}	74.85 ± 8.96^{A}	69.12 ± 7.18^{A}
	AT	60.17 ± 4.63^{a}	6.75 ± 0.24^{Bb}	8.16 ± 0.49^{Bb}	10.14 ± 1.04^{Bb}
Hp (mg/mL)	BT	0.412 ± 0.06	0.442 ± 0.08^{A}	0.524 ± 0.06^{A}	0.608 ± 0.09^{A}
	AT	0.316 ± 0.06^{a}	0.028 ± 0.01^{Bb}	0.046 ± 0.02^{Bb}	0.084 ± 0.03^{Bb}

^{a,b} :Values with different superscript letters within a row differ significantly at P<0.05.
^{A,B} :Values with different superscript letters within a column differ significantly at P<0.05.
BT: Pretreatment; AT: Posttreatment.

Discussion

High-concentrate diets often contain high levels of fermentable carbohydrate and low levels of fiber to maximize energy intake. Feeding a diet with low NDF content or large changes in dietary composition often result in a higher relative risk of SARA. Ration formulation totally involves a balance between acid and buffer production. The results of some studies (11, 12) have shown the effectiveness of acarbose at controlling pH under conditions of SARA. Similarly, in the present study, administration of acarbose has consistently increased ruminal pH (P<0.05) in all groups immediately after feeding, on h 4, 8 and 12 when compared to control group. It was reported that, the increased ruminal pH was associated with the effect of acarbose on slowing the rate of degradation of starch to glucose, thereby reducing the rate of VFA production (4). Also it was determined in the present study that, the doses of 2 or 3 mg acarbose were more efficacious than 1 mg dose of acarbose to prevent SARA. Because, ruminal pH levels were found to be statistically higher in groups 2 and 3 than group 1 (P<0.05). In the current study, total VFA and molar percentages of propionate and butyrate increased (P<0.05), whereas percentage of acetate decreased (P<0.05) due to acarbose addition when compared with control group. These responses are similar to those reported by McLaughlin et al. (4) and Nagaraja et al. (22). When rumen pH is maintained at a higher level, than rate of rumen VFA production tends to reduce. Similar to the ruminal pH, administration doses of 2 or 3 mg acarbose were found to be statistically more efficacious (P<0.05) than 1 mg dose of acarbose on reducing total VFA under conditions of SARA. Also, the molar percentages of propionate and butyrate for the cows in all treatment groups were higher than controls (P<0.05). This is because of the effect of acarbose on reducing glucose availability (23). These results are expected because supplemental acarbose stimulates the presence of lactic acid-utilizing bacteria, which produce propionate. Differences for molar percentages of propionate, butyrate and acetate in acarbose administrated groups were noted between prefeeding (0 h) and postfeeding (4, 8, 12 h) sampling times, but no differences occurred between 4, 8 and 12 h postfeeding. In general, lactate levels do not increase markedly until the pH drops below 5.2 (10). In the present study, prefeeding lactate levels were between 18-23 nM in the groups but after acarbose administration, ruminal levels of lactate were extremely low and below the level of detection (< 1 nM).

Animals respond to trauma, tissue injury, or infection by activating the acute phase response (24). This response includes the production of APPs, such as SAA and Hp in the liver (24). Hence, an elevation at Hp and SAA levels was previously suggested as useful parameters for controlling SARA (13, 15, 16). Grain-induced SARA increased the concentrations of both SAA and Hp in peripheral blood of steers, albeit that the increase in Hp was greater in steers that had been adapted to a 60% concentrate diet than in steers that received an all forage diets before the SARA induction (25, 26). Studies by Gozho et al. (27) and Khafipoor et al. (28) showed that grain-induced SARA also increased SAA in lactating dairy cows. In the current study, prefeeding serum levels of Hp and SAA were statistically higher in all groups (P<0.05) due to SARA. After administration of acarbose, the decreases in serum Hp and SAA levels were found to be statistically significant (P<0.05) when compared with controls. This significant drop in APPs in the present study shows that, acarbose might be very effective in activating the repair process necessary to return the organism to normal function.

As a conclusion, supplementing the diet of dairy cows, especially at the doses of 2 or 3 mg of acarbose/kg of BW improved ruminal function and accelerated the repair process of the organism and had strong effects on reducing incidence of SARA. But it is obvious that, control of fiber-content and ration quality is more effective and less expensive than the use of α-amylase and glucosidase inhibitors.

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