In vivo Alterations in Ruminal Parameters by *Megasphaera elsdenii* Inoculation on Subacute Ruminal Acidosis (SARA)

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***Abstract*—**SARA is a common and serious metabolic disorder in early lactation in dairy cattle and in finishing beef cattle, caused by diets with high inclusion of cereal grain. This experiment was performed to determine the efficacy of *Megasphaera elsdenii*, a major lactate-utilizing bacterium in prevention/treatment of SARA *in vivo*. *In vivo* experimentation, it was used eight ruminally cannulated rams and it was applied the rapid adaptation with the mixture of grain based on wheat (80% wheat, 20% barley) and barley (80% barley, 20% wheat). During the systematic adaptation, it was followed the probability of SARA formation by being measured the rumen pH with two hours intervals after and before feeding. After being evaluated the data, it was determined the ruminal pH ranged from 5.2-5.6 on the condition of feeding with 60 percentage of grain mixture based on barley and wheat, that assured the definite form of subacute acidosis. In four days SARA period, *M. elsdenii* (10^{10} cfu ml^{-1}) was inoculated during the first two days. During the SARA period, it was observed the decrease of feed intake with *M. elsdenii* inoculation. Inoculation of *M. elsdenii* was caused to differentiation of rumen pH ($P<0.0001$), while it was found the pH level approximately 5.55 in animals applied the inoculation, it was 5.63 pH in other animals. It was observed that total VFA with the bacterium inoculation tended to change in terms of grain feed ($P<0.07$). It increased with the effect of total VFA inoculation in barley based diet, but it was more stabilized in wheat based diet. Bacterium inoculation increased the ratio of propionic acid (18.33%-21.38%) but it caused to decrease the butyric acid, and acetic/propionic acid. During the rapid adaptation, the concentration of lactic acid in the rumen liquid increased depending upon grain level ($P<0.0001$). On the other hand bacterium inoculation did not have an effect on concentration of lactic acid. *M. elsdenii* inoculation did not affect ruminal ammonia concentration. In the group that did not apply inoculation, the level of ruminal ammonia concentration was higher than the others applied inoculation. *M. elsdenii* inoculation did not changed protozoa count in barley-based diet whereas it decreased in wheat-based diet. When it is generally evaluated, it is seen that *M. elsdenii* inoculation has not a positive impact on rumen parameters. Therefore, to reveal the full impact of the inoculation with different strains, feedstuffs and animal groups, further research is required.

Keywords*—In vivo*, subactute ruminal acidosis, *Megasphaera elsdenii*, rumen fermentation.

I. Introduction

SUBACUTE RUMINAL ACIDOSIS (SARA), is a problem commonly observed during early lactation period in high producing dairy cows and at the end of feeding periods in livestock animals. It occurs when diets is changed from high-forage to high grain without giving enough time for adaptation, to meet the demands for increasing feed requirement. In addition, SARA triggers development of other metabolic diseases and causes loss of fertility; therefore, it leads to serious economic losses in intensive livestock production.

Megasphaera elsdenii, is a bacteria present in the rumen of animals fed with high grain rations and in rumen of young animals, it is capable of fermenting 97% of lactic acid [1]-[4]. Studies in *vivo* and *in vitro* have reported that increasing the population of lactic acid using bacteria such as *M. elsdenii* in the rumen during conversion to rations containing highly concentrated feed helps regulate rumen fermentation and prevent accumulation of lactic acid [1], [5]-[9]. It has been shown that when the proportion of concentrated feed in the ration is increased from 50% to 90%, *M. elsdenii* given orally improves feed consumption and prevents lactic acidosis [10]. Increasing *M. elsdenii* population in rumen seems to be a more natural practice compared to use of ionophore antibiotics; thereby providing a more acceptable means for consumption of meat and dairy products with regard to consumer health [11].

II. Materials and Methods

In this study, 8 Akkaraman ram aged 3-3.5 years (76.2±19.5 kg) were used, which had ruminal cannula. Animals were kept in their individual places and given fresh and clean water during the study. For adaptation to forage, alfalfa hay given to animals *ad libitum* in the first place during 15 days. In the study, 2 different grain mixtures were used that were based on barley and wheat. Chemical composition of the feed mixtures that were used in the study are presented in Table I.

After adaptation to forage, grain mixture at 20%, 40% and 60% rates were given on 1st, 4th and 7th days at a level that is 1.85 fold of maintenance ration and gradual adaptation was applied. With the initiation of adaptation period, animals were monitored for SARA by measuring pH levels in the ruminal fluids every day, once before feeding and with 2 hours intervals following feeding. After determining that SARA has developed, animals were grouped into two in the study with two different feed mixtures. During SARA period, animals were continued to be fed with 60% grain feed. During the first two days of SARA period, 2 hours after morning feeding, 100 ml TSB (Tryptic Soy Broth) containing approximately $2.4x10^{10}$cfu/ml bacteria (ATCC 17753) was inoculated to the

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animals in the study group via cannula. The same dose of bacteria was administered again on the second day at the same time.

TABLE I
CHEMICAL COMPOSITION OF ALFALFA AND GRAIN MIXTURES; %DM

	DM	CP	Ash	EE	NDF	ADF	NFC
BGBM	88.76	12.33	5.05	2.35	21.60	7.03	58.66
WGBM	89.04	14.27	5.46	2.23	20.10	6.94	57.90
Alfalfa	88.33	19.98	11.26	2.93	33.90	32.00	31.93

BGBM: Barley Grain-Based Mixture (80% Barley-20% Wheat), WGBM: Wheat Grain-Based Mixture (80% Wheat-20% Barley), CP: Crude protein, EE: Ether Extract, NDF: neutral detergent fiber, ADF: Acid detergent fiber, NFC: Non-fiber carbohydrate

After conversion to grain mixture following adaptation to forage, on 1st and 3rd day of each ration combination (20%, 40% and 60%), ruminal fluid samples were obtained via cannula at 4th and 8th hour after morning feeding during SARA period, in order to analyze VFA (gas chromatography, Shimadzu, Model 15-A), NH_3-N [12] and lactic acid [13] and to perform protozoa count [14].

DM, CP, EE and Ash analyses in the grain mixtures and in alfalfa hay that were used in the study were made according to analysis methods as stated in AOAC [15] (Table I). NDF and ADF levels were measured according to the method described by [16] using TheAnkom^{200} Fiber Analyzer device.

A. Statistical Analysis

For construction of models in data analysis, processes were arranged according to non-complete (2x3) factorial design. Main effect of grain feed (barley vs. wheat), main effect of grain feed ratio (20%, 40% and 60%) and interaction between these two effects were regarded as absolute factors of the mixed model; animals consuming these grain feed were regarded as random factor. Additionally, time effect was regarded as fixed factor, since measurements were carried out in different days and at different times. For this reason, data in factorial design were subjected to repetitive measurement system and split-plot model was developed. Results was accepted as significant when $P<0.05$, and as tendency when $0.05<P<0.10$.

Grain feed and bacteria inoculation levels ($10^{0, \text{ and } 10}$ cfu *Megasphaera elsdenii*) were analyzed similarly in repetitive measurements at different days and time periods in 2 x 2 factorial design (two-way ANOVA). Results were accepted as significant when $P<0.05$ and as tendency when $0.05<P<0.10$ [17].

III. RESULTS AND DISCUSSIONS

One of the most important observable indications of subacute acidosis is the reduction in feed consumption. Increased consumption of grain feed that contain high proportion of easily fermented carbohydrates cause decreased ruminal pH, which leads to reduction in feed consumption. Dry matter intake (DMI) has been reported to be decreased in dairy cows in which SARA was induced by barley-wheat mixture [18], [19]; it has also been reported that by feeding animals with feeds like barley and wheat that increase acid production up to a ratio of 50% of DMI, animals could tolerate excess produced acid and did not change their DMI [20]. It has been stated that day-to-day fluctuations in feed consumptions are related with feed that increase acid production, and can be evaluated as an indicator for SARA [21], [22]. In this study, while there was no difference between grain mixture types regarding DMI, it was observed that DMI showed tendency to decrease when the proportions of both grain feed were increased.

Administration of propionic acid to rumen causes a feeling of fullness, number of meals and amount of consumed feed at each meal, hence, DMI decrease [23]. The reduction in DMI after *Megasphaera elsdenii* inoculation ($P<0.09$) (Table I) is thought to be related with the change in fermentation towards propionic acid (Table II). During SARA period *M. elsdenii* inoculation is ineffective for increasing pH above 5.6, therefore, it is thought that suppressive effect of low pH on feed consumption is not eliminated and no favorable effect is observed.

It was reported that the herds can be regarded as SARA (+) if ruminal pH is below 5.5 in more than 3 of the 12 cows' sample [24]. In this study, each 20% increase in feed mixtures based on barley and wheat caused decrease in ruminal pH. It was also determined that there were fluctuations in pH values measured before feeding and with 2 hours intervals after feeding during the whole day until the end of testing. Ruminal pH varies depending on consumption of fermentable carbohydrates; utilization and absorption of produced acid and secretion of buffer materials, and fluctuates during the day [25].

In this study, during feeding with 60% feed mixture based on barley and wheat during adaptation period, mean pH was determined as 5.75 and 5.85 (Table II) During SARA period, ruminal pH was determined to be 5.56 and 5.62, respectively. Previous studies report minimum pH as 5.8-5.0 [26] or 5.0-5.6 [27] during SARA that is induced with high proportion of grain feed, which indicate that our results as 5.56 and 5.62 are within the normal limits. Differences in ruminal pH between animals are caused by the differences in salivary production, VFA absorption rate, and passage rate of fluids through rumen and VFA metabolism [28]. Variations of ruminal pH between animals during feeding with highly concentrated feed [22], [29], [30] were also encountered in this study.

It is interesting that *Megasphaera elsdenii* inoculation in SARA induced with barley and wheat mixture does not have favorable effect on ruminal pH, but pH starts to increase towards 4th day of SARA period with the inoculation administered on the second day. In SARA induced animals, *M. elsdenii* inoculation has been reported to reduce minimum ruminal pH and lactic acid concentration significantly; however, it has also been reported that *M. elsdenii* inoculation has no effect on ruminal pH [31].

TABLE II
ALTERATIONS IN THE DRY MATTER INTAKE, RUMEN PH AND AMMONIA, LACTIC ACID AND PROTOZOA COUNTS DEPENDING ON THE *M. ELSDENII* INOCULATION WITH GRAIN MIXTURE AND RATIOS

Trial Period	Grain Mixture	Ratios of Grain Mixture / Bacterial inoculation	DMI, kg	pH	Ammonia, mmol/L	Lactic Acid, mmol/L	Protozoa Counts (ml)
Adaptation	Barley	20	1.77	6.14	11.53	0.59	3.3×10^5
		40	1.69	5.94	8.98	0.50	7.2×10^5
		60	1.63	5.75	9.92	0.65	1.5×10^6
	Wheat	20	1.69	6.25	10.87	0.36	5.6×10^5
		40	1.66	6.10	12.37	0.45	1.2×10^6
		60	1.51	5.84	12.55	0.72	1.3×10^6
SEM			0.07	0.02	0.37	0.06	0,05
SARA	Barley	-	1.7	5.62	10.09	0.86	1.1×10^6
		+	1.68	5.50	9.12	0.98	1.0×10^6
	Wheat	-	1.69	5.64	12.59	0.79	1.8×10^6
		+	1.43	5.60	11.66	0.68	7.1×10^5
SEM			0.08	0.02	0.59	0.09	0.06
ANOVA							
Adaptation							
GM			0.12	0.0001	0.0001	0.164	0.001
GMR			0.06	0.0001	0.27	0.001	0.0001
GM*GMR			0.84	0.02	0.0001	0.061	0.002
SARA							
GM			0.12	0.006	0.0001	0.032	0.716
B			0.09	0.0001	0.11	0.92	0.0001
GM*B			0.14	0.115	0.98	0.19	0.003

DMI: Dry Matter Intake; GM: Grain Mixture; GMR: Grain Mixture Ratio, B: Bacteria.

At adaptation period, during feeding with 60% barley and wheat based mixtures, total VFA concentration does not show difference according to the type of grain feed, which indicates that barley and wheat have similar degradation rates (Table III). While 47.6% and 61.3% of wheat is degraded in rumen at the end of 4 and 8 hours, 42.3% and 58.3% of barley is degraded at the given times [32].

Increased rates of propionic and especially butyric acid (Table III) that promote epithelial growth, by increasing the proportion of grain feed during adaptation period [30], [33]-[36] is thought to prevent SARA development by facilitating better regulation of barrier functions of ruminal epithelium [37] and passage rate of metabolites through ruminal wall [38]-[40] and increase in absorptive capacity.

It has been determined that increased number of protozoa in rumen promote butyric acid production by causing a decrease in lactic acid accumulation [41], and proportion of butyric acid decreased when number of protozoa decreased [42]. In this study, it is thought that increased proportion of butyric acid (Table III) together with increased proportion of grain may be related with the increase in number of protozoa (Table I), *Megasphaera elsdenii* that was inoculated in SARA does not have favorable effect on number of protozoa, and maybe it was due to the reduction in proportion of butyric acid. Aikman et al. [43] reported that *M. elsdenii* inoculation promoted propionic acid production. Increased production of propionic acid, which is a glycogenic precursor, reduces energy loss by decreasing methane production [44]. In lactating animals, 67% of glucose synthesis is made from propionic acid [45]. For this reason, increased proportion of propionic acid by *M. elsdenii* inoculation in this study (Table III) is thought to be beneficial considering that it can alleviate the disturbances as a result of negative energy balance at the beginning of lactation.

Acid production in rumen increases in parallel with the level of consumed carbohydrate [46]-[48]. In this study, lactic acid concentration increased with increased proportion of grain, which is a reflection of this fact (Table I).

It is reported that lactic acid utilizing microorganisms in ruminal fluid (*Megasphaera elsdenii, Selenomonas ruminantium* etc.) start to grow beginning from the first days of conversion to concentrated feed [2], [9], [49].

During SARA, lactic acid is converted to volatile fatty acids by lactic acid utilizing bacteria; therefore it does not accumulate in the ruminal fluid [50], [51].

Average lactic acid level in the rumen based on barley and wheat during this period was reported to be 0.86-0.79 mM, therefore, it is observed there was no lactic acid accumulation. Decreased ruminal pH in subacute acidosis was reported to be a result of increased VFA concentration [52], [53]. Continuous increase in VFA in this study suggests that VFA accumulation may be responsible of pH reduction in SARA. Additionally, no important effect of *M. elsdenii* inoculation on lactic acid was seen in the study.

TABLE III
ALTERATIONS IN VFA PARAMETERS DEPENDING ON THE *M. ELSDENII* INOCULATION WITH GRAIN MIXTURE AND RATIOS

Trial Period	Grain Mixture	Ratios of Grain Mixture/ Bacterial inoculation	Acetic Acid*	Propionic Acid*	Isobutyric Acid*	Butyric Acid*	Isovaleric Acid*	Valeric Acid*	Total VFA,mM	As:Pr**
Adaptation	Barley	20	62.95	18.89	1.83	11.96	2.09	2.29	1.54	3.38
		40	60.75	18.63	1.98	13.6	2.66	2.36	1.34	3.34
		60	57.55	19.95	2.18	16.15	2	2.17	1.28	3.05
	Wheat	20	59.85	18.58	2.63	13.38	3.05	2.52	1.25	3.3
		40	56.55	19.15	2.73	15.6	3.26	2.71	1.32	3
		60	56.97	17.22	2.65	17.39	3.18	2.59	1.4	3.45
SEM			0.66	0.45	0.14	0.51	0.22	0.14	0.04	0.1
SARA	Barley	-	54.26	21.38	1.47	18.19	1.93	2.78	1.31	2.71
		+	53.58	24.37	1.54	16.68	1.73	2.1	1.44	2.33
	Wheat	-	57.57	15.28	2.18	19.62	2.71	2.65	1.46	3.97
		+	56.92	18.38	2.29	17.24	2.61	2.56	1.45	3.28
SEM			0.76	0.81	0.13	0.5	0.11	0.19	0.04	0.16
ANOVA										
Adaptation										
GM			0.0001	0.02	0.0001	0.0001	0.0001	0.005	0.09	0.9
GMR			0.0001	0.8	0.36	0.0001	0.15	0.52	0.23	0.25
GM*GMR			0.02	0.001	0.43	0.75	0.43	0.8	0.0001	0.001
SARA										
GM			0.0001	0.0001	0.0001	0.05	0.0001	0.39	0.02	0.0001
B			0.39	0.0001	0.5	0.0001	0.16	0.04	0.12	0.001
GM*B			0.9	0.95	0.86	0.39	0.61	0.12	0.07	0.33

DMI: Dry Matter Intake; GM: Grain Mixture; GMR: Grain Mixture Ratio, B: Bacteria. *mol/100 mol, **Acetic acid/Propionic acid

It was reported that due to decreased pH and decreased number of cellulolytic bacteria that use ammonia nitrogen together with the increased proportion of concentrated feed in ration [54], ammonia concentrations rise [41], [55], [56]. In this study, ammonia level showed difference depending on the type of grain mixture, but *M. elsdenii* inoculation following SARA did not have an effect on ammonia levels. Similar results were obtained with the studies that reported number of protozoa in the ruminal fluid increased logarithmically as the proportion of grain increased during adaptation period [49], [57]-[59] and number of protozoa decreased as pH dropped after development of SARA [59].

Considering that number of protozoa decreased as ruminal pH decreased [55], it can be said that *Megasphaera elsdenii* inoculation decreased ruminal pH and hence, the number of protozoa (Table I). Additionally, it is thought that tendency of the ruminal pH towards increase after second *M. elsdenii* inoculation may also be observed in the number of protozoa.

IV. CONCLUSIONS

The finding that there was not any difference in ruminal lactic acid levels, but a decrease in ruminal pH after *Megasphaera elsdenii* inoculation could be related to numerical increase in total VFA production. Decreased number of protozoa after inoculation is also thought to be due to decreased ruminal pH. In this study, although there was no statistical difference in most of the chosen parameters, tendency of pH toward increase, increase in total volatile fatty acids and especially propionic acid, decrease in As:Pr ratio after second *M. elsdenii* inoculation suggests it may have significant beneficial effects.

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