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Article

An inline reticulorumen pH as biomarker of cow's reproduction and health status

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Abstract: study hypothesis is that interline registered pH of a cow's reticulum can be used as an indicator or biomarker of its health and reproductive status. The main objective of the study was to examine the relationship of pH using the biomarkers of the automatic milking system (AMS) and some parameters of a cow's blood components. The following four main groups were used to classify the cow's health status including; 15-30d. Postpartum, 1-34d. After insemination, 35 d. after insemination (not pregnant) and 35d. (Pregnant). Using the reticulum pH assay, the animals were categorized as pH <6.22 (5.3% of cows), pH 6.22-6.42 (42.1% of cows), pH 2.6-6.62 (21.1% of cows) and pH >6.62(10.5% of cows). Using milking robots, milk yield, its fat protein, lactose level, somatic cell count and the electron conductivity were registered. Other parameters assessed include the temperature and pH of the contents of reticulorumens. The assessment of the aforementioned parameters was done using specific smaX-tec boluses. Blood gas parameters were assessed using a blood gas analyzer (EPOC, Canada). The study findings indicated that pregnant cows have high pH during insemination than the non-pregnant cows. It was also noted that cows with low fat-protein ratio, lactose level, and high SCC had low reticulorumen pH. They also have the lowest blood PH. It was also noted that with the increase of reticulorumen pH, there is an increased level of blood potassium concentration, high hematocrit, low sodium, and carbon dioxide saturation.

Keywords: blood gas, reticulorumen, precision livestock farming (PLF), automatic milking system (AMS).

1. Introduction

The first widely adopted application of precision livestock farming (PLF, years before the term PLF The individual electronic milk meter remains the most adopted application used for the precision of livestock farming (PLF) status [1]. The term PLF coined in the early 1970s and '80s. The other most commonly used parameters of PLF include the use of commercialized behavior that is based on estrus detection [2], rumination tags [3] (4544-4554), and use of online-time milk analyzer [4]. The sensors in these applications provide useful data that can be used by farmers to identify livestock that needs special care before the health condition progresses. Therefore, it is essential to use technological applications that give reliable and accurate data to ensure proper management of livestock farming. This is a critical aspect and so, transforming data to actionable solutions must be an accurate process [5] (pp.403-425). One of the most accurate data used for continuous monitoring of an individual life stock health status is the reticuloruminal pH (RRpH). The advantage of utilizing this data is due to its ability of diurnal recording. Various scientific investigations have used the continuous measurement of ruminal Ph to assess livestock health status [6] (pp.202-207). The technique entails the use of a memory chip is inserted in the livestock's rumen, and to retrieve the data, it has to be physically removed or use of an external cable to transmit data to an external unit.

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According to Cantor [7] (p.164), the use of reticulorumen temperature is an effective measure to predict livestock health status such as dairy herd water intake. Cantor argues that real-time observation of reticulorumen pH and temperature in fresh dairy cows are effective in assessing the risk of subclinical ruminal acidosis (SARA) because they provide an opportunity to evaluate the prophylactic effect of treatment strategies applied [8](pp.245-250). Antanaitis [12] argues that some blood parameters and dairy cow's rumination time can be used as biomarkers for accurate diagnosis of subclinical acidosis and ketosis. However, there is limited information on how the two parameters can be used to assess the disease, so future studies comparing the data findings using many animals. Over the last few decades, there has been a dramatic decrease in dairy cows' fertility rate due to various preventable causes [10] (18-27). The reticuloruminal pH data can also be used to predict the reproductive health of livestock [9] (pp. 23-26). Dairy cows with altered rumen metabolism (that is low pH) have low fertility rate. Therefore, using reticuloruminal pH is a great predictor of a dairy cow's reproductive success. However, more studies on the role of reticuloruminal pH in determining cow's reproductive health are needed [9] (pp.23-26). Alzahal et al. assessed the ruminal temperature and pH of dairy cows and their association in predicting dairy cows' nutritional and health status [13] (PP.3568-3574). Similar studies conducted by Cooper-Prado et al. reported that ruminal temperature lowers a day to parturition [14] (pp.1020-1027). Optimum fermentation of diet and fiber digestion is achieved at ruminal pH between 6.0 and 6.4. At this pH level, the cellulolytic bacteria permits effective digestion of fiber, which is inhibited in pH levels below 6.0[15] (pp.5697-5701). Therefore, the decrease in ruminal pH increases acidity, which in turn increases the temperature of the obamasum [16] (pp.771-776). Thus, using the two parameters, one can predict the health status of a cow.

The two parameters/data is gathered using wireless sensor nodes often attached to the animal. The wireless sensors are then attached to wireless –health monitoring systems. The analysis of the data collected can be used to assess, detect and prevent numerous livestock diseases. Another method of collecting data is the use of rumen fluid samples, whereby the sample collected using an oral-ruminal probe or rumen fistula. [18] (Pp.4759-4773). Rumen pH and temperature parameters fluctuate. However, the collection of rumen fluid samples is avoided when possible because it causes distress to the research subjects [19] (pp.39). With the technological advancements, new noninvasive technologies such as the use of intra-ruminal boluses have been developed to collect pH and temperature data to monitor cow's intra-ruminal metabolism. However, there is limited information on how interline registered reticulorumen pH can be utilized as a biomarker of assessing cows' health status and their reproductive systems. The study hypothesizes the possibility that interline registered reticulorumen pH can accurately predict cow's reproduction and health status. The main objective of this study is to examine the relationship of reticulorumen pH with biomarkers and make a comparison with those of automatic milking system (AMS) and blood biomarkers to determine dairy cow's reproduction and health status.

2. Materials and Methods

2.1. Location and experimental design

The experiment is conducted from 20190201 to 20190901 on a dairy farm located in the Eastern part of Europe 56 00N. 24 00 E. About 95 Lithuanian Black and white dairy cows that matched the selection criteria were identified. The inclusion criteria were cows that had two or more lactations. the cows must be identified as clinically healthy, the temperature of 38.8 degrees Celsius 5-6 rumen motility every three minutes, no signs and symptoms of laminitis, metritis or mastitis. The research subjects were taken to a loose housing system, where they were fed using a constant feeding rotation during the entire research time. The nutritional balance was maintained to ensure that their physiological needs were adequately met. The TMR comprised of 30% corn silage, 4% hay grass, 50% grain mash concentrate and 10% grass silage. This diet was formulated using NRC 2001 guidelines of a 550kg Holstein cow producing 35kg/d. The composition ration was as follows, DM

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(%) -48.8, NEL (Mcal/kg) 1.6, NDF, ADF, NFC and CP percentage of DM were as follows, 28.2, 19.8, 38.7 and 15.8 respectively. Using this aforementioned mixed ration, the research subjects were fed twice per day at 1000hrs and 2000hrs. 2kg/d of concentrates was used to feed the cows at the milking site. The average of BCS used was 3.45 (±0.25).

2.2. Measurements

Using the SmaX-tec boluses were used to assess the contents of cow reticulorumens Ph and temperature. The device is preferred for this study because of its ability to display real-time Ph and temperature data. Using the instruction manual, boluses were put into the cow's reticulorumens. The data was collected using specific antennas of the SmaX-tec device. The reticuloruminal pH evaluation was done using an indwelling wireless data transmitting system (smaXtec animal care GmbH, Grax, Austria). The entire system is controlled by a microprocessor. After conversion using the AD converter, The data collected stored in an external memory chip. The device size is small enough to permit oral administration to an adult cow. More so, it is shock-proof to rumen fluid. At the beginning of the study, Ph probes were calibrated using ph4 and ph7 buffers.

Lely Astronaut ® A3 milking robots were used for milking the cows. The robots were also used to register the rumination time (RT) (min/d), yield MY (kg/d), bodyweight BW (kg), lactose ration (%), milk fat-protein ratio (F/P), milk electrical conductivity of all quarters of udder (front left and right (EC1 and EC2 respectively), rear left and right EC3 and RR respectively in Ms/cm, and conception of concentrates. The samples of blood gas were obtained and stored in an ice bath until processing is done. Using Epoc blood gas analyzers (EPOC, Canada), the following parameters were obtained including base excess in blood (BE), partial carbon dioxide pressure (PCO2), partial oxygen pressure (PO2), bicarbonate (Chco3), Hydrogen potential (pH), total carbon dioxide carbon (TCO2), base excess in extracellular fluid (Beef), Sodium (Na), Calcium (C), Potassium (K), hematocrit (HCT), chlorides (cl), hemoglobin concentration (cHgb) and lactate (Lac).

2.3. Animals and experimental condition

The dairy cow's reproductive system was classified as follows (Tab1):

Table 1. Creation of experimental groups

Group	Days/Status of reproduction	n	%
I	15-30 d. postpartum	35	36.8
II	1-34 d. after insemination	20	21.1
III	35 d. after insemination (non-pregnant)	20	21.1
IV	35 d. after insemination (pregnant)	20	21.1
Total		95	100.0

According to reticulorumen pH assay experimental animals were divided into four classes 1. pH <6.22 (5.3% of cows), 2. pH 6.22-6.42 (42.1% of cows), 3. pH 2.6-6.62 (21.1% of cows) and 4. pH >6.62(10.5% of cows). Oestrus was identified with specific devices in this study measuring activity in steps, and rumination time (min/d) (by increasing activity and decreasing rumination time) monitored by herd management program Lely Astronaut ® (24/7). The research subject was considered oestrus if it presented with the following signs, restlessness, type and amount of mucous discharges, extent of their alertness, tail raising, and congestion of the mucous membrane around the vulvar. The uterine tone was assessed using rectal palpation. About 12 hours after the oestrus sign has been presented, the research subjects were inseminated using frozen semen. Successful implantation and pregnancies were confirmed using an Easy scan ultrasound device (IMV imaging, Scotland) once at day 30 to 35. The pregnant cows were grouped in a different group from the non-pregnant cows.

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2.4. Data analysis and statistics

Statistical data analysis was conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software. The data was then presented using descriptive statistics and normal distribution analyses such as Kolmogorov –Smirnov test. The statistical relationship between reticulorumen pH and AMS biomarkers, body weight (BW), activity of cows, milk yield (MY), Milk fat-protein ratio (F/P), somatic cell count in milk (SCC), milk lactose content, and all the four udder quarters electrical conductivity was shown using Pearson correlation. To effectively analyze SCC variables, they were converted to SCClog 10. The analysis of the linear relationship between reticulorumen Ph and analyzed AMS was done using Pearson. The multiple comparisons of groups means were calculated using Tukey's test. A probability below 0.05 was considered reliable (P-Value < 0.05).

All dates were registered during the investigation day, except for pregnant and non-pregnant cows whose data was registered during the insemination day.

Study approval number – 012858.

3. Results

We determined that the average pH of the reticulorumen was 6.47 ± 0.016, the temperature of the reticulorumen was 38.779±0.020 °C, the rumination time was 455.26±6.052. The average milk productivity of cows was 40.41±0.724 kg, BW - 648.37± 13.265 kg, and the ratio of fat to protein in milk was 1.17± 0.013.

3.1. Reticulorumen pH as a biomarker of reproduction status of cows

Analysis of the reticulorumen pH of cows by reproductive status showed the highest average value of this indicator in group IV (Fig. 1A) - 2.13% higher compared to group I, 0.76% higher compared to group II and 1.37% higher compared to group III. According to multiple comparison of means all differences between groups of cows by reproductive status were statistically significant (P<0.05).

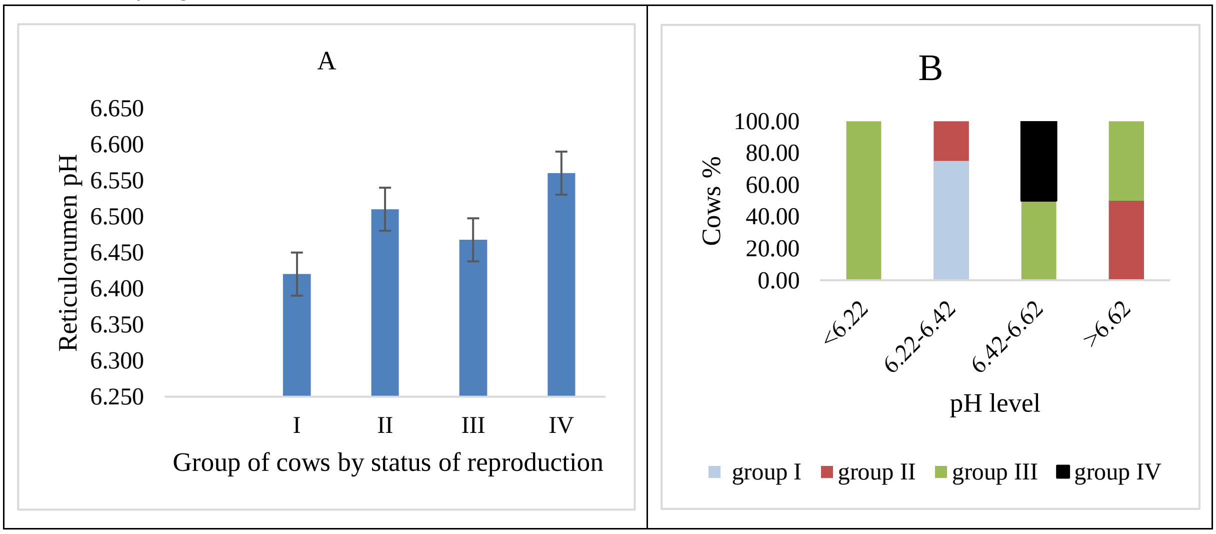

Fig. 1. Analysis of reticuloromen pH in cows by status of reproduction.

We found that all pregnant cows (group IV) belonged to the third class according to their reticulorumen pH range from 6.42 to 6.62 (Fig 1B).

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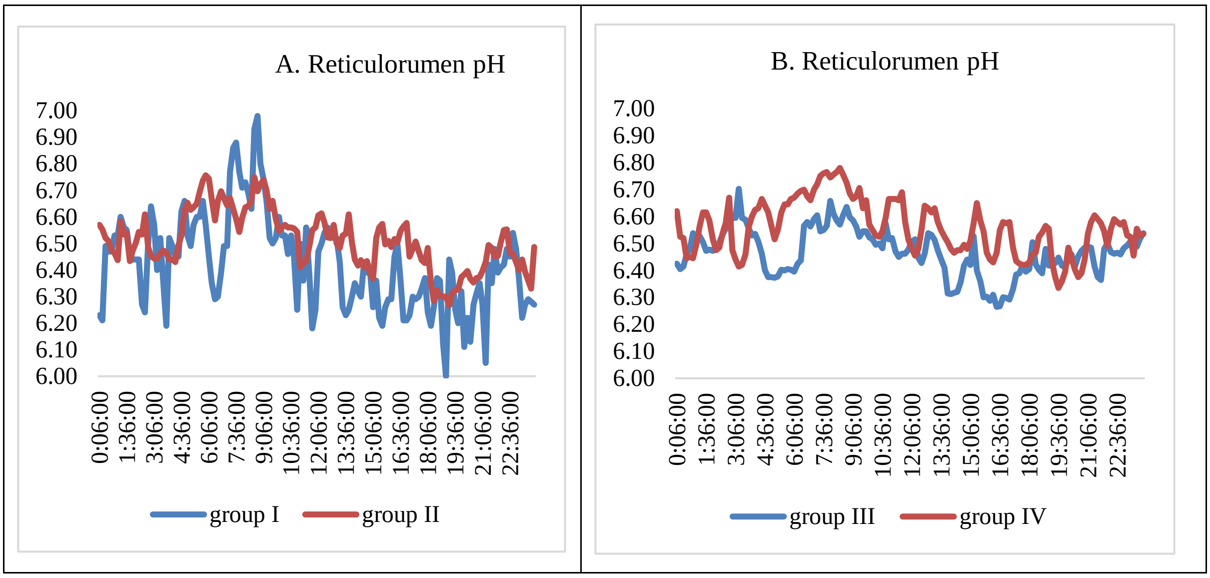

Fig. 2. Reticulorumen pH changes during 24 h by reproduction status of cows

The data in Fig. 2 showed that the cows of group I (15 - 30 days postpartum) showed more fluctuating reticulorumen pH values per day compared to other groups. After comparing the reticulorumen pH in non-pregnant and pregnant cows 35-90 days after insemination, we can see a higher level of this indicator in pregnant cows.

3.2. Reticulorumen pH as a biomarker of health status of cows

The average activity of cows in the reticulorumen pH class 1 was 3.5% lower compared to class 4 and 14.3 - 14.96% lower compared to classes 1 and 3. In cows of the indicated class 3, we determined the highest temperature of the reticulorumen, in class 4 - the lowest temperature (0.07 ° C lower). The differences in arithmetic means were not statistically significant (Table 2).

Table 2. Influence of reticulorumen pH and reproductive status on AMS indicators and milk traits of cows

Reticulorumen pH class	AMS parameters (M, SE)			AME parameters (M, SE)		
1	Activity (steps/hour)	10.24^{a}`	1.239	Fat (%)	3.58^{a}	0.187
2		10.30^{a}	0.506		4.58^{b}	0.076
3		8.96^{a}	0.620		3.93^{a}	0.094
4		9.27^{a}	0.876		3.93^{a}	0.132
1	Reticulorumen temperature (°C)	38.78^{a}	0.078	Protein (%)	3.37^{a}	0.057
2		38.76^{a}	0.032		3.58^{b}	0.023
3		38.79^{a}	0.039		3.43^{a}	0.028
4		38.72^{a}	0.055		3.37^{a}	0.040
1	BW (kg)	756.00^{a}	61.710	F/P	1.06^{a}	0.048
2		593.67^{b}	25.193		1.28^{b}	0.020
3		630.75^{a}	30.855		1.15^{a}	0.024
4		630.00^{a}	43.636		1.17^{a}	0.031
1	RT (min/d)	487.00^{a}	24.947	Lactosis	4.53^{a}	0.028

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2		423.50 [b]	10.185	(%)	4.61 [b]	0.011
3		436.75 [b]	12.474		4.59 [a]	0.014
4		478.50 [a]	17.640		4.56 [a]	0.020
1	MY (kg/d)	37.50[a]	2.214	SCC (tousd/ ml)	124.00 [a]	222.028
2		41.07 [b]	1.067		105.83 [a]	90.643
3		37.13 [a]	1.307		135.25 [a]	111.014
4		49.85 [c]	1.849		95.00 [a]	156.998

[a,b,c] Values within a column with different superscripts differ significantly at *P*<0.05

In the class 2 we found the lowest level of milk EC (68.5 - 70.5), in the other classes -statistically significant higher (from 70.5 to 72 mS/cm, P<0.05) (Fig.3).

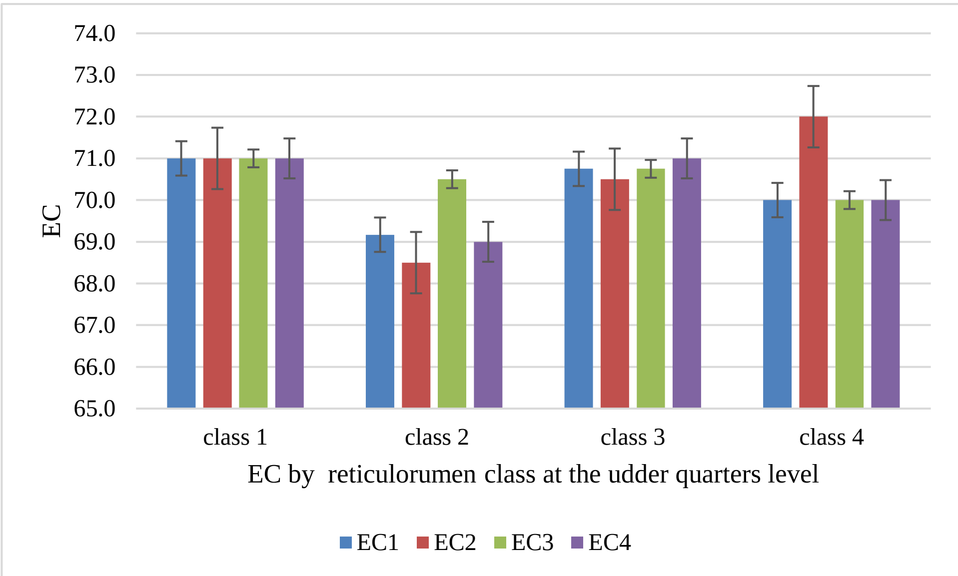

Fig. 3. Electrical conductivity of milk (mS/cm) at the udder quarter level

Reticulorumen class 2 had a lower (P<0.05) RT (3.12% lower compared to class 3, 12.99% lower compared to group 4 , and 15% lower compared to class 1). The study showed that the highest levels of milk fat and milk protein and the optimal F/P in the second class were evaluated. In class 1, we found the lowest ratio of milk fat to protein and the lowest concentration of milk lactose. We determined the lowest SCC in the milk of class 4 and class 2, the highest - in class 3 and class 1 (Table 2). On the other hand, class of cows with the highest milk SCC showed the highest electrical conductivity in milk at the udder quarter level (Fig.3).

3.3. Correlations of reticulorumen pH with biomarkers from AMS

Correlation coefficients between reticulorumen pH and biomarkers from AMS are presented in the Figure 3.

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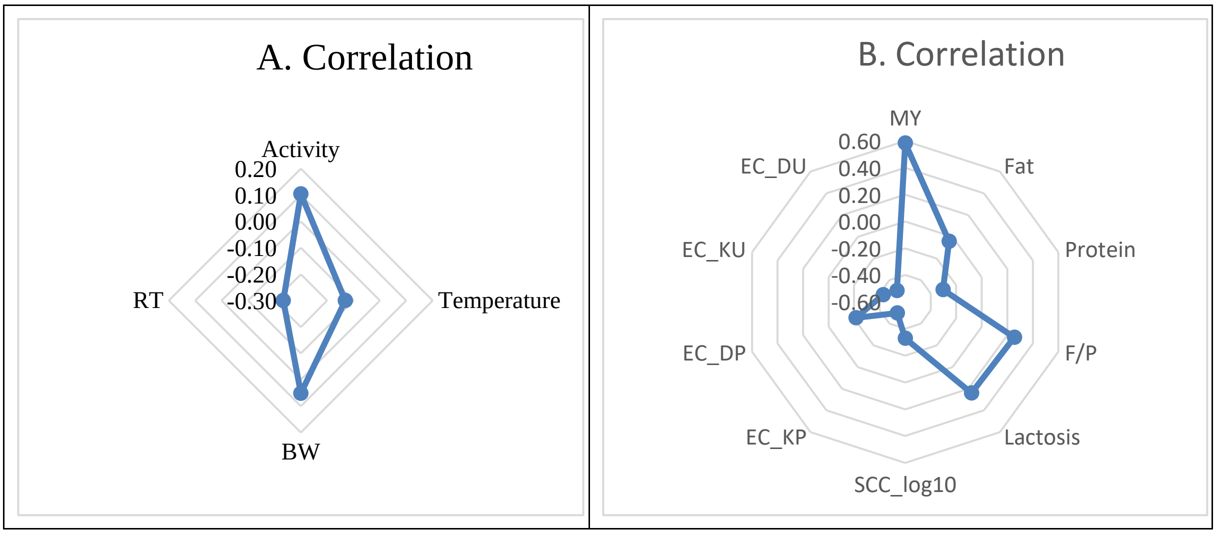

Fig.3. Reticulorumen pH correlations with biomarkers from AMS. RT – rumination time; BW – body weight; SCC – somatic cell count; EC – electrical conductivity of milk at the udder quarter level (DU - rear right, KU - rear left, DP - front right, KP - front left)

Reticulorumen temperature and RT weakly negative related with reticulorumen pH (r=-0.131-0.234) and weakly positively correlated with BW and activity of cows (r=-0.051-0.104). MY (r=0.583, P<0.001), milk lactose (r=0.240, P<0.05) and F/P (r=0.250, P<0.05) positively related with reticulorumen pH, negatively related with milk protein (-0.304, P<0.01), SCC (-0.329, P<0.05) and EC (-0.213-0.498, P<0.05-0.01) and milk fat (-0.042).

Table 3. Influence of reticulorumen pH level on blood indicators of cows

Reticulorumen pH class	Blood parameters (M, SE)			Blood parameters (M, SE)		
1	pH	7.38^{a}	0.016	Na	137.00^{a}	0.601
2		7.43^{b}	0.005		137.13^{ab}	0.212
3		7.42^{b}	0.008		137.25^{ab}	0.3
4		7.43^{b}	0.011		136.00^{ac}	0.425
1	pCO2	49.20^{a}	2.204	K	3.90^{a}	0.11
2		45.20^{b}	0.779		4.10^{a}	0.039
3		45.13^{b}	1.102		4.00^{a}	0.055
4		40.55^{a}	1.558		4.30^{b}	0.078
1	pO2	49.90^{a}	19.062	Ca	1.24^{a}	0.016
2		67.11^{a}	6.740		1.13^{b}	0.006
3		61.45^{a}	9.531		1.14^{b}	0.008
4		52.00^{a}	13.479		1.22^{a}	0.011
1	cHCO3-	29.30^{a}	1.288	TCO2	29.20^{a}	1.257
2		30.23^{ab}	0.455		29.90^{ab}	0.445
3		29.03^{a}	0.644		28.78^{a}	0.629
4		27.00^{ac}	0.91		26.75^{ac}	0.889
1	BE	4.20^{a}	1.372	Hct	24.00^{a}	0.884

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2	(ecf)	5.98 [ab]	0.485		23.75 [a]	0.313
3		4.48 [a]	0.686		26.00 [b]	0.442
4		2.70 [ac]	0.97		27.00 [b]	0.625

[a,b,c] Values within a column with different superscripts differ significantly at $P<0.05$

The highest blood pH level was determined in reticulorumen class 2 and 4, lowest in class 1 (P<0.05). On the contrary, in the class 1 we estimated the highest pCO2 and lowest pO2 and Ca level. In the class 4 was found the lowest cHCO3-, BE (ecf), TCO2, Na and the highest level of K and HCT (Table 3).

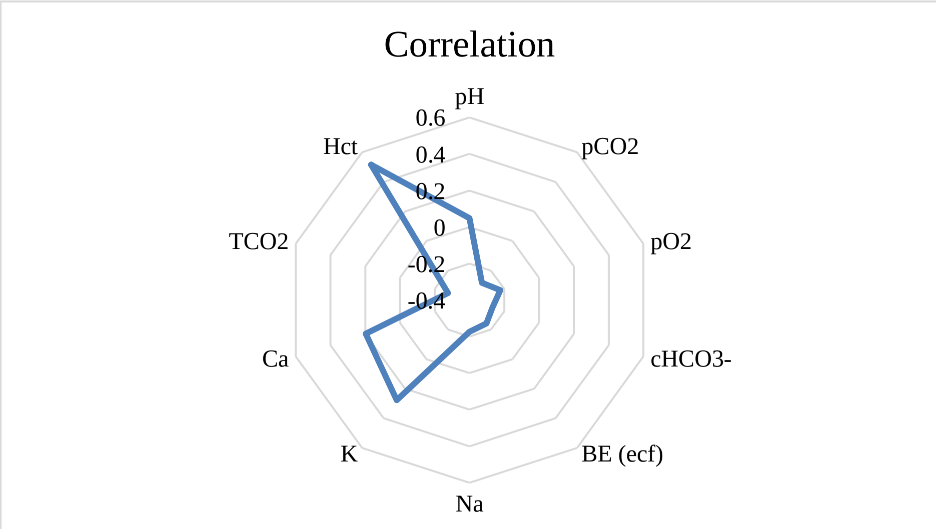

Fig. 4. Reticulorumen pH correlations with blood indicators

Reticulorumen pH statistically reliable positively correlated with blood K (p<0.01) and Hct (p<0.001), negatively – with pCO2, TCO2 (p<0.01) and with pO2, cHCO3-, BE (ecf) and Na (p<0.05). Data are presented in Fig.4.

4. Discussion

4.1. Reticulorumen pH as indicator of cow's reproduction success

The current study indicated that pregnant cows tend to have higher reticulorumen PH during insemination time than the non-pregnant cows. The study findings also indicated that dairy cows that have disturbed rumen metabolism have a low chance of conceiving. Therefore, the study highlights that reticuloruminal pH can be used effectively as a predictor for a dairy's reproductive health. According to Inchaisri et al [21] (pp.1183-1190), pH significantly influence conception during insemination. Arguably, low pH in the reticulorumen increases the temperature of the reticulorumen and obamasum. From the study, the average temperature of the reticulorumen during post insemination till 170 d was considerably high than in non-pregnant cows [9] (pp. 23-26). It was observed that the vaginal temperature before estrus was considerably higher than during the post-ovulation period [22] (pp. 2368-2373). During Estrus, the average temperature in the reticulorumen increases.

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4.2. Reticulorumen pH and health status of cow's

The available literature indicates that the assessment of ruminal pH as an optimum measure to evaluate the risk of SARA due to the variation of the dairy cow's rumen pH [23] (pp. 3777-3785). The study findings indicate that dairy cows react uniquely to low pH values of the rumen. Therefore, each cow has differing susceptibility to SARA [24] (pp. 21-31). Rumination activity and fermentation processes are interconnected. Thus, reduced rumination activity causes lower production of saliva buffering, thereby increasing risk for SARA [25] (pp.458-477). The increased rumination activity observed after the calving period is due to the high feed intake during the post-pregnancy process. The accelerated passage rate caused a reduced rumination activity of DMI. Contrary to Pahl et al findings, it was observed that treatment did not affect the rumination patterns of the dairy cows [26] (PP.6935-6941). It was observed that dairy cows chew per minute and bolus rumination reduced considerably during the last days before calving and the last days after calving. Similar observations have been reported by Schmitz et al [25] (pp. 458-477).

The study findings indicated that cows with lower RR pH had low milk fat protein ratio, low lactose concentration, and a high SCC. They also had a low blood ph. Available literature indicates that low ruminal pH triggers the lysing and death of gram-negative bacteria found in the rumen. The action causes an increase in the concentration of lipopolysaccharides, which in turn causes a trigger the increase in the concentration of systemic inflammatory markers such as cytokines, haptoglobin and acute protein serum Amyloid A [27] (pp. 7115-7124). It is well known that the reticulum has a high pH level than the rumen. Therefore, SARA detection thresh holds should be designed in a manner that identifies the localized pH of the reticulum [28] (pp.201-205). The current standards for SARA detection involve the use of high-resolution kinetics of rumen pH sensors. However, it was observed that the addition of the buffering agents to a high concentrate diet was effective in preventing milk fat concentration. [31] (pp. 2760-2769). This is because it re-established an optimum pH level in the rumen and reticulum.

Feed composition determines the milk fat ratio [30] (pp. 22-28). The dairy cows under investigation had low milk fat-protein concentration in most of the test days, which indicated that the energy level of the number of feeds obtained is generally low [32] (pp. 89-103). This is one of the signs observed in cows presenting with sub-acute rumen acidosis [33]. Dairy cows that have been diagnosed with SARA and non-acute ruminal acidosis generally tend to have a depression of milk-fat percentages [34] (pp.46-53). However, because the disease poses different action on milk fat content per cow, the findings on low milk fat content concerning feeding composition in most bulk tank testing remain unclear [35] (pp.1005-1028). The pH of the ruminal fluid was found below. This is because the microbes in the rumen break down carbohydrates into short-chain fatty acids at a faster rate than the rumen's absorptive rate, outflow and buffering activity [36] (pp.21-31). The reduction of the microbial populations in the rumen causes reduced fiber digestion [37] (pp. 421-423). Consequently, the feed intake reduces [38] (pp. 1399-1403) and also causes a reduction in milk fat production [39] (pp.1060-1070). The altered unsaturated fat bio-hydrogenation processes in the rumen, liver abscess, systemic and localized tissue inflammation in the rumen's papillae and SARA are the key causes of lameness and horn lesion [40] (pp. 966-970). Owen's et al [41] (pp. 275-286) argues that chronic and acute acidosis occurs due to the ingestion of diets that contain readily fermented carbohydrates in excess. As a dairy animal adapts to high rich concentrates of feeds in their feeding yards, it causes acute acidosis which becomes chronic as the yard feeding continues. In the acute acidosis phase, the ruminal acidity and osmolality cause elevated acids and glucose accumulation, which in turn causes increased damages in the rumen and intestinal wall due to high blood pH and dehydration. These events if not well managed can be fatal.

According to the study findings, an increase in RR pH causes an increase in Hct and blood K, and a decrease in BE, Na, and CO2. According to Giensella et al [42], it is vital to perform blood gas analysis as it is a valuable tool especially during the diagnosis of acidosis. The analysis gives a great

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insight into the extent of acidosis using a noninvasive approach than the rumen. According to a study conducted by Gokce et al [43] (pp. 121-127), animals with additional pathological disorders such as respiratory diseases like pneumonia tend to display an altered acidotic response. In this study, it was noted that PCO2 differed significantly during the different stages of SARA, which suggested an indication of acute respiratory acidosis. PO2 was observed to decrease statistically during SARA, and it is argued that pathology is likely increased consumption of the vascular O2. In this case, decreased PO2 values are associated with increased anaerobic metabolism and O2 consumption [43] (pp.121-127). Metabolic disturbances wage initially in a hidden form and their information is associated with problems of fermentation processes in the rumen. It is evident that nutrient conversion is the key precursors of milk and is largely dependent on rumen fermentation [44] (pp. 542-546). The functional ability of the mammary gland is directly correlated with the dairy cow's health status and so, the milk ingredients reflect the level of total metabolism [45]. Therefore, the biochemical markers in the milk depict the accurate metabolic status of dairy cows.

5. Conclusions

The present study concludes that the interline registered pH of a cow's reticulum can be used as an indicator or biomarker of its health and reproductive status. In pregnant cows, the reticulorumen pH is considerably high during insemination as compared with the non-pregnant cows. Cows with lower RR pH have the lowest milk fat ratio, lactose concentration, and high SCC. High RR pH increased blood concentration of blood K and HCT but caused a reduction of CO2, BE and Na. Therefore, reticulorumen pH can be used effectively to predict a cow's reproductive and health status.

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