Study code: 2014/04
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FIELD TRIAL WITH SODIUM BICARBONATE IN DAIRY COWS

FINAL REPORT

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2.- General informations

2.1.- Study details

Title:	Field trial with sodium bicarbonate in dairy cows.
Study code No.:	2014-04
Test substance(s):	sodium bicarbonate (Bicar®Z)
Target animal category:	Italian Friesian dairy cows
Test facility:	Centro di Ricerche per la Zootecnia e l'Ambiente (CERZOO) S.r.l., Via Castellarino, 12 – 29122 Piacenza, Italy, Tel. +39 (0)523 506102
Study site:	Agrogi di Giuseppe Ruggeri, Cascina Malgherosse 13, 25029 Verolavecchia
Sponsor:	Solvay Chimica Italia SpA, Via Piave, 6 – 57013 Rosignano Solvay (LI) Italy, Tel. +39 (0)586 766623.

2.2.- Key study personnel

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Sponsor Representative: Ing. Giordano Zappelli, Solvay Chimica Italia SpA, Via Piave, 6 – 57013 Rosignano Solvay (LI) Italy, Tel. +39 (0)586 766623

3.- Authorization for animal study

The study was authorized by Italian Health Ministry according to the article 31 of the Italian D.Lgs 26/2014 covering the accommodation and care of animals used for experimental and other scientific purposes: authorization n. 180/2015-PR issued on March 24, 2015.

4.- Introduction

Buffers are frequently used in diets for lactating dairy cows. The main purpose is to counteract the acid load coming from acidotic high starch and low fiber diets being fed to animal, particularly in high producing early lactating animals. Because of the considerable amount of fermentable substrate entering the rumen (forages and grains) subclinical condition of acidosis might develop and worsen if not adequately faced with consequences throughout the entire lactation thus reducing the amount of milk produced by the cow. Up to now there are few parameters to monitor the risk of acidosis and one tool available to the farmer is the inclusion of buffers into the diet. Moreover, the buffering capacity of the ration is usually not measured and the rumen acid load is unknown. Then, in typical diets in Italy for lactating dairy cows sodium bicarbonate is added in amounts ranging from 80 to 150 g/head/day to buffer the rumen environment.

5.- Objective of the trial

The aim of the study was to verify the effect of increasing amount of a sodium bicarbonate buffer (Bicar®Z) in diets for high yielding dairy cows on milk yield and milk quality, health conditions and reproductive performances.

6.- Materials and methods

The study was carried out in a commercial dairy herds with 800 cows in milking in a free stall (Agrogi di Giuseppe Ruggeri, Cascina Malgherosse 13, 25029 Verolavecchia) between March and August 2015. The herd is equipped with Afifarm®, Afimilk® and Afiact® systems allowing for individual data recording for daily milk yield, animal activity for heat detection and health status monitoring. Four groups of multiparous cows in milking (120 cows each) were used for the trial (two as control and two as treated groups). All cows received the same basal diet (Table 1) with either 150g/head/day (Control) or 300g/head/day (Treated) Bicar®Z as buffer. The lower level of Bicar®Z was the one already in use in the diet of the commercial dairy and no changes were made on diet formulation except for the increased amount of sodium bicarbonate for the treated group. Cows entered the groups at random after calving (10-15 days in milk) and all cows calved between March and April 2015. At the end, 160 multiparous dairy cows were involved in the trial.

The level of the Bicar®Z for the treated group was increased to 400g/head/day starting May 25th due to the high climate temperature. The cows were monitored for the first 20 weeks in lactation. At the end of the trial 11 cows were excluded from the experiment because of group crossing whereas 149 cows (65 control; 84 treated) were considered. Feed intake was monitored daily on a group base (two groups/level of Bicar®Z inclusion) and expressed on a week base.

Total mixed ration (TMR) samples were collected every week for chemical analysis. Samples were dried at 55°C in a ventilated oven until a constant weight, then ground to pass a 1-mm screen (Thomas-Wiley laboratory mill, Arthur H. Thomas Co., Philadelphia, PA) and stored at -20°C for later analysis. Upon analysis, frozen samples were thawed at room temperature, then analyzed in duplicates according to the Association of Official Analytical Chemists (AOAC, 1995) for DM (gravimetric loss of free water by heating at 105 °C for 3 h, method 945.15), CP (Kjeldahl N x 6.25, method 984.13) and ADF without residual ash (method 973.18). The NDF was analyzed with the addition of a heat-

stable amylase (activity = 17400 Liquefon units/ml, Ankom Technology) and corrected for residual ash (Mertens, 1997) without sodium sulfite with the aid of an Ankom 2200 Fiber Analyzer (Ankom Technology, Macedon, NY). Starch was measured by polarimetry (Polax 2L, AtagoR , Tokyo, Japan) according to European Community official method of analysis (European Community, 1999).

The temperature and relative air humidity was measured once a week for each treatment group and the temperature humidity index (THI) was obtained (Noaa, 1976).

Animals were milked three times a day and the individual daily milk yields were recorded. Individual milk samples were collected every 20 days and immediately analyzed (Laboratorio Regionale ARAL, Crema-Italy) for fat, protein, lactose, casein and urea contents. Blood and urine samples were collected on dry cows at 30 days (+/- 5 days) from expected calving and at 30 days (+/- 5 days) in lactation. Cows were blood sampled by jugular vein puncture before the morning meal (0730 h). Blood samples were collected into Li-heparin (17 U of heparin/ml of blood) Vacutainer tubes (Vacutainer systems, Belliver industrial estate, Plymouth, UK), and immediately placed in an ice bath until being centrifuged at 3,000 x *g* for 15 min. Then, plasma was collected and stored at -20°C until analysis.

Plasma metabolites were analyzed (Università Cattolica del Sacro Cuore) by a clinical auto-analyzer (ILAB 600; Instrumental Laboratory, Lexington, MA) for glucose, cholesterol, total protein, albumin, globulin, aspartate aminotransferase (GOT/AST), gamma glutamyl transpeptidase (GGT), bilirubin, alkaline phosphatase (ALP), haptoglobin, nonesterified fatty acids (NEFA), β-hydroxybutyric acid (BHBA), creatinine, calcium, inorganic phosphorous, magnesium, sodium, potassium, chlorine and zinc contents.

Once collected urine samples were stored at -20°C until analysis for pH determination and mineral contents (calcium, magnesium, sodium, potassium, phosphorous and chloride).

Rectal grab fecal samples were taken at random from 20 animals/group at 30 +/- 5 days in lactation for pH determination and volatile fatty acids (VFA) contents (Fussell and McCalley, 1987).

7.- Statistical analysis

Data were tested for normality with the Shapiro-Wilk test before statistical analysis. Outlying data were investigated and removed. The Wood's incomplete gamma function (1967) was fitted to the dataset made of individual daily milk yield.

The function was as follow:

$$Y_t = at^b e^{-ct}$$

Where:
Y_t = is the milk yield at days in milk (t),
a = initial milk yield,
b = rate of increased milk until peak,
c = rate of decline peak after peak
e = neper number.

The week at peak (b/c), peak yield ($a(b/c)^b e^{-b}$) and persistency (-(b+1)log(c)) were calculated from curve fitting parameters. The model parameters were estimated by nonlinear least squares using proc nlin of SAS (SAS Institute Inc., Cary, NC).

Data measured over time (milk yield and composition) and on the same experimental unit were statistically analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). The experimental unit was the animal treated alike. The statistical model included the fixed effect of treatment, time of measurement and their interaction, and the random effect of cow. The time of measurement within cow was considered as repeated measure and the covariance structure compound symmetry provided the best fit for the data with the lowest Akaike's information criteria. The model was:

$$Yijk = \mu + \alpha i + \beta ij + \gamma k + (\alpha \times \gamma)ik + eijk,$$

where Yijk is the dependent variable at time k on the jth subject assigned to treatment i, μ is the overall mean, αi is the fixed effect of treatment (i = 1 to 2), βij is the random effect for the subject j assigned to treatment i; γk is the fixed effect of time, (α × γ)ik is the interaction between treatment and time and eijk is the residual error.

Data measured once on the same animal (urine, plasma and fecal samples) were subjected to ANOVA using the GLM procedure of SAS according to the model described below:

$$Yij = \mu + \alpha i + eij,$$

where Yij is the dependent variable on the jth subject assigned to treatment i, μ is the overall mean, αi is the fixed effect of treatment (i = 1 to 2), and eij is the residual error.

Differences between means were accepted as significant if $P<0.05$.

8.- Results and discussion

Individual daily milk yield were fitted with the Wood's model and results are shown in Table 2 and Figure 1. There were no significant differences among the equation coefficients (initial milk yield, rate of increase until peak and rate of decline after peak) and among the calculated parameters (week at peak, yield at peak and persistency). The persistency is a dimensionless parameters and it is useful for comparison among different studies. The woods parameters were within the expected parameters for considered animals. When looking at the daily milk yield, (sampled every 20 days during lactation) the treated animals produced an average of 1.6 kg more milk than the control group (Figure 1) which in turn means 224 kg more milk over a 20 week period.

The dry matter intake measured on a week base is shown in Figure 2. For the considered period, the treated animals had an average of 1.1 kg more dry matter intake. The value can be considered reasonable in view of the numerically higher average milk yield leading to a feed efficiency of 1.45 (kg milk/kg D.M. intake). The temperature humidity index in Figure 2 depicts a growing trend. The green part of THI can be considered safe for the animal, whereas the red one could lead to negative effects on milk yield and dry matter intake. A THI of 72 could be considered a threshold value, however in our condition with high producing animals the threshold value can be lowered to 65. As the THI increases the animals reduced the feed intake, with lower reduction for the treated group. Nevertheless, as the THI gets high toward the end of the experiment the DM intake is similar between groups.

The milk yield and quality is shown in Table 3. The value of milk yield is obtained from the average milk yield of the last six milking from the day of sampling included (every 20 days). The treated group had an higher ($P < 0.05$) milk yield compared with control animals (38.2 *vs*. 40.3 kg,

respectively for control and treated groups). When corrected for the 4% fat content, the milk yield of the treated groups was still higher (+ 1.8 kg), even though not significant (due to the high variability of the fat value), compared with the control group. This was due to the slight numerically lower fat content. Parameters of milk quality were similar among groups except for the lactose yield which was significantly higher for the treated group (1.85 *vs*. 1.98 kg, respectively for control and treated groups). The higher lactose yield is a consequence of the numerically higher lactose content of the milk along with the higher milk yield of the treated group which could be related to the higher feed intake of these animals yielding to more glucose available for the mammary gland.

Metabolic parameters and minerals measured on plasma are shown in Table 4. The high level of Bicar®Z does not seem to impact the measured metabolic parameters and minerals. The higher ($P<0.05$) levels of albumin and bilirubin along with the similar value observed for the GOT/AST confirm a better health status of treated animals compared with control animals. The lower level of albumin, along with the lower cholesterol (the latter not significant) could be a consequence of reduced liver synthesis sparing the liver activity for haptoglobin and ceruloplasmin synthesis which are related to reduced performance, inflammatory condition and metabolic disorders. Indeed, this condition could also have confounding effect in BHBA levels interpretation, being BHBA higher ($P<0.05$) in control animals and suggesting a possible energy deficiency or a higher body reserve fat mobilization.

Since the higher feed intake treated animals had an higher absolute protein intake which could lead to higher urea level in plasma. However, the urea level were similar among groups denoting a lower body amino acid oxidation for treated animals.

Even though differences arise between groups for magnesium, sodium and chlorine concentration in plasma, only for magnesium it could be addressed a relation with dietary availability. Nevertheless, in a dairy farm with limited or absence of error in animal feeding, the related blood variation could be of ambiguous interpretation, and this is particularly true when values are within the normal range of variability.

The minerals contents and pH in urine collected form dry and lactating animals are reported in table 5. The inclusion of high amount of Bicar®Z did not impact the mineral contents in urine.

The analysis of fecal samples collected during the experiment depicts a better gastro intestinal tract condition for the treated animals compared with the control (Table 6). In particular, treated animal had a lower ($P < 0.05$) fecal acid load (7.03 vs 5.75 Mmol/100g feces, respectively for control and treated group) suggesting a better ruminal and intestinal carbohydrates utilization determining less fermentable substrate reaching the hind-gut. This means a better hind-gut status and less risk of inflammatory processes of the intestinal mucosa.

The number of pregnant animals and the average days open were improved in the high Bicar®Z group compared with the control group (44% vs. 56% for pregnancy and 134 ± 43 vs. 124 ± 48 for days open, respectively in the CTR and Bicar®Z group).

9.- Conclusion

The inclusion of higher amount of Bicar®Z in the diet during the first 140 days of lactation increased the milk yield with positive effects on some hepatic health parameters. There were no negative effects on minerals contents in urine whereas fecal parameters were improved suggesting less fermentable organic matter reaching the lower tract of the intestine.

The reproductive efficiency (i.e. percent of pregnant animals and days open) was improved in the high Bicar®Z group compared with the control group.

10.- Tables and Figures

Table 1 – Ingredient and chemical composition of the base diet used in the study

	Base diet
Ingredients (% DM)	
Corn silage	40
Grass hay	10
Alfalfa hay	10
Cotton seed, whole	7
Protein supplement	11
Corn/barley mix	20
Mineral and vitamin	2
Chemical composition	
Starch	27
NSC	41
NDF	30.2
Crude protein	16.0
Calculated	
Forage	40
NEl, Mcal/kg	1.69

Table 2 –Wood's incomplete gamma function fit over individual daily milk yield for control and treated groups[1]

	Control	Treated	P
Initial milk yield, kg	29.2	29.7	0.63
Rate of increase until peak	0.37	0.38	0.87
Rate of decline after peak	0.05	0.05	0.76
Week at peak	7.9	8.1	0.32
Peak yield, kg	42.8	44.5	0.14
Persistency	4.19	4.23	0.34

[1]Control = 150 g/head/day Bicar®Z; Treated = 300-400 g/head/day Bicar®Z

Table 3 - Milk yield and quality among control and treated groups[1]

	Control	Treated	P
Milk, kg	38.2	40.3	< 0.05
4% fat corrected milk, kg	36.3	38.1	0.11
Fat, g/100 ml	3.71	3.68	0.71
Protein, g/100 ml	3.00	2.95	0.38
Lactose, g/100 ml	4.87	4.93	0.45
Fat, kg	1.41	1.46	0.30
Protein, kg	1.14	1.19	0.12
Lactose, kg	1.85	1.98	< 0.05
Urea, mg/dl	20.20	21.94	0.20
Casein, g/100 ml	2.30	2.27	0.60

[1]Control = 150 g/head/day Bicar®Z; Treated = 300-400 g/head/day Bicar®Z.

Table 4 - Metabolic parameters in blood samples collected form dry and lactating animals within the control and treated groups[1]

Blood parameter	Dry	Lactating		SEM	Reference range	
		Control	Treated		Dry	Lactating
Glucose, mmol/L	3.60	3.46	3.46	0.078	3.64-4.06	3.30-3.90
[2]Cholesterol, mmol/L	2.71[a]	4.87[b]	5.27[b]	0.065	2.19-3.06	3.84-5.81
Urea, mmol/L	3.80[a]	4.82[b]	4.67[b]	0.195	2.74-4.31	4.41-5.97
Ceruloplasmin, µmol/L	2.47[a]	2.97[b]	2.82[b]	0.099	2.18-3.11	2.19-3.15
Total protein, g/L	80.10[a]	85.26[b]	85.60[b]	1.368	73.9-82.2	77.0-84.9
Albumin, g/L	34.84[a]	34.43[a]	37.06[b]	0.493	34.6-36.2	33.3-35.0
Globulin, g/L	45.26	50.82	48.54	1.647	38.5-48.9	41.5-51.2
[2]GOT/AST, U/I	92.51[a]	116.00[b]	125.09[b]	0.055	50.6-72.1	67.1-89.6
[2]GGT, U/I	29.04[a]	38.29[b]	40.51[b]	0.067	21.0-30.9	25.3-34.5
[2]Bilirubin, µmol/L	0.84[a]	1.33[a]	1.85[b]	0.136	< 1	< 1
[2]ALP, U/I	64.40[b]	42.43[a]	45.29[a]	0.071	34.1-57.3	34.5-55.8
[2]Haptoglobin, g/L	0.20	0.30	0.17	0.280	< 0.1	< 0.1
[2]NEFA, mmol/L	0.09[a]	0.11[a]	0.16[b]	0.088	0.13-0.37	0.12-0.38
[2]BHBA, mmol/L	0.47[a]	0.67[b]	0.52[a]	0.043	0.19-0.33	0.23-0.45
Creatinine, µmol/L	105.70[b]	85.48[a]	86.90[a]	1.676	101.4-128.9	82.8-98.5
Calcium, mmol/L	2.54	2.49	2.53	0.025	2.42-2.61	2.38-2.59
Inorganic phosphorous, mmol/L	2.14[a]	1.91[b]	1.70[b]	0.063	1.72-2.20	1.57-2.17
Magnesium, mmol/L	0.94[a]	0.95[a]	1.04[b]	0.016	0.88-0.96	0.91-1.03
Sodium, mmol/L	145.58[c]	141.99[b]	139.91[a]	0.393	142.1-147.9	141.1-145.5
Potassium, mmol/L	4.18[a]	4.01a[b]	3.95[b]	0.062	3.86-4.62	3.67-4.64
Chlorine, mmol/L	105.08[c]	102.02[b]	100.40[a]	0.462	105.1-110.0	100.2-106.6
Zinc, µmol/L	15.21[b]	11.03[a]	11.97[a]	0.509	13.7-18.5	11.5-16.3

[1]Control = 150 g/head/day Bicar®Z; Treated = 300-400 g/head/day Bicar®Z.
[2]The analyses were conducted on natural logarithmic scale and SEM is on natural logarithmic scale
Means with different superscripts differ for $P < 0.05$

Table 5 – Minerals contents and pH in urine collected form dry and lactating[1] animals

Parameter	Dry	Lactating		SEM	Reference	
		Control	Treated		Dry	Lactating
Calcium, mmol/L	1.04	0.58	0.58	0.227	0.5-3.2	0.5-3.2
Magnesium, mmol/L	5.79[b]	4.96[a]	4.91[a]	0.179	8-26	8-26
Sodium, mmol/L	19.33[a]	31.08[b]	41.69[b]	3.359	10-65	10-65
Potassium, mmol/L	245.52[b]	192.76[a]	172.47[a]	12.825	<200	>150
Phosphorous, mmol/L	0.04	0.01	0.01	0.021	0.8	0.8
Chloride, mmol/L	54.91[b]	33.89[a]	35.73[a]	4.619	22-100	22-100
pH	8.08	8.13	8.13	0.018	7-7.8	8-8.3

[1]Control = 150 g/head/day Bicar®Z; Treated = 300-400 g/head/day Bicar®Z.
Means with different superscripts differ for $P < 0.05$

Table 6 - Volatile fatty acids (Mmol/100g feces) and pH on fecal samples collected from control and treated animals[1]

Parameter	Control	Treated	P
Acetic acid	4.83	4.02	0.07
Propionic acid	1.10	0.92	0.09
Butyric acid	0.90	0.63	0.02
Valeric acid	0.09	0.07	0.03
Iso-butyric acid	0.06	0.06	0.92
Iso-valeric acid	0.06	0.05	0.21
Total acids	7.03	5.75	0.05
C2/C3	4.43	4.37	0.77
(C2+C4)/C3	5.25	5.05	0.38
pH	6.71	7.01	0.05

[1]Control = 150 g/head/day Bicar®Z; Treated = 300-400 g/head/day Bicar®Z.

Figure 1 – Lactation curve in control and treated groups as from the Wood's incomplete gamma function parameters

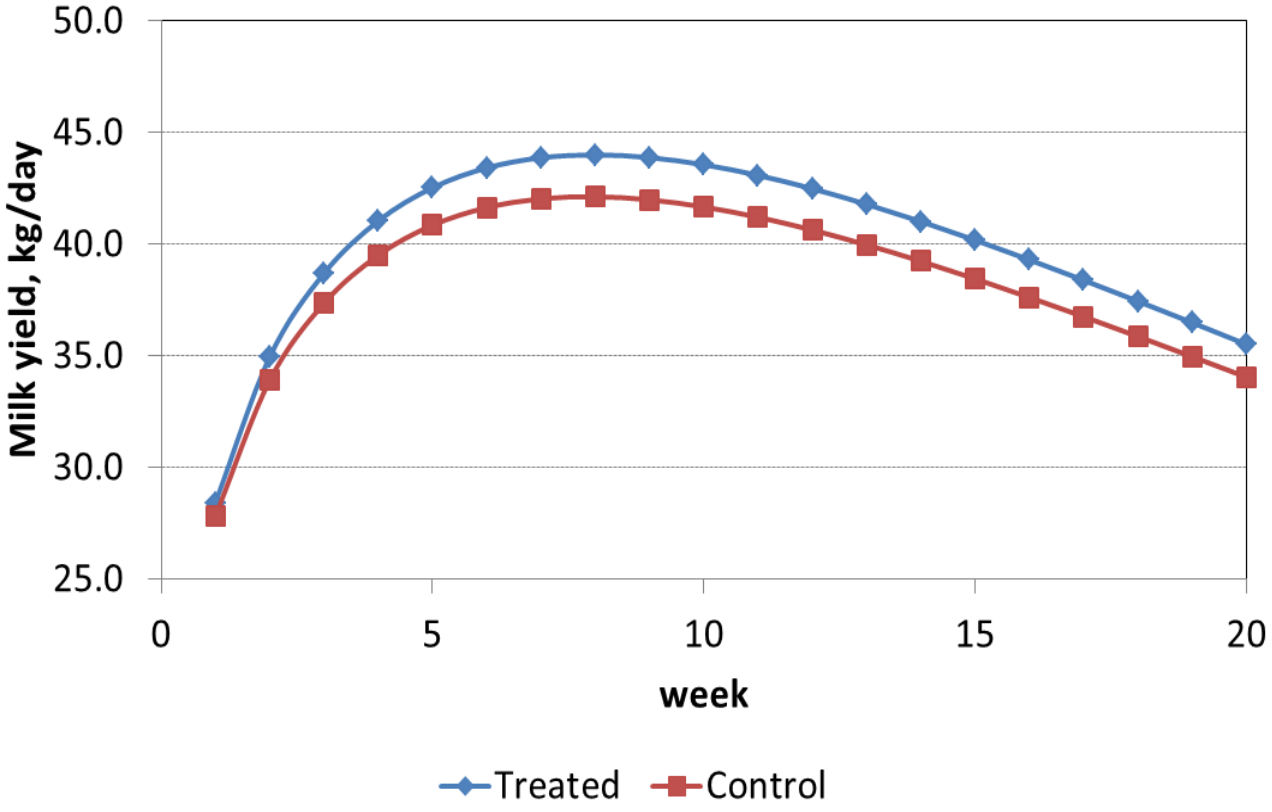
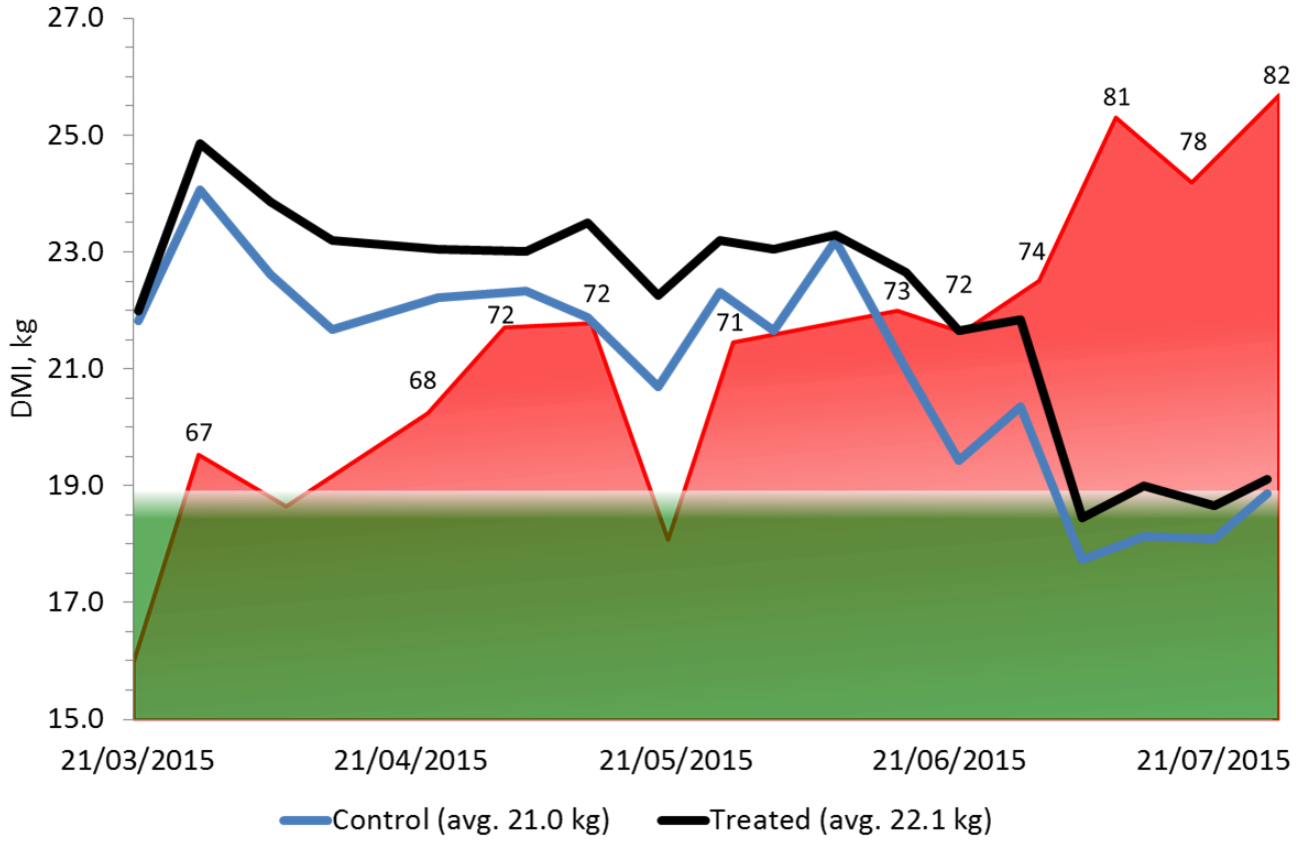


Figure 2 – Dry matter intake (kg) for the control and treated group (lines) during the experimental period and temperature humidity index (THI) over a week base measurement



11. - Study timing and approval of the final report

Start of the experimental phase of the study: March 21, 2015

End of the experimental phase of the study: July 21, 2015

Responsible of the project:
Dr. Giorgio Fusconi _____ (Date) _____ (Signature)

Study Director:
Prof. Francesco Masoero _____ (Date) _____ (Signature)

12.- The document was acknowledged by the Test Facility Manager

Dr. Mauro Morlacchini _____ Date _____ Signature

13. -The document was agreed by the Sponsor

Responsible for the Sponsor:
Ing. Giordano Zappelli _____ Date _____ Signature