Acute phase proteins in cows induced with SARA

Research to the response of the acute phase protein with a subacute rumen acidosis in cows caused by too little structure in the feed

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Abstract

Subacute rumen acidosis (SARA) is considered to be one of the most important nutritional diseases in dairy cattle. There have been a lot of studies into how SARA is induced by changing the diet abruptly. For instance some studies examined the effects when the concentration of feed was increased in comparison to the roughage. In other researches the particle size of alfalfa was studied.

The objective of this study was "to determine the response of acute phase proteins on subacute rumen acidosis, as caused by reducing the physical structure of the diet of dairy cows". To do this the particle size of the diet was changed from 20 cm to 5 cm in grass silage and from 5 cm. to 1 cm. in wheat straw. This study included two groups of three cows, which had diet change at different times. Before and after the diet change the rumen pH, the rumen LPS concentration and the acute phase proteins (APP) were measured. Next to that several performances of the cows were measured. These included: feed intake, water intake, rumination time, rumen motility, milk yield and milk composition. No changes were seen after the diet change in all these parameters. Only one cow displayed an increase in APP, but its rumen LPS was not increased at the same time and the pH was not recorded, due to failing of the equipment. So it might be possible that the acute phase response was caused by an inflammation in another part of the body. All in all the diet change did not cause SARA in the investigated cows according to the measurements. Recommendations for future studies on this object are, to have a bigger diet change, take more cows and measure the APP every day instead of every three days. This way changes due to measurement mistakes can be ruled out.

Introduction

Subacute ruminal acidosis (SARA) is defined as an intermittent fall of ruminal pH to non-physiological levels. The definition for SARA differs. In this study the following definition has been chosen; Daily episodes of low rumen pH below 5,6 for at least three hours per day. This is a form of rumen acidosis which is considered to be one of the most important nutritional diseases amongst dairy cattle[1].

When a cow does not have rumen acidosis, the bacteria in the rumen are in equilibrium. The cow regulates this itself by controlling the pH levels in the rumen with its saliva, the composition of product in the rumen and the availability of nutrients. The saliva regulates the pH with its buffering properties. The saliva is produced continuously and the production increases while eating and rumination[2]. SARA occurs after switching to a faster fermenting diet. The benefit of the faster fermented diet is that the

ingredients are available more directly. With this the cow has to ruminate less frequently and can eat more. The reason for this is that the feed has less structure and, thus takes up less space in the rumen. The availability of ingredients causes the acceleration of the growth of fermenting bacteria, including the lactic producing ones. Consequently, this causes a disequilibrium of the ruminal environment in terms of flora and mucosa[3]. As a result of the growing population of the lactic bacteria, there is an accumulation of lactic acid and an alteration of the mix of the volatile fatty acids. This causes a rapid decline of the pH in the rumen. Normally, saliva can prevent the pH levels dropping too low for a long period of time. However, the production of salvia does not increase enough in the case of SARA, because the cow ruminates less frequently.

The acidification, caused by the lactic bacteria and domination of the *Streptococci*, contribute to an even stronger acidification of the rumen, which in turn causes bacteria and protozoa to die[2, 4]. A component of the cell wall of the Gram-negative bacteria is Lipopolysaccharide (LPS)[4]. This component is released from the bacteria after they die. So the amount of free LPS increases in the rumen. The LPS may move to the blood through the ruminal wall or further on in the gastric system, where it causes an inflammation[5, 6]. It is not yet understood, whether LPS is taken up in an active transcellular process or in a paracellular route [7].

SARA has been linked to a significant increase in certain acute phase proteins (APP) in the blood[8-11]. These includes: Serum amyloid alpha (SAA) and haptoglobin (Hp). The main functions of these APPs are:

- SAA: binding of lipoproteins and taking care of a faster clearance by the liver hepatocytes[11], to extracting cholesterol from cells and facilitating the transport of cholesterol[12], binding and activating neutrophils and macrophages[13] and an increase in phagocytosis of coliform bacteria by macrophages[14].
- Hp: binding hemoglobin and selectively antagonizing the effects of LPS in vitro by suppressing monocyte production of tumor necrosis factor-alpha, Interleukin 10 and interleukin 12[11, 15].

As previously mentioned SARA is considered to be one of the most important nutritional diseases of dairy cattle[1]. The only difficulty is to identify a cow suffering from SARA. The question is whether it is possible to measure the APP to know whether a cow has SARA.

A few studies investigated the effect of SARA after changing the feed of the cows. They did this by feeding an increased ratio of grain compared to roughage. The result of

that was that the feed was too low in NDF[9, 16-18]. SARA proved to be induced this way in these studies.

Besides an unbalanced proportion of concentrate and roughage, there are other conditions with high a risk of causing SARA.

One of these is the effect of the physical structure of feed[19]. For instance, the physical structure of the feed was changed, by replacing alfalfa hay with alfalfa pellets in a case study. This study showed a significant difference in APP, when the structure in feed is gradually changed from alfalfa hay to alfalfa pellets[19].

Moreover, Li investigated the difference between grain and physical structural induced SARA in 2012[9]. The physical structure that was reduced was alfalfa. It was concluded that only SARA induced by grain caused an increase in the concentration of LPS in blood.

In a study of Beachemin et al. (2003) it was noticed that cutting alfalfa hay and silage to a shorter length caused the rumen to be pH below 5,8 for a longer period of time[20]. Also Krause et al. (2002) studied the effect of particle size on rumen pH[21]. He took alfalfa of 13,6 mm. for the long particle size and 3,6 mm. for the short particle size[22]. The outcome of his study was that the average pH decreased from 6,02 to 5,81.

In this study, the size of the diet is also changed to a smaller physical structure. However, the difference with previously mentioned studies is that the physical structure of grass is reduced instead of alfalfa. By changing this diet it is investigated whether a small physical structure of the feed has an influence on the concentration of APP. Assuming that the abrupt transition from a feed with standard physical structure to one with a substantially smaller physical structure triggers SARA. Besides APPs the iron concentrations are measured, because Baydar and Dabak (2014) concluded in their article that the iron concentration could be a diagnostic tool for acute inflammation in cattle[23].

The practical use of this study is to investigate, whether it is possible to predict a subacute rumen acidosis with the concentration of Hp, SAA or iron. Furthermore, it is investigated which parameters change when SARA is present. That is why the following parameters are closely monitored: rumination, rumen motility, water and feed intake, blood acute phase response, milk yield and composition.

The objective of the study

The objective of this study is "to determine the response of acute phase proteins on subacute rumen acidosis, caused by reducing the physical structure of the diet of dairy cows".

This objective will be investigated with the following hypotheses: One of them is that diminishing the structure of the feed causes a reduction in the average pH of the rumen. Furthermore, a reduction in the average pH of the rumen causes an increase in the concentration of LPS in the rumen and an increase in the concentration of LPS in the rumen causes an increase in the concentration of haptoglobin and Serum amyloid alpha in the blood. The feed intake and water intake is expected to increase and rumination activity decreases when the structure of the feed is reduced (figure 1).

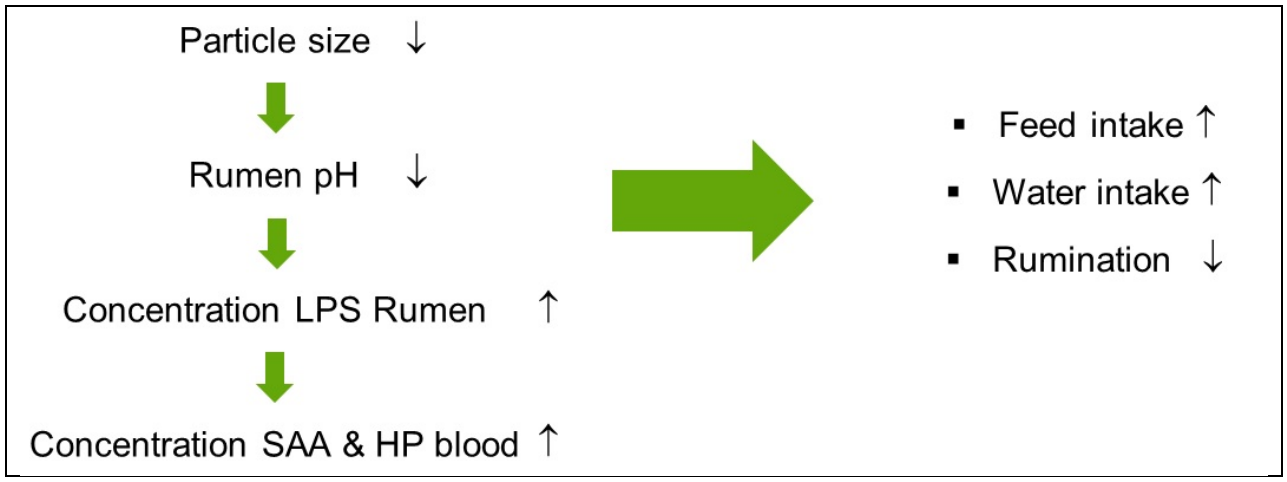


Figure 1 Diagram of the hypotheses in this study.

Materials and methods

Animal diet and procedures of the experiment

Six rumen fistulated Holstein-Friesian dairy cows were housed in individual tie stalls with individual feeding troughs. Average milk yield was 31.4 ± 2.5 kg/day and their days in milk ranged between 39 and 590. Their weight was 702 to 711 kg. The cows were divided into two groups, group 1 and group 2, of three cows each. The transition of group 1 was three weeks before the transition of group 2 (figure 2). That was the only difference between the groups. Each group had a transition from the control diet to the experimental diet, in which the experimental diet had the same nutrient and ingredient composition as the control diet.

Therefore, the only change was in size of the feed. In the control feed the grass silages and wheat straws had roughly a length of respectively 20 cm and 5 cm. To induce SARA the lowest possible length was taken, before turning the diet to pulp, because that would change the elementary characteristics of the diet. In the experimental diet the grass and straw had roughly a length of 5 and 1 cm. The diets were given over a period of three weeks.

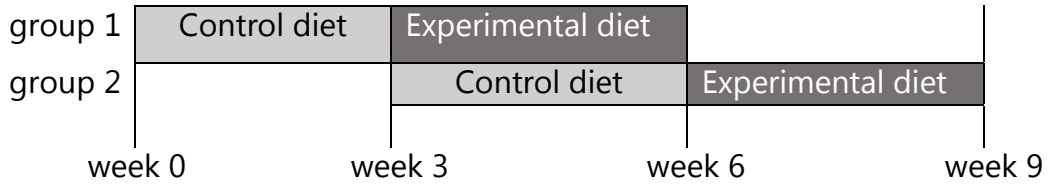


Figure 2 Weeks which diet was given

Table 1 Composition of diet

Ingredient	**Kg Product**	**Ingredient**	**Kg Product**
Grass silage[1]	20,60 kg.	Correction concentrate (meal)	3,00 kg.
Corn silage	11,50 kg.	Limestone	0,22 kg.
Wheat straw[2]	1,20 kg.	Salt	0,11 kg.
Production concentrate (pellet)	7,00 kg.	Rupromin USA	0,14 kg.

[1] Grass silage had a length of 20 cm. in the control diet and a length of 5 cm in the experimental diet

[2] Wheat straw had a length of 5 cm. in the control diet and a length of 1 cm in the experimental diet

Table 2 Nutrition diet

Nutrient	**Percentage of dry matter[1]**	**Nutrient**	**Percentage of dry matter[1]**
Crude fat	3,60 %	Calcium/Phosphorus	0,24 %
Crude fiber	18,80 %	Magnesium	0,27 %
Ash	9,20 %	Sodium	0,48 %
Starch	15,80 %	Potassium	1,90 %
Sugar	8,00 %	Chlorine/Chloride	0,79 %
NDF	37,50 %	Sulphor	1,70 %
ADF	21,98 %	VEM[2]	953
ADL	2,42 %	Rumen bypass protein	6,00 %
Calcium	1,01 %	DVE[3]	9,10 %
Phosphorus	0,43 %	OEB 2007[4]	2,60 %

[1] dry matter of the whole diet was 53,5%

[2] feed unit for milk production

[3] intestinal digestible protein

[4] rumen degradable protein balance. According to the Dutch CVB system of 2007

Rumen pH analysis

Rumen pH was measured using a pH bolus of eCow during the whole experiment. The bolus was secured to the rumen fistula, so the probe stayed in the rumen during the

whole period of the experiment. The boluses were calibrated before they were inserted into the cow and were set to a 5 minutes measurement interval.

Rumen motility

The motility of the rumen was measured using a rumen motility meter developed by eCow (Decon, UK). The motility was measured using two pressure probes, which were inserted in the rumen and reticulum. The pressure was measured every two seconds. With this data graphs of measured pressure were drawn, in which the pressure patterns of the rumen were counted. The pressure waves were measured two days before to two days after the diet change in all cows for a day. However, this measured at different moments because only one motility meter was available to measure all cows.

Rumen LPS

Rumen fluid of group 2 was sampled using a sterile syringe and cooled down with ice. The fluid in the syringes was transferred into 4 sterile eppendorf cups. These were centrifuged to eliminate particles and non-free LPS at a speed of 10000 g. for 30 minutes. So only the LPS not bound to bacteria cell walls went into the sample. This supernatant was aspirated with a sterile needle and syringe. Then the substance was filtered using a sterile filter (syringe filter units (0,22 µm), disposable, Durapore® PVDF) of Millex (Billerica, MA, USA) and transferred to two new sterile eppendorf cups (Eppendorf® Biopur® Safe-Lock microtubes 2 ml.). These cups were heated for 30 minutes in an incubator at 100˚C. The cups were cooled down at room temperature for ten minutes before storing them at -20 ˚C in the freezer.

The samples of group 1 were collected in non-sterile containers. These containers were emptied in small containers, which were stored at -20 ˚C in the freezer. After being stored for a few weeks the rumen fluid was defrosted and taken with a syringe. After that the same method as in group 2 was used. The reason why another method was used in group 1 was that the instruments had not been available during the diet change of the first group. The frozen samples were investigated with the *Limulus* amebocyte lysate assay (Lonza, Walkersville,MD) at "Biocheck" (Leipzig, Germany).

Blood measurement

Blood samples were collected from the jugular vein one day before and one and four days after the dietary switch with a serum container. The blood samples were allowed to clot at room temperature for 30 minutes, after which they were centrifuged at a speed of 3.000 RPM for 12 minutes. Then that serum was transferred to multiple Eppendorf cups. These cups were frozen and sent to "GD animal health service" (Deventer, Netherlands). The samples were tested on concentrations of Hp ('Phase™ haptoglobin colometric assay, Tridelta Development Ltd., Maynooth, Ireland), SAA ('Eiken' SAA latex agglutination test, Mast house Ltd., Merseyside, UK) and iron (Ferentest, bioMerieux inc.,SA) using ultraviolet-visible spectrophotometry.

Rumination

This was measured using "Rumiwatch" of "Agroscope" (Bern, Switzerland) and "Lely QWES-HR" of "Lely" (Maassluis, Netherlands). The Lely "QWES-HR" measured the average rumination each day for the experimental period. With this information it was measured how long the cows were ruminating each day during the experiment. This was done by a collar, which measures whether the cow is ruminating with a microphone. The Rumiwatch measured, with a halter, the position of the head and the pressure when the cow chews. With these measurements the Rumiwatch can register whether the cow is eating, drinking, ruminating or doing something else. These measurements were used to see what the cow was doing during the pressure waves, recorded with the rumen motility meter. This halter was used at each cow one day before and one day after the diet change at the same time the rumen motility was measured.

Feed and water intake

Feed intake and water intake were measured daily during the whole experiment. The water intake was measured with a meter in the water supply of each cow to get an average water intake for each day. The cows were fed once a day in the morning. The feed was weighted before it was given and the remaining was removed and weighted again the following day.

Milk yield and composition

The cows were milked twice daily. The amount of milk given was recorded. The milk was analyzed weekly. The analyses included; percentage of fat, proteins and lactate in the milk, the concentration of urea and the somatic cell count. In the week of the diet change, the change in diet came three days after the milk analyses.

Statistical analyses

Data of the values of LPS were compared with the observed iron concentrations and the APP values. This was done with the R^2 of the trend line.

Results

pH data was missing for cows 2, 4 and 6, due to the fact that the pH probes malfunctioned and did not record the pH on these dates. In cows 4 and 6 there were no data of the pH before and after the diet change respectively. In cow 2 there was no data on the nine days before the day of the diet change. Cow 2 had the same diet eleven and ten days before the diet change as the two last days before the diet change. Therefore, the pH data of these dates were used instead of the data of two and one days before the diet change. As shown in figure 3 after feeding all cows, only cow 2 showed a decrease while the others showed an increase of pH. After four hours the pH decreases to the value at the beginning, whereas it increased towards the end of the period. At the end it slowly decreased again (figure 3).

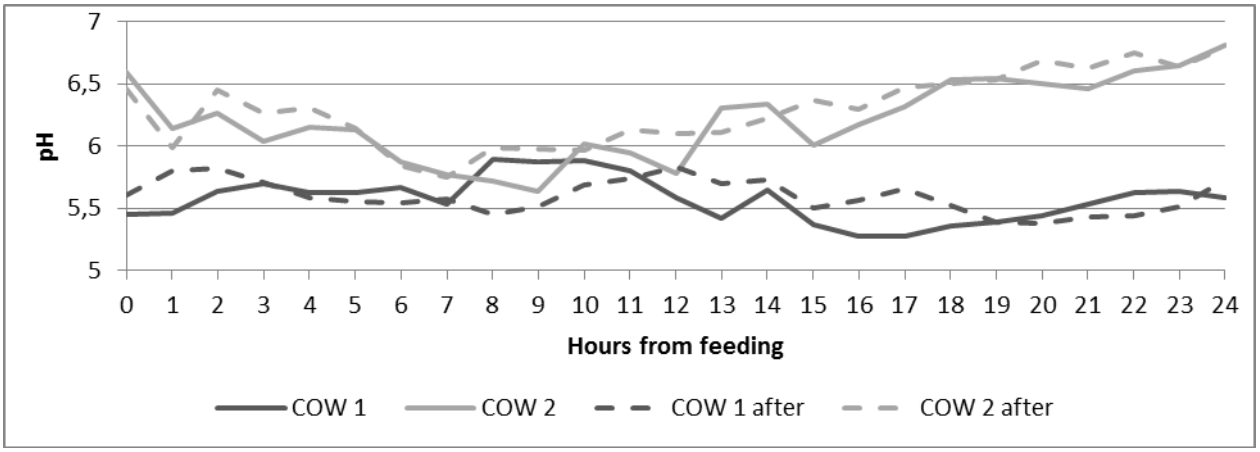
Figure 4 shows that the average pH levels did not change significantly. This seems to be the case for each individual cow. Consequently the average pH levels of cows 1,2,3 and 5 increased from 5,78 to 5,82 (table 3).

Table 3 Average of daily pH levels of each cow

Cow	**Average pH level of cow before diet change**	**Average pH level of cow after diet change**
COW 1	5,57	5,61
COW 2	6,19	6,24
COW 3	5,87	5,87
COW 4		6,17
COW 5	5,49	5,57
COW 6	5,73	
Average cow 1,2,3 and 5	***5,78***	***5,82***

Regarding the average pH level in each cow, the graphs show no difference in pH levels after the diet change. Only the graph of cow 4 displays an increase in pH levels after the diet change, but of that cow the data from before the diet change is missing (figure 5).
The graph of the pH levels compared to time of cow 1 and 5 is high before and after the diet change. After the diet change cow 3 and cow 4 also have a ruminal pH under 5,6 for more than 180 min. a day (figure 6).

A.



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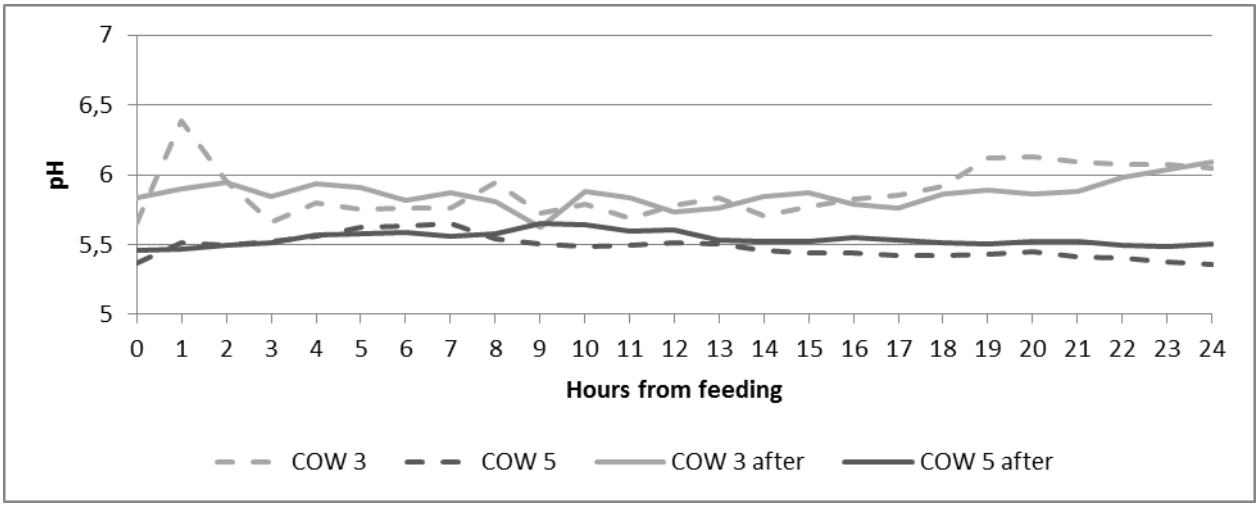

Figure 3 The average was taken of 2 days before and 2 days after the diet change. The cows were fed at 0 hour. A) pH of cows 1 and 2. For cow 2 the average of 11 and 10 days before the diet change was taken. B) pH of cow 3 and 5

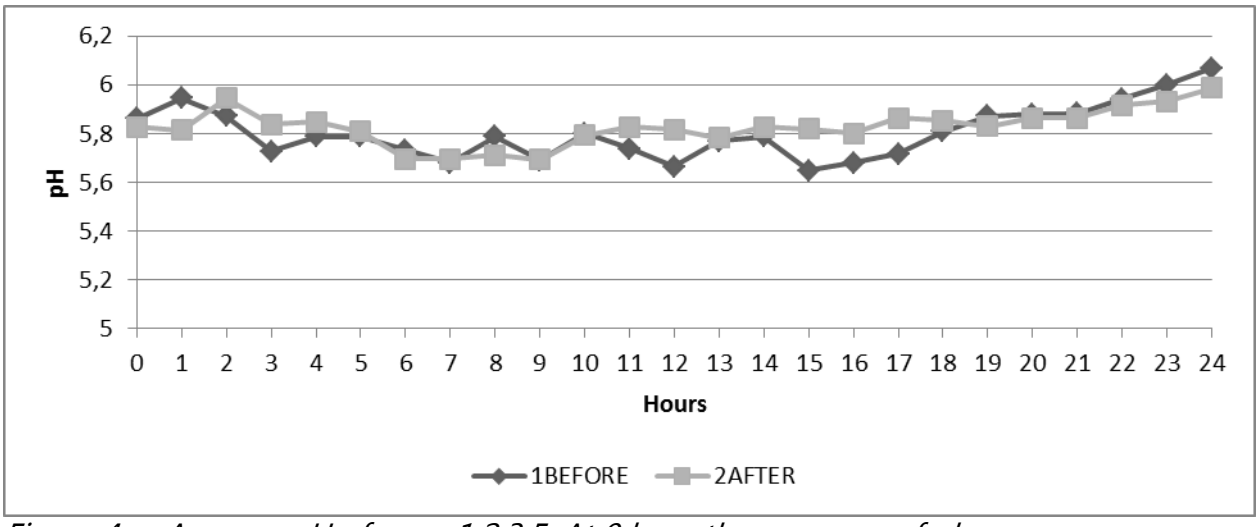

Figure 4 Average pH of cows 1,2,3,5. At 0 hour the cows were fed

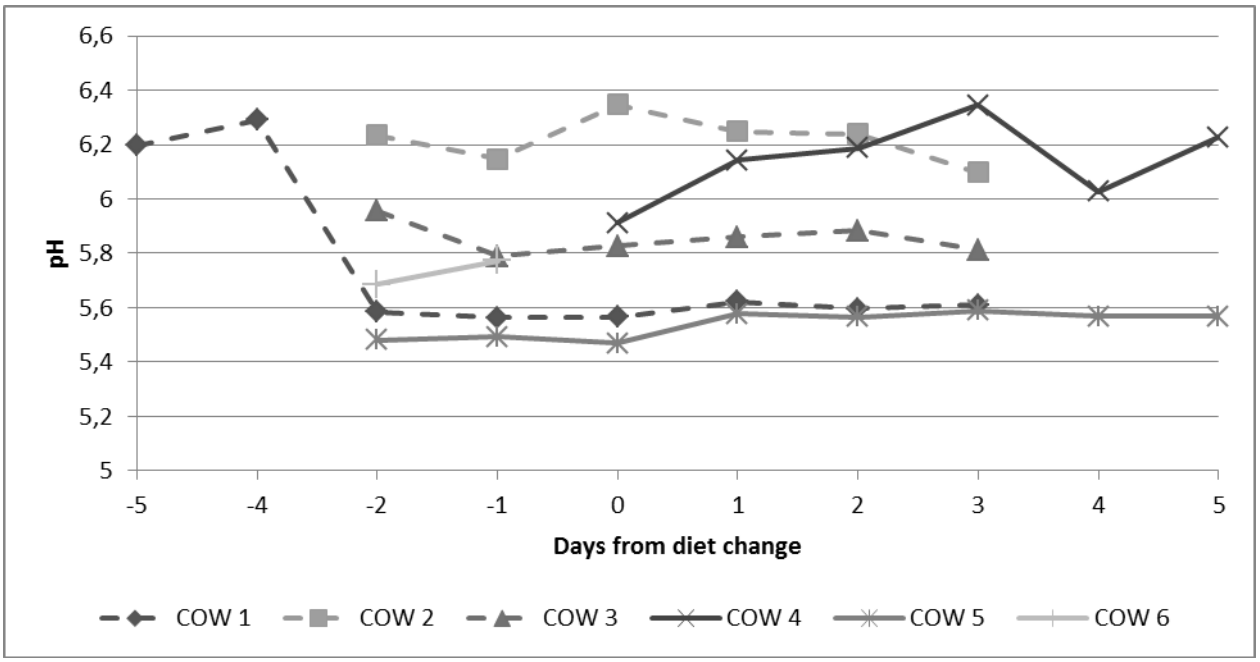

Figure 5 Average pH every day. The diet change took place at day 0

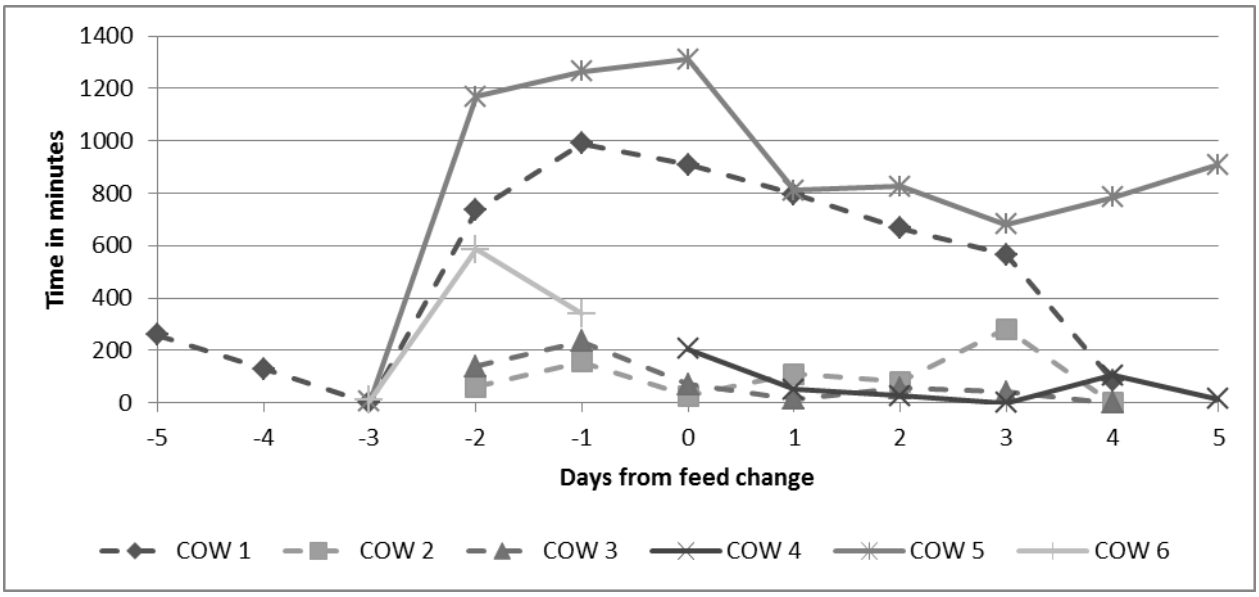

Figure 6 Time pH was under 5,6

Acute phase proteins

The concentration of LPS declined in most of the cows after one day, except for cow 5 in which the LPS increased (figure 7). On average it declined with 15.492,6 EU/ml. in the 5 cows. After the first day only the second group was measured. In this group the LPS increased in two cows and decreased in another one after which the concentration came back to almost the same concentration levels as before the diet change. The average difference between before the diet change and after the diet change was 6.173 EU/ml.

The concentration Hp hardly changed in most of the cows over the period it has been measured (figure 8A). However Cow 1 displayed a decrease in the concentration. Cow 4 showed a dramatic increase in Hp concentration after the first day. The Hp concentration was 1,13 mmol/L in that cow at day four.

The SAA concentrations in cows 1, 3, 4 and 6 were getting higher after the first day of the diet change, whereas the concentration of cow 4 decreased (figure 8B).

In addition, iron concentrations stayed almost the same (figure 9). The average was 28,95 µmol/ml. in the concentration of the cows, with the exception of cow 4, which initially showed an increase and after that a decrease.

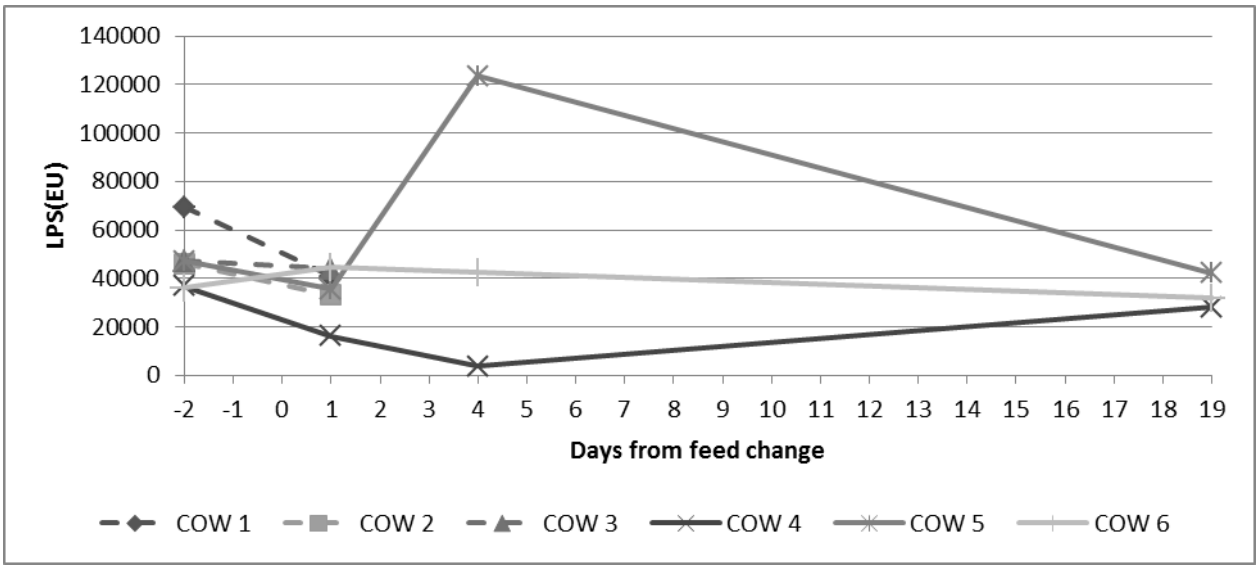

Figure 7 Lipopolysaccharide (LPS) concentration. The diet change took place at day 0.

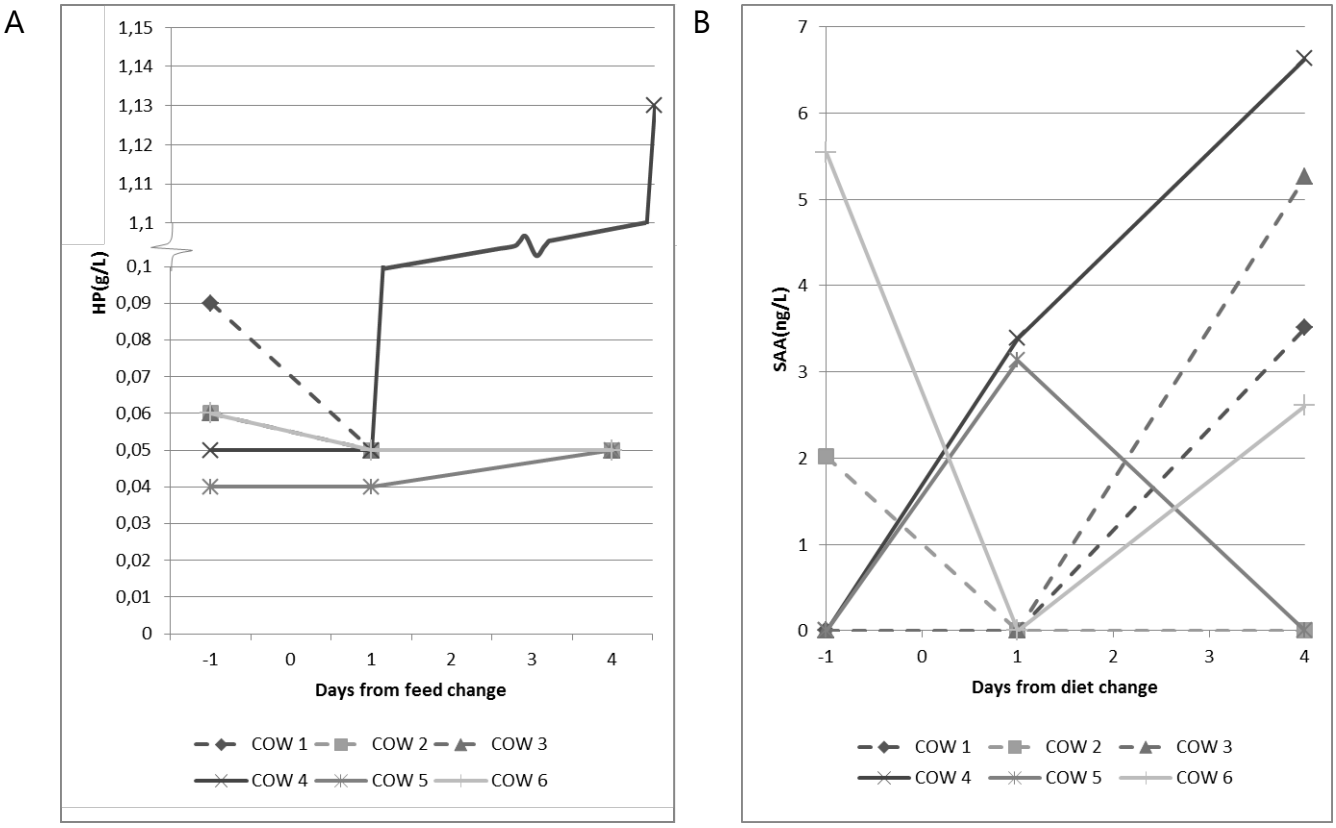

Figure 8 Acute phase response: A) Haptoglobin and B) Serum amyloid alpha (SAA) concentration. The diet change took place at day 0 in both.

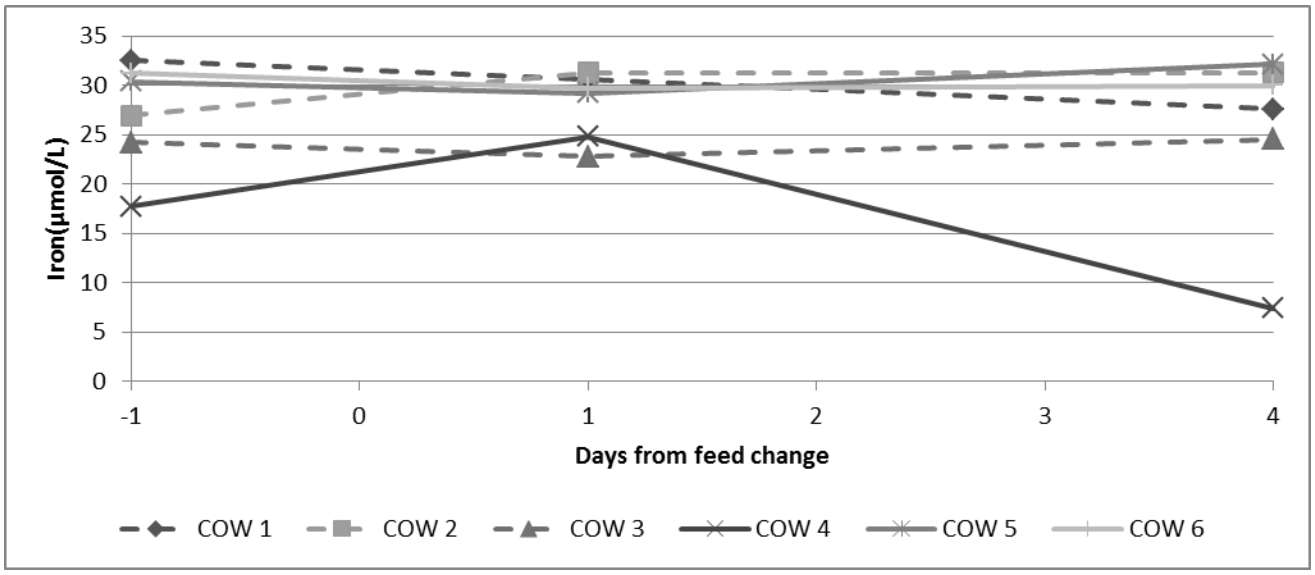

Figure 9 Concentration Iron. The diet change took place at day 0.

Comparison acute phase proteins

To evaluate whether the LPS influenced the APP, the LPS is compared with SAA and Hp. There was no influence seen of LPS on the APP (figures 10 and 11). Hp is compared to iron. Here was no influence seen with low concentration of Hp. Only when the concentration Hp increased the iron concentration decreased (figure 12).

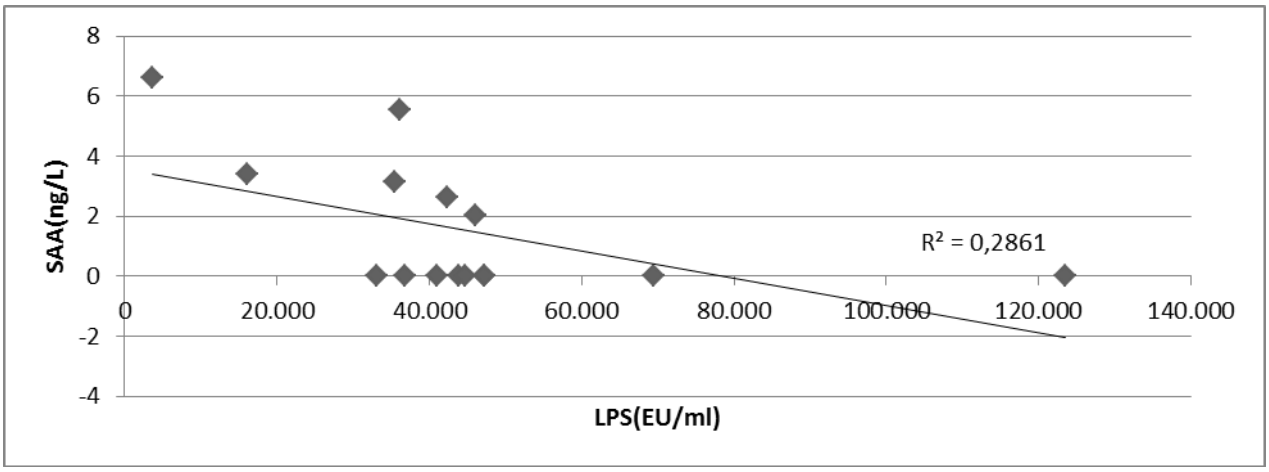

Figure 10 Correlation between serum amyloid alpha (SAA) and Lipopolysaccharide (LPS) in different cows

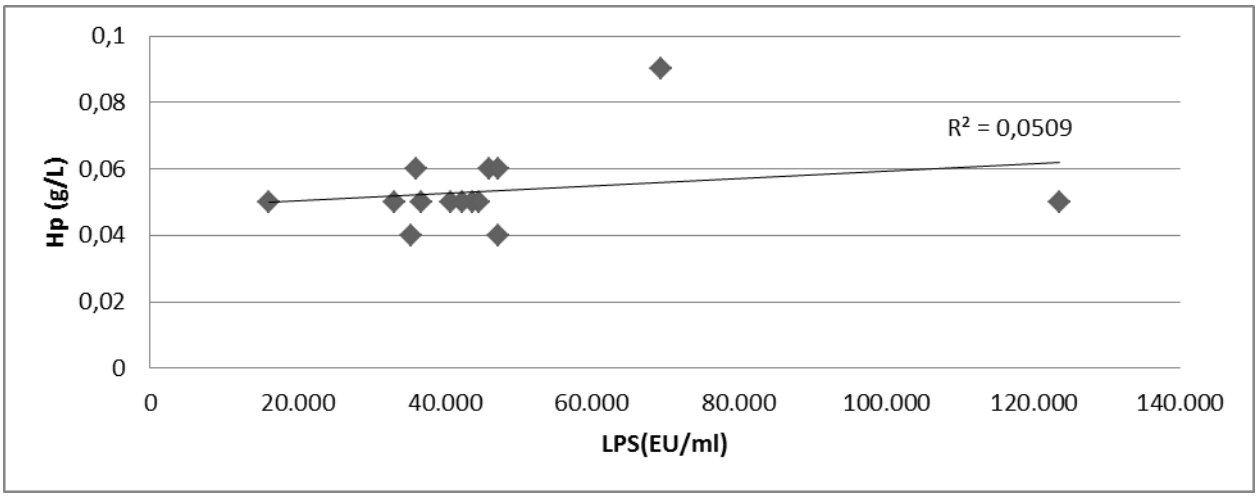

Figure 11 Correlation between haptoglobin (Hp) and Lipopolysaccharide (LPS) in different cows

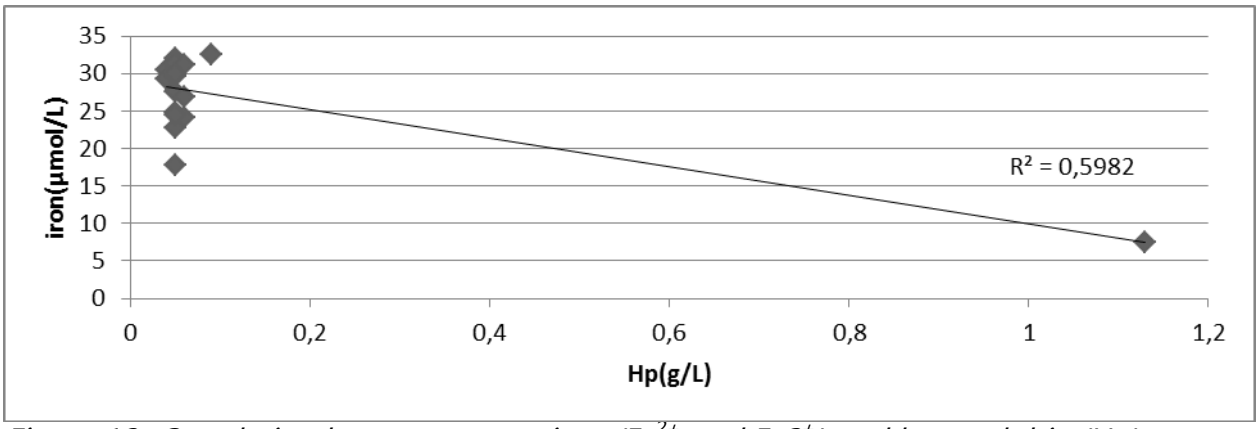

Figure 12 Correlation between serum iron (Fe^{2+} and $Fe3^{+}$) and haptoglobin (Hp)

Rumination

The rumination minutes decreased a lot in two cows (figure 13). The rumination cycles in five minutes, differs not more than 1 cycle in every cow (figure 14). Figure 14 shows the average of rumination cycles during eating, rumination and other activities. As can be seen in table 4 there is no large differation in changes between the different activities.

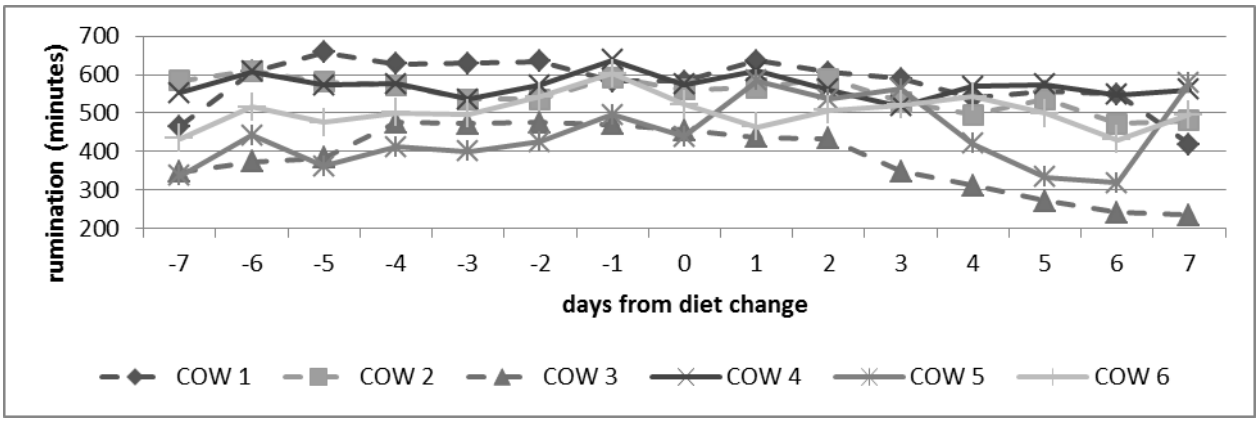

Figure 13: Average of number of minutes spent to ruminate in each cow. The diet change took place at day 0.

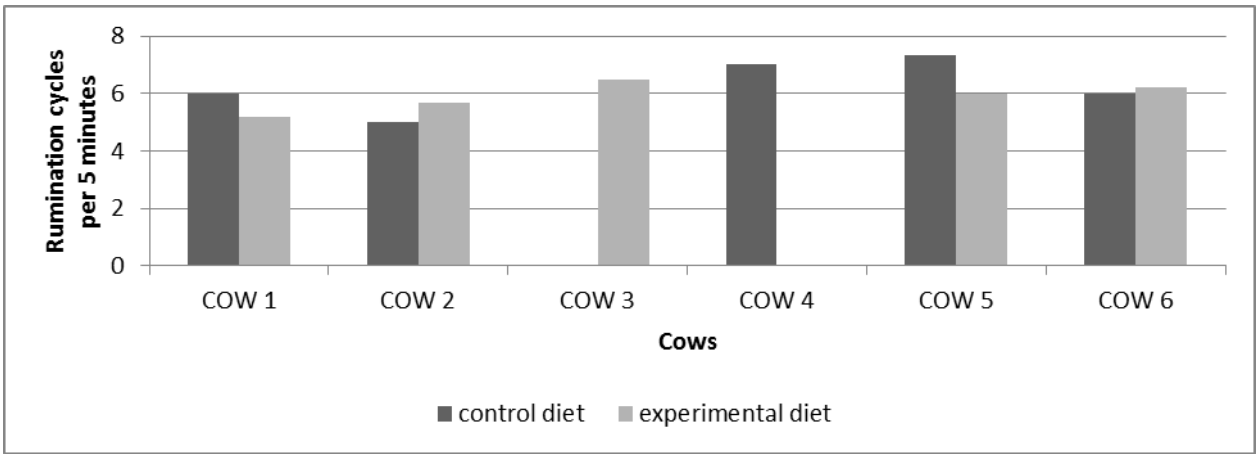

Figure 14 Average of rumination cycles before and after diet change in each cow. In cow 3 and 4 there was no accurate data.

Table 4 Average rumination cycles per activity (in which other means other than rumination or eating). The cycles in eating were not recorded in cow 3. "Before" means in this situation before the diet change and "After" is after the diet change.

	Rumination		**Eating**		**Other**	
	Before	*After*	*Before*	*After*	*Before*	*After*
COW 1	4,5	5	7	4,5	6,5	6
COW 2	4	5,5	5	5	6	6,5
COW 3		5				8
COW 4	7		7		7	
COW 5	8	5,5	7	6,5	7	6
COW 6	7	6	5,5	6	5,5	6,5

Water and feed intake

Water intake does not show to be clearly affected by dietary treatment. Only the water intake is considerably less after six days. This lowering in intake is also seen a week before the diet change and does not appear to be a stable parameter in any way. Therefore it is plausible this decrease is not due to the diet change (figure 15). Moreover, dry matter intake does not show to be affected by treatment (figure 16).

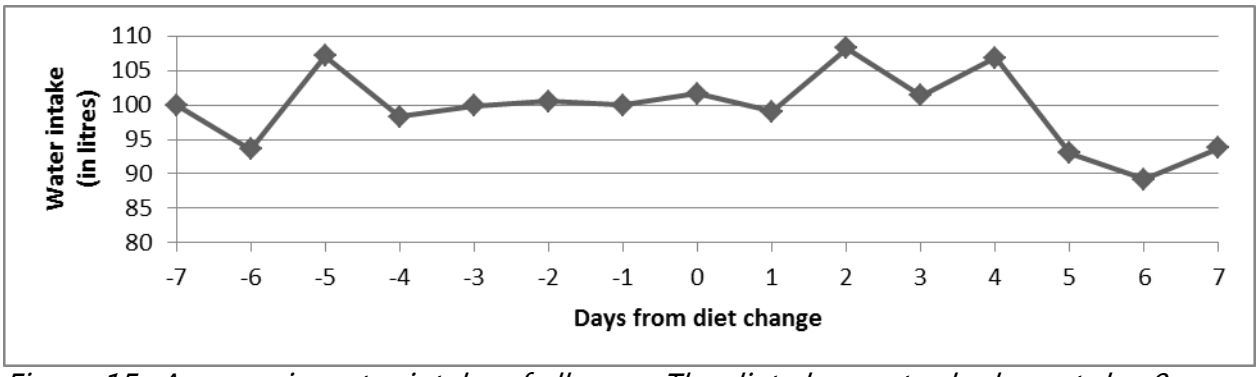

Figure 15 Average in water intake of all cows. The diet change took place at day 0.

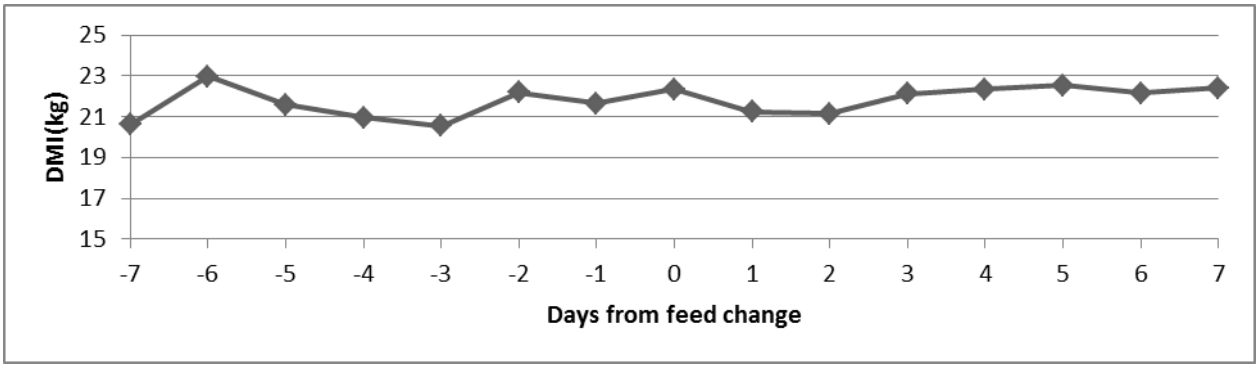

Figure 16 Average of dry matter intake (DMI) of all cows. The diet change took place at day 0.

Milk yield and composition:

The milk yield graph only shows an increase three days before the diet change, but around the diet change there were no changes noticeable, except for a small elevation (figure 17).

All the cows displayed a decrease in milk urea. The amount of lactose in the milk did not change much. It went from an average of 4,55% to 4,52%. The other compositions stayed the same in most of the cows. Only cow 4 had a decrease in milk protein and an increase in milk fat. Cow 6 had a high number Somatic cell count in the milk before the diet change. This value decreased every week, only after the diet change the rate of the decreasing was less steep (figure 18 to 21).

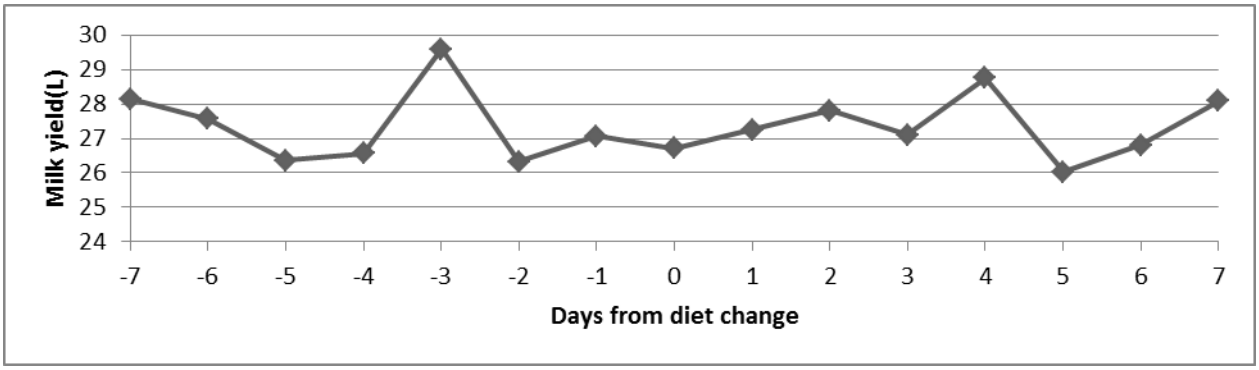

Figure 17 Average of milk yield. The diet change took place at day 0.

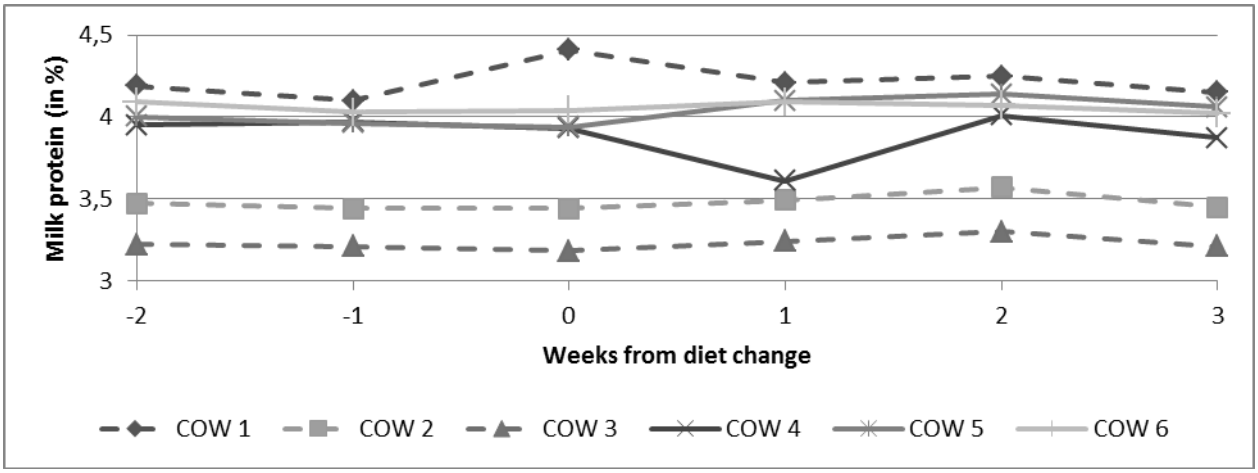

Figure 18 Percentage protein in milk. The measurement in week 0 was 4 days before the diet change.

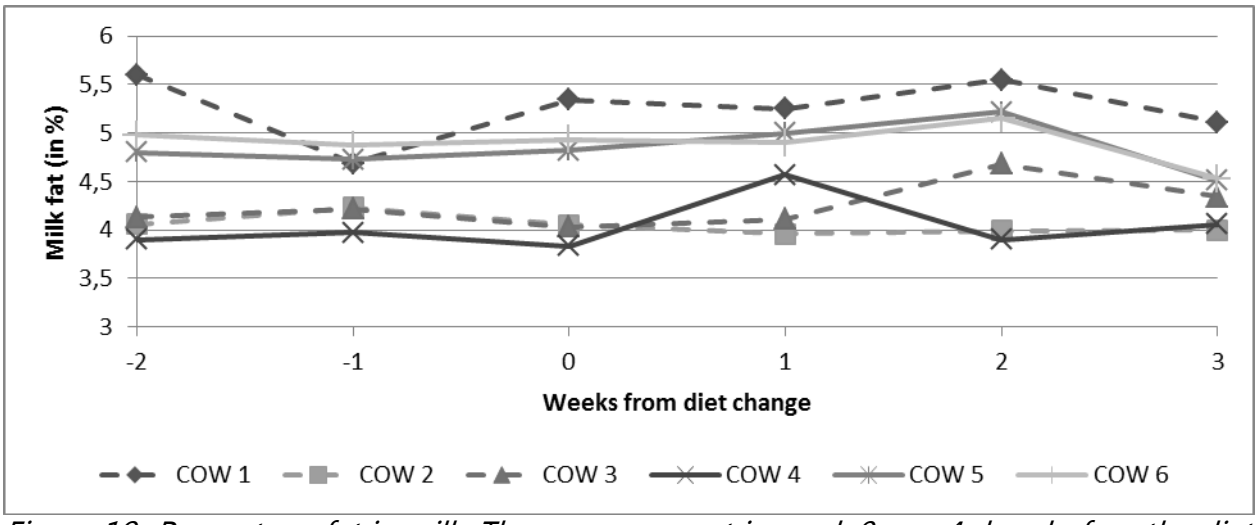

Figure 19 Percentage fat in milk. The measurement in week 0 was 4 days before the diet change.

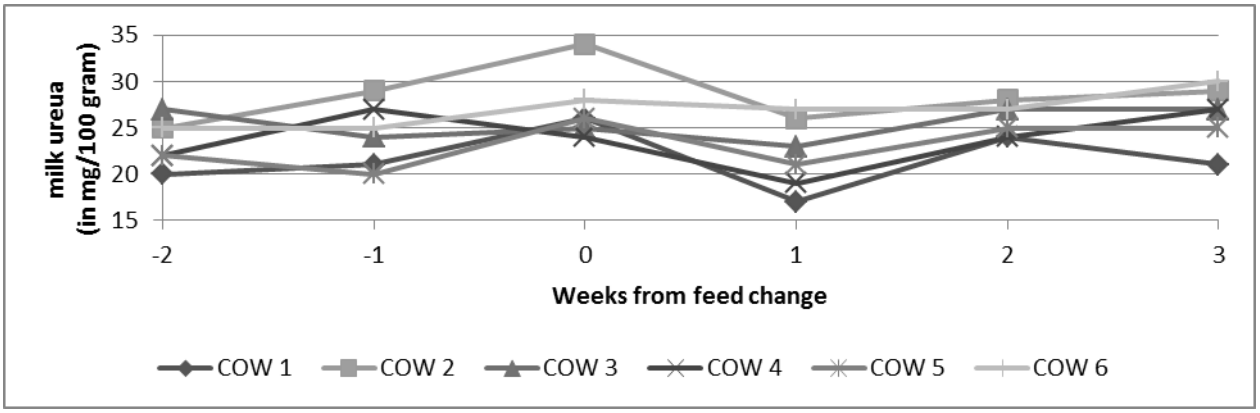

Figure 20 Urea in milk. The measurement in week 0 was 4 days before the diet change.

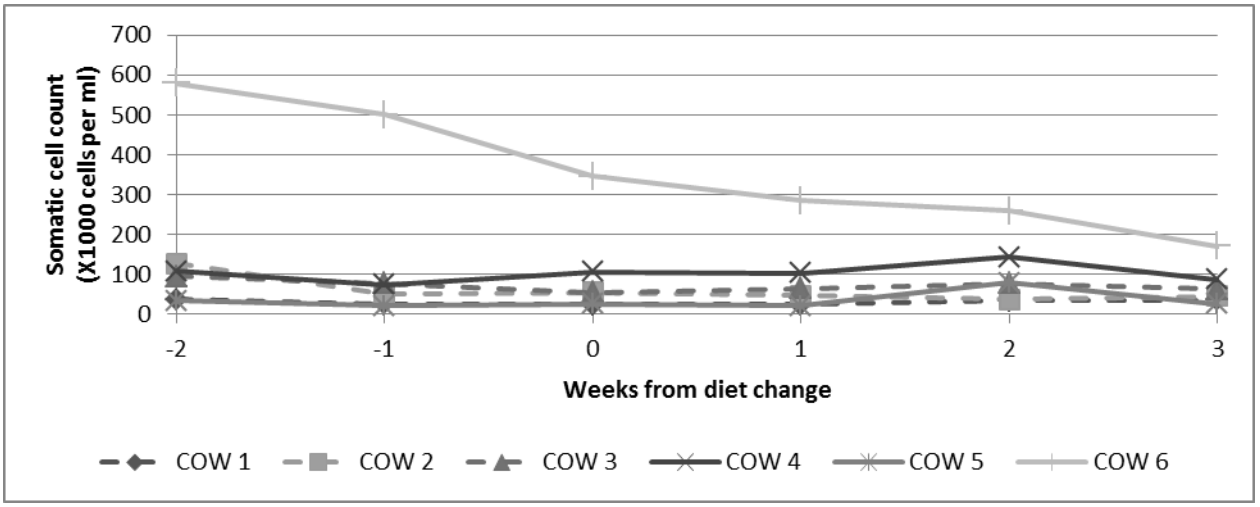

Figure 21 Somatic cell count in milk. The measurement in week 0 was 4 days before the diet change.

Discussion

Academically, there seems to be no general agreement on the definition of SARA. Garret (1999) described SARA as a disease in which the rumen pH drops below 5,5, while Beachemin (2003) takes pH 5.8 as the threshold so that there is a greater change on ruminal acidosis[20, 24]. Gozho (2007) adds a time element and states that the pH level has to be under 5,6 for at least 180 minutes[17]. This definition is also applied by Khafipour (2009) [19]. This study uses the description of Khafipour to define whether a cow suffers from SARA, because it is supported by both Gozho (2007)and Khalifour(2009) and seems to be the common ground between Beachemin (2003) and Garret (1999) concerning pH levels.

When SARA appears after a diet change, the pH levels are reduced and stays at pH <5,6 for over 180 minutes. Such a pH pattern is for instance reported in studies in which the ratio of concentrations of the feed was changed compared to the roughage. In these studies the time for the control test have a range of 15 to 118 minutes; the tests in which the diet change was applied have a range of 171-594 minutes[9, 16, 25-29].
A similar effect is reported in two studies, of Li (2012) and Colman (2013) in which the structure of the feed is altered by pelleting the diet. In these studies the figures were respectively 56 and 135 to 225 and 490 minutes [9, 25].

Although the structure of the diet was changed in this research, this change was significantly smaller as compared to the Colman, Li procedure. Instead of comparing standard with pelleted roughage, this current experiment just compared standard roughage versus roughage of reduced particles. Part of this study has been to determine the effect of this relative small difference in diet changes on the development of SARA.

From the results as shown in figure 5 it can be concluded that no change in the time the pH stays below pH 5,6 is measured, as compared to the standard diet before the diet change. This means SARA did not occur. Probably the cows could cope with the alteration of the feed. However, the performance of each individual cow differs quite a lot. In cow 1 and cow 5 the pH stayed at pH <5,6 for over 180 minutes, comparing before and after the diet change (figure 6).

It is assumed these differences can be explained by deviancies in the pH probe. This assumption is made as the same probe has been used for cow 1 and cow 5. No other signals have been observed that could make the pH levels that low, such as that the pH did not exceed pH 6 even during feeding and stayed at same level before the diet change as well after it. So the diet change did not have an influence on the pH in these cows. These cows had the same bolus. An explanation is that the bolus was calibrated incorrectly. Also the average pH did not change a lot (table 3).

The purpose of this study was to evaluate whether SARA, as a result of a diet change, would affect the concentration of LPS and APP. As no SARA was determined this could not be tested. However, the effect of the diet change on the concentration of the APP and LPS could be examined. In various published studies the values of LPS in rumen fluid ranged between 3.715 EU/ml and 42.122 EU/ml in the control cows. The values after the diet change were reported as being more than 3 times as high and ranging from 12.589 EU/ml to 168.391 EU/ml.[9, 16, 17, 19, 26]. In this study however, the LPS just changed a fraction in most cows as can be seen in figure 7. The explanation for this is that the gram-negative bacteria did not die in large numbers, because the pH stays above 5,6. The values measured before the diet change ranged from 36.852 EU/ml. to 69.500 EU/ml. In this study the values stayed below 60.000 EU/ml. so there seems to be no influence.

Regarding the APP in figure 8, the results from this study stayed in the same range as the control cows of other studies. In studies in which the concentration of SAA was investigated, the concentration ranged between 1,3 and 286,8 ng/L. in the control group. After the diet change it became between 33 and 499ng/L.[16, 17, 26, 27, 30, 31].

In this study, the SAA stayed below 10 ng/L also after the diet change (figure 8B). Moreover Hp levels stayed in the same range as the control cows. In the control cows Hp levels between 0 and 0,53g/L were measured. After the diet change the Hp levels increased more than 2 times in cows with a significant increase compared to the control cows[10, 16, 17, 19, 26-28, 30-33]

As in the current study the Hp levels stayed below 0,1 g/L in the cows in this research, there was not witnessed any significant change in most of the cows after the diet change (figure 8A). The iron concentration stayed on the same level in most of the cows. This is a range between 20 and 35 µmol/L (figure 9). In the study of Baydar and Dabak (2014) the average of the concentration decreased from 26,78 µmol/L in controls to 7,82 µmol/L in cows with mastitis. Instead of defining the concentration of both the ferric as well as ferrous iron(which is done in the current study), the study of Baydar and Dabak only monitored the concentration of Ferric iron[23].

Although the rumination and water and feed intake displayed a few fluctuations they did not change after the diet change (figure 13 to 16). Only the rumination minutes decreased after the diet change in two cows. This decrease can be induced by the diet change or more likely by a normal fluctuation. There were some differences in the values of the rumination cycles between the different activities. The reason for this is that the rumination cycles were counted in only two periods of five minutes per activity before and after the diet change, because the measurements of the motility meter did not show the normal ruminal pressure patterns all the time. As a reference to the normal patterns the book of Reece et. al. (2004) is used[2].

Also the milk yield and composition, except for urea, does not change after the diet change in most cows. The small lowering of the concentration of urea was seen in all cows. Normal values of milk urea range between 18 and 40 ml/100 gram[34], so the urea levels stayed in the range of normal cows. One of the largest contributor of urea in the blood is the rumen ammonia nitrogen [35]. So changes in the rumen can contribute to the lower urea concentration. One of the changes in the that can contribute to a higher concentration of urea is changes in the pH of the rumen, due to a higher ratio of NH_4^+ relative to NH_3. As in NH_3 can pass the ruminal wall passively and NH_4^+ could not [35]. The logical explanation could be that lowering of the structure causes a lowering of the pH in the rumen, which causes the lowering in the urea of the milk. Although as postulated earlier, there was no change measured in the pH of the rumen after the diet change.

Only cow 4 had elevated APP after the diet change; the concentration of ruminal LPS did not increase (figures 7 to 9). The explanation for this is the APP could have reacted to something else. This could be an indication for an inflammation of the rumen or another part of the body. The fat concentration of the milk increased and the protein concentration decreased in that period in that cow too. The decreasing of milk fat and increasing in milk protein is quite odd, because it is postulated that milk fat is decreasing and milk protein is increased when the structure of the diet is lowered[2, 36]. This is another clue that the increase in APP is not caused by SARA.

It can be that the higher value of SAA in cow 8 is caused by the relative high somatic cell count in the milk. The SAA can be increased.

The graphs 10 to 12, which show the comparison between LPS and APP and Hp and Iron show that the R^2 is low. This value is not reliable, because this study used a small number of cows and only a small number of measurements were taken from each cow. With this it is assumed the same concentration of LPS gives the same concentration of the APP and iron in different cows. It is questionable this is true, based on the other results of this study. With the number of measurements it was not possible to get a valuable trend line in each cow. Also it was not possible to do further statistics, because of the number of cows used in this study and the variation between cows.

Conclusions

In this study hardly any changes were witnessed in most of the parameters. The pH levels did not react to the diet change and the LPS and APP did not reach as high as in other studies. Furthermore, other cow performances did not alter after the diet change. Thus, it can be concluded that lowering the physical structure of the feed, as in this study, does not cause a reduction of the average pH and an increase of LPS in the rumen.

In addition, this study could not determine whether it is possible to predict the presence of SARA in cows by the concentration of Hp, SAA or iron, because SARA did not occur. To induce SARA the diet change has to be more dramatic. For instance the physical structure could be made smaller by cutting the grass and wheat straw in smaller particles. Other recommendations would be to take more samples of the APP and LPS, because their concentrations can fluctuate.

In conclusion, the used size of the feed doesn't induce SARA and thus new parameters to predict its presence could not be established. Therefore, one recommendation for future studies is that the diet change has to be changed more drastically to see whether cows get SARA and what the determination parameters are. Moreover, the group of cows used in the study should be made larger in future experiments, because this study only considered a relatively small groups and the cows in them differ a lot from each other. Finally, the drop of the urea after the diet change could be a topic worth investigating.

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