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Automatically monitoring of dietary effects on rumination and activity of finishing heifers

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**Running head: Rumination and activity of beef heifers**

**Title:** Automatically monitoring of dietary effects on rumination and activity of finishing heifers

**E. Giaretta, A.L. Mordenti, G. Canestrari, A. Palmonari, A. Formigoni**

Departement of Veterinary Medical Sciences (DIMEVET), Via Tolara di Sopra, 50, 40064, Ozzano dell'Emilia, BO, Italy

Corresponding author: Elisa Giaretta, elisa.giaretta3@unibo.it

## **Abstract**

Rumination and activity behaviors are important welfare indexes in beef cattle housing. The main objective of this study was to assess if SCR automatic collars are able to reliably assess the rumination and activity patterns of beef heifers. For this purpose, individual rumination time and activity (RT and AT, respectively) were continuously recorded using an automatic neck collar system (Hr-Tag, SCR Engineers Ltd, Israel) on the three trials performed. For the Experiment 1, 60 Italian crossbred heifers were randomly assigned to one of two experimental diets for 9 months: the CORN-SILAGE diet (CS), which included 50% forage on dry matter (DM) basis (43% corn silage, 7% wheat straw) and the hay diet (HAY), with 57% forage on DM basis (28.5% grass hay, 28.5% alfalfa hay). Heifers consuming HAY diet showed greater ( $P < 0.05$ ) RT (min/day) and AT (bits/day), compared to CS diet. RT per kg of Dry Matter and per kg of aNDFom intake were similar in the two experimental groups, while RT per kg of peNDF intake was greater ( $P < 0.05$ ) in the CS group compared to the HAY one. Daily rumination and activity trends were significantly different among CS and HAY group. In the second experiment 16 beef heifers were randomly allocated in two homogeneous pens, containing 8 animals each one, and two non-homogeneous ones, where 16 animals were placed in more time steps, with different stocking density. The AT of non-homogeneous pens was significantly higher respect to the homogeneous ones, suggesting a

distress condition for values higher than 309 bits/day AT. In the third experiment RT and AT of 3 animals with respiratory disease were collected using the SCR system and compared with AT and RT of the health animals. Sick animals presented significantly lower RT and higher AT than the health ones. The cut-off to distinguish sick from health heifers were set to 537 bits/day AT and 381 min/day RT. In conclusion, this study demonstrated that SCR automatic collars can reliably monitor different rumination and activity behaviors of beef animals in various management conditions and different health status.

## Introduction

In recent years, multiple devices have been developed and implemented by the dairy industry to automatically monitor behavior and physiological parameters (Rutten et al., 2013; Barkema et al., 2015), while few trials were made in beef cattle area. Physical activity levels and rumination time are two parameters that are currently available for monitoring dairy cow health, and it has already been demonstrated that rumination and activity alterations could be associated to oestrus and to clinical and subclinical health disorders (Calamari et al., 2014; Gáspárdy et al., 2014; Stangaferro et al., 2016). Monitoring activity combined with other variable detections has been used to characterize pre-partum behavior, and predict calving in dairy cows (Borchers et al., 2017). In addition, the automatically recording of activity and rumination, and their correlation with animal performance, were assessed to measure the effects of different rations on ruminal function (Adin et al., 2009) and to detect early onset of lameness (Van Hertem et al., 2013) and mastitis (Stangaferro et al., 2016). Nevertheless, the use of automatic and continuous monitoring of rumination and activity time is not yet commonly used in beef farms. Rations used in finishing cattle are commonly characterized by a low content of forages and a high use of concentrates in order to increase the energy density of the diet and consequently the weight daily gain. In these conditions the rumination

behavior should be impaired; the possibility to record continuously this parameter could help to detect easily and precisely the single sick animal inside of the group. Continuous monitoring of behavioural and physiological parameters can aid early detection of sick animals, allowing immediate and targeted therapy (Weary et al., 2009).

The automatic collar system (Hi-Tag; SCR Engineers Ltd., Netanya, Israel) is based on the acoustics signals of rumination, thanks to a sensory microphone able to detect any passage of feed bolus. In addition, the collars also continuously measure activity, which is defined as presence or absence of head/neck movements (Schirmann et al., 2009; Schirmann et al., 2013; Gentry et al., 2016). Rumination collars (SCR tag) have recently been used in beef cattle in order to study the relationship between different dietary treatments, digestion, rumination and activity behaviors (Gentry et al., 2016; Weiss et al., 2017). A recent study of Marchesini et al., (2018) used rumination and activity data as health status and performance indicators in beef cattle during the early fattening period.

Further studies are necessary to better know the rumination and activity patterns in finishing beef cattle housed in different conditions. The objective of this study was to monitor rumination and activity of finishing heifers underwent two different dietary treatment (Experiment 1) and allocated either in homogeneous or non-homogenous pens (Experiment 2). In addition, rumination and activity patterns of heifers in different health status were studied (Experiment 3).

## Materials and Methods

The Scientific Ethic Committee on Animal Experimentation of the University of Bologna examined and approved the experimental protocol (n.: 4783-X/10 All: 17) used in this study.

### Experiment 1.

For the first trial, sixty Italian crossbreed heifers were divided in two homogenous experimental groups, with similar characteristics of age (approximately 7 months) and body weight (BW) ( $200.9 \pm 30.6$  kg, mean  $\pm$  SD). After an 8-weeks adaptation period, animals were randomly allocated to 6 pens, each containing 10 heifers (space available/animal =  $4.5 \text{ m}^2$ ), located in the same housing facility. Heifers were individually weighed at the beginning and at the end of the trial, in order to calculate average daily gain (ADG) and the gain to feed ratio (G:F). Pens were then randomly assigned to 1 of 2 treatments (Table 1). Heifers were fed once per day at 07:30 in the morning and received *ad libitum* diet based on a specific amount of DM delivered, in order to have no residues in the manger one hour before the next delivery. Access to fresh water was allowed continuously. Diets were prepared as total mixed rations (TMR) with a horizontal mixer wagon (Zago 13-m<sup>3</sup>, ZAGO srl, PD, Italy) equipped with a weighing scale. The corn-silage group (CS) received a basal diet composed of corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and a protein supplement (6%). The HAY group received a hay diet, based on higher percentage of roughage, composed of alfalfa hay (28.5%) and grass hay (28.5%). Moreover, the diet included earlage (28.5%), wheat hulls (9.5%), and a protein premix (5%). Animals were slaughtered by pen, at an average BW of  $426.74 \pm 36.4$  kg, following the standard weight slaughtering procedures. Individual slaughter weights were taken in the abattoir immediately before the slaughtering procedure. Harvest was completed at the local abattoir in the Department of Veterinary Medical Science of Bologna in 1 wk period.

The individual rumination time (RT) and activity time (AT) were continuously recorded during the last 90-days of the trial, for both groups of animals, using an automatic neck collar system (Hr-Tag, SCR Engineers Ltd, Netanya, Israel). Animals were checked daily to verify the right collar position. Data from individual tags were transferred to the system software (Dataflow, SCR Dairy) automatically every 20 min *via antenna* located in the barn. Rumination and activity time (RT and AT, respectively) were recorded by the software as daily means (rumination minutes or activity bits during a day) and as 2h intervals in order to study daily trends.

### ***Feed analysis***

TMR samples were collected at the beginning of experimental period, and then twice a month. Feed samples were dried in a forced air oven (M700-VF, MPM instrument, Bernareggio, IT) at 60°C for 48 h to determine DM content. Particle size distribution of the dry diets was determined using a Penn State Particle Separator (PSPS. Mertens, 1997; Heinrichs, 2013), and peNDF was calculated by multiplying the percentage of particles retained above a 4 mm screen times the aNDFom content. For chemical analysis, dried diets were ground separately in a Cyclone mill (1-mm screen; model SM100; Resch GmbH, Haan, Germany). Feed samples were analyzed for ash, determined after 3 h combustion at 550°C in a muffle furnace (Vulcan 3-550, Dentsply Neytech, Burlington, NJ); aNDFom (Mertens, 2002; Palmonari et al., 2017); acid detergent fiber (ADF); acid detergent lignin (ADL), and crude protein (CP) (AOAC, 1990).

### ***Diet Characteristics and Fiber Particle Size***

Chemical composition of the two experimental diets is described in Table 1. The percentages of CP, aNDFom, ADF in HAY diet were higher than in CS diet, while the CS diet had a higher starch content. Particles separation are presented in Table 2. The proportion of particles that were retained on the top of 19 mm and 8 mm sieves was greatest ( $P < 0.05$ )

for HAY diet, while the CS diet contained the greatest proportion ( $P < 0.05$ ) of small particles ( $< 4$  mm) compared with HAY one. The physical effectiveness factor, given by particles longer than 4 mm (sum of particles retained on the top 3 sieves of the PSPS) and estimated peNDF were greater ( $P < 0.05$ ) for the HAY diet compared to the CS one.

## **Experiment 2. Homogeneous and non-homogeneous groups**

In the second experiment 32 animals were housed for 6 months in four pens in order to study activity and rumination behavior under different environmental conditions. Sixteen heifers were randomly allocated in two homogeneous pens (for age and BW), containing 8 animals each one; animals were divided in two homogeneous groups for age (approximately 7 months) and BW ( $200.9 \pm 30.6$  kg, mean  $\pm$  SD). Other sixteen heifers were randomly allocated in different times in two non-homogeneous pens, with different stocking density. Animals were placed in these two boxes in more steps: at the beginning of the experiment (t1), 3 during the second month (t2) and during the fifth month (t3) of the trial. All 32 animals from homogeneous and non-homogeneous pens were fed with the same diet (the CTR one). During the second trial, individual rumination time (RT) and activity time (AT) were continuously recorded for all four groups of animals using the automatic neck collar system (Hr-Tag, SCR Engineers Ltd, Netanya, Israel).

## **Experiment 3. Detection of health status**

In the third experiment, rumination and activity behaviours of 3 beef cattle with respiratory disease (sick animals) were collected using the SCR system and compared with AT and RT of healthy animals (of the experiment 1). RT and AT of sick animals were continuously recorded until the death of the animal, occurred for the symptoms worsening.

## ***Statistical analysis***



Data were analyzed by R version 3.4.0 (R Core Team). After checking for normal distribution, chemical and physical composition of diets were analyzed by a t-Student test. Pen was used as the experimental unit for the Experiment 1, while animal was used as the experimental unit for the Experiment 2. and 3. Regarding the experiment 1, data concerning animal performance (ADG, G:F, slaughter weight and age) were analyzed by the mixed models including treatment (diet) as the fixed effect and the initial weight as the covariate. Rumination and activity behaviors were analyzed by the mixed models. To analyze daily rumination time (expressed as min/day, min/kg DM, min/kg aNDFom and min/kg peNDF) and daily activity (bits/day) the fixed effects of treatment (diet), day, and their interactions were included in the initial models. Pen was considered a random effect. Rumination and activity raw data (min/2h and bits/2h, respectively) were analyzed by the mixed models followed by the Tukey *post hoc* test for multiple comparison. The initial model included the fixed effects of treatment (diet), time (2h) and their interactions. Pen was considered a random effect. The final model for each parameter of interest was selected by backward elimination of explanatory variables with  $P > 0.05$ . Data are expressed as least square mean (LSM) and the standard error of the LSM (SEM). An effect was considered significant at  $P < 0.05$ .

As regards the experiments 2 and 3, the animal was considered the statistical unit. Receiver Operating Characteristic (ROC) curves were generated to discriminate homogeneous and non-homogenous groups (exp. 2) and to differentiate sick from healthy animals (exp. 3), using RT and AT cut-off. To analyze daily RT and AT of homogeneous and non-homogeneous pens (exp. 2), pen and period (t1, t2 and t3) were included in a mixed model as fixed effects, while animal by day was considered the random effect. RT and AT of sick and health animals (exp. 3) were studied by a mixed model including the health status of animal as fixed effect and the animal by day as random effects.

## Results

### Experiment 1

#### *Feedlot performance*

ADG, G:F, initial and slaughter BW, housing period, and the age at slaughter of feedlot cattle are presented in Table 3. No significant differences were observed for length of housing period and age at slaughter between the two experimental groups. The CS group had a higher slaughter BW and ADG ( $P < 0.05$ ) than the HAY one. G:F was lower ( $P < 0.05$ ) in HAY heifers compared to CS group.

#### *Rumination and Activity*

The daily RT and AT were significantly ( $P < 0.05$ ) different between the two experimental groups. As shown in Fig. 1, daily RT of HAY group was significantly ( $P < 0.05$ ) higher than that observed in the CS group (473.75 vs 405.22 min/day, SEM = 3.90, respectively). A significant difference was also observed for daily AT, which was higher in the HAY group compared to the CS one (619.31 vs 553.12 bits/day, respectively, SEM = 5.27). Significant differences ( $P < 0.05$ ) were observed for rumination time per kg of DM and peNDF intake, while there were no differences in rumination time per kg of aNDFom (Table 4).

Considering RT throughout the day (min/2h), significant differences were observed within treatment, and between the two experimental groups, as shown in Fig. 2. About 4 h after feed delivery, the results demonstrated a significantly increase of RT (+15 min/2h on average respect to 08:00 h) in both experimental groups ( $29.10 \pm 10.0$  min/2h in CS group and  $30.60 \pm 9.14$  min/2h in HAY one). Moreover, in HAY group rumination was maintained significantly ( $P < 0.01$ ) higher ( $29.60 \pm 9.55$  min/2h average) for the following 6 h (from 12:00 h to 18:00 h). On the other hand, a significant ( $P < 0.01$ ) rumination decrease ( $29.10 \pm$

10.0 to  $19.75 \pm 8.35$  min/2h) resulted in CS group from 12:00 h to 16:00 h, followed by a rapid increase ( $P < 0.05$ ) in the next 2h interval ( $26.52 \pm 10.82$  min/2h at 18:00 h). RT progressively increased during night time (from 20:00 h to 04:00 h in CS group, and from 18:00 h to 04:00 h in HAY group). Moreover, the increment (from 20:00 h to 04:00 h) was significantly ( $P < 0.01$ ) greater in HAY compared to CS group ( $43.66 \pm 15.63$  vs  $51.77 \pm 20.23$  min/2h on average for CS and HAY group, respectively). A considerable ( $P < 0.01$ ) RT decrease was observed from 04:00 h to 08:00 h in both experimental groups, until the next feed delivery ( $16.75 \pm 8.88$  min/2h and  $15.04 \pm 9.97$  min/2h in CS and HAY groups, respectively, at 08:00 h).

As regards the AT throughout the day (bits/2h), significant ( $P < 0.01$ ) differences were observed within treatment and between the two-dietary treatment (Fig. 3). In both experimental groups, the activity was significantly ( $P < 0.05$ ) lower during the night time hours (average  $38.23 \pm 9.51$  bits/2h and  $36.03 \pm 11.04$  bits/2h in CS and HAY groups, respectively, from 22:00 h to 04:00 h) and began to increase from 04:00 h. During the day (from 10:00 to 20:00), the averaged AT was significantly ( $P < 0.01$ ) higher in the HAY group than in the CS one ( $52.52 \pm 12.94$  bits/2h and  $69.88 \pm 19.28$  bits/2h for CS and HAY, respectively).

## Experiment 2

Figure 4 shows the activity trend of homogeneous and non-homogeneous pens. As visualized on the Figure 4, AT suddenly increased after the introduction of new animals into the pen, and remained higher than homogeneous pen, still the entrance of the other heifers, which determined an additional increment of all animals AT. Indeed, significantly differences ( $P < 0.0001$ ) were observed on AT among homogeneous and non-homogeneous groups ( $511.68 \pm 78.67$  vs  $768.32 \pm 193.82$ , respectively). In addition, the CV was highest in non-homogeneous pens (0.25 vs 0.15 in homogeneous ones) demonstrating a great AT

variability among heifers of this experimental group. Significant interactions ( $P < 0.001$ ) resulted among period and pen (Figure 5). As concerns non-homogeneous pens, significant increments of AT from t1 to t2, and subsequently from t2 to t3, were observed; while a significant reduction of AT, from t1 and t2 to t3, resulted in non-homogeneous pens. The ROC curve (Fig. 5) showed the best AT cut-off differentiating homogeneous pens from non-homogeneous ones at 609.50 bits/day (area under the curve 0.92; 95 % CI 0.91 to 0.93; sensitivity 0.81; specificity 0.91). No significant differences were observed on RT between the two experimental groups ( $471.93 \pm 66.80$  vs  $474.35 \pm 71.54$ ).

### **Experiment 3.**

RT was significantly ( $P < 0.01$ ) lower in sick animals, compared to the healthy ones ( $267.91 \pm 81.01$  vs  $456.65 \pm 94.69$ , respectively). Significant ( $P < 0.01$ ) differences on AT data were observed among healthy and sick animals ( $518.92 \pm 112.64$  vs  $580.61 \pm 89.44$ ). The ROC showed the best AT cut-off, to differentiate sick from healthy heifers, at 537 bits/day (area under the curve 0.70; 95 % CI 0.64 to 0.76; sensitivity 0.85; specificity 0.48) (Fig. 6). Regarding RT data shown in Figure 7, the best cut-off, to discriminate sick and health animals, was set at 381 (area under the curve 0.93; 95 % CI 0.90 to 0.95; sensitivity 0.88; specificity 0.83).

### **Discussion**

The aim of Experiment 1 was to validate SCR collars on beef animals, underwent two different dietary treatments. Significant differences have been observed in daily RT (min/day) between the two experimental groups (Table 4). RT was significantly higher in HAY group rather than in the CS one. Whereas peNDF is defined as the portion of NDF that requires further mastication to allow passage out of the rumen (Mertens, 1997), this

increment could be justified by the significantly higher percentage of peNDF in HAY diet in respect to the CS one. However, the RT relative to peNDF is significantly lower in HAY group than in CS one, suggesting that other variables in addition to peNDF may influence the ruminal behavior.

RT in terms of min/d, in both experimental groups, was higher than what observed in Gentry et al (2016) study, while was similar in terms of min/kg peNDF. Thus, the difference in daily RT (min/day) between CS and HAY group was not enough to justify the significantly higher amount of peNDF in HAY diet, which appeared less efficient than the one in CS group. According to Mertens (2002), to maximize RT and ADG the dietary peNDF value should be 15% of ration DM, with a range from 12 to 18%, while Fox and Tedeschi (2002) recommend that feedlot diets contain 7 to 10% peNDF. peNDF was defined by Mertens (2002) as the percentage of the NDF retained on a screen with 1.18 mm openings after dry sieving. Thus, considering that peNDF calculated in our study was based on the amount of fiber retained on the top of 4 mm sieve, the values that we obtained were higher compared with the two recommendations: 17.01% and 24.75% in CS and HAY diet, respectively. Indeed, we observed both in the CS and HAY groups lower ADG and G:F ratio compared with other studies, such as Gentry et al. (2016). A linear decrease of RT per kg of peNDF as particle size increased was also observed by (Beauchemin and Yang, 2005). Moreover, in Gentry et al. (2016) study, RT appeared to be more sensitive to particle size than to sieving techniques, and no differences in RT per kg of peNDF were observed. Bonfante et al. (2016) study demonstrated that different peNDF percentages in the diets of Holstein heifers influence rumination time, but have no effects on rumen temperature and rumen pH, suggesting that other variables contribute to rumen physiology. In our study, the presence of wheat straw in CS diet (as 7% of DM), may have caused the increase of RT per kg of peNDF, which resulted significantly greater than in the HAY group. These results are

in line with Farmer et al. study (2014) which demonstrated that the addition of chopped wheat straw to lactating cows diets resulted in greater time spent chewing and eating per kg of peNDF consumed, while the time spent chewing, eating, and ruminating were not affected by reducing dietary forage. Also Mertens et al. (1997) reported that straw elicited approximately 1.74 times the chewing response of alfalfa or grass forage of similar long particle size. The importance of forage type and quality was also highlighted by Fustini et al. studies (2011, 2017), which demonstrated that straw and different alfalfa cuttings influence ruminating behaviors as well as the DMI. These results suggest that further researches are needed to compare measurement techniques to calculate the appropriate peNDF requirements in beef finishing diets, and to study the effect of different forage sources and levels in the diet on ruminal behavior.

The significant differences observed in RT per kg of DM could be explained by the greater rumination time relative to the similar DMI. Rumination per kg of NDF was similar between the two experimental groups. Other authors (Beauchemin and Yang, 2005; Gentry et al., 2016) observed no differences in rumination per kg of NDF for dairy cow and beef cattle, respectively, consuming different amount or various particle sizes of forage.

The diets used in our experiment also differ for CP and starch contents, which could have an influence on ruminal behavior. Previous studies demonstrated indeed that variations in intake of dietary fractions modulated rumination patterns (Byskov et al., 2015; Mendes et al., 2015; Farsuni et al., 2017). Further studies are necessary to better evaluate the influence of each dietary component on ruminal behavior in beef cattle. Considering RT and AT trends throughout the day, significant differences were observed between the two experimental groups, and during the day within treatment. Daily rumination trend was similar to what observed by Gentry et al. (2016), but the time spent in rumination for each 2 h time block was greater in the current study. As shown also by Gentry et al. (2016), a slight increment of

RT was observed about 4 h after feed delivery, followed by a great increase during the nighttime in both experimental groups. From 08:00 h to 10:00 h the time spent in rumination was maintained significantly lower respect to the rest of the day, probably because of the restriction of TMR that induced the animal to eat immediately after the morning delivery, and to considerably reduce their rumination activity for the next 2 h. Based on the current data, it would be of great interest to evaluate rumen behavioral changes in a different feed regiment, such as *ad libitum* intake. Furthermore, as reported by Cavallini et al. studies (2017, 2018) the total daily rumination did not change, while daily rumination and feeding patterns (RT/2 h) changed in dairy cows fed on *ad libitum* intake. Moreover, the increase of RT from 4 h after feed delivery and during nighttime was significantly higher in HAY group compared to the CS one, which showed a more fluctuating trend.

The AT daily mean was significantly higher in HAY group than in CS one. Moreover, as expected, daily AT (bits/2 h) was significantly lower during the nighttime in both experimental groups, while it was significantly higher ( $P= 0.01$ ) during the day in HAY group compared to the CS one. To date, there are a few studies about beef cattle activity, and none of them observed any differences in AT between different feeding groups (Gentry et al., 2016).

Variations of activity parameter are difficult to explain, since activity represents the sum of a range of different behaviours, including eating, drinking, walking and social interactions (Weary et al., 2009). These peaks of activity within a day may coincide with the number of episodes of feed intake each day, since bulls fed a high-concentrate diet spent less time eating and took shorter meals than bulls fed more concentrate diets (DeVries et al., 2005; Mialon et al., 2008)

The aim of the Experiment 2 was to set an AT cut-off in order to distinguish homogenous from non-homogeneous pens. As largely demonstrated, the social context has a

major effect on behavior. The social relationships can be agonistic (dominant-subordinate) or affiliative (sociability) (Neave et al., 2018). As demonstrated by Zobel et al. (2011), beef heifers in confined housing systems adopt different social strategies to respond to highly competitive feeding environment. In that study, measures of feeding behavior were used to detect different animal strategies characterized by engaging in competition or avoidance of agonistic interactions. In addition, Gutmann et al. (2015) study on dairy cows showed that long-term familiarity had a stronger effect on the intensity of social relationships, i.e. regarding investment of time and energy, than very recent shared experience. Thus, keeping well-acquainted animals together may contribute to a stable inner structure of a herd and thus promote animal welfare. According to our results, the average AT of non-homogeneous pen significantly increased when unknown animals were added into a preformed group. On the contrary, the average AT of homogeneous pen significantly decrease after three months of housing (Figure 4). On one hand, the increment of AT on non-homogeneous pen may indicate distress situations determined by the entrance of unknown animal, which probably led to increase animal competition and group rearrangement. On the other hand, the long-term social interactions between animals of homogeneous pens determined a greater animal tranquility, which contributed to the decrease of the average AT. These different activity trends between homogeneous and non-homogeneous pens demonstrated that environmental changes cause different heifers responses and corroborate the formation of affiliative relationships among individuals of gregarious species, as extensively demonstrated by other authors (Plusquellec and Bouissou, 2001; Miranda-de la Lama and Mattiello, 2010; Raussi et al., 2010). These results suggested that AT could be an interesting and valid indicator of animal welfare, particularly when they are housed in confined systems. The automatically monitoring of AT, could contribute to better understand social interactions between animals and distress conditions. Through the ROC curve we were able to distinguish non-



homogeneous pens from the homogeneous one, defining an AT cut-off of 609.50 min/day. The abnormal general activity of non-homogeneous pens was thus detected over this cut-off. Further researches are needed to better investigate the relationships between general activity and other variables, such as lying, feeding and ruminating behaviors, with the use of different technologies, in order to understand the physiological or altered activity pattern of beef cattle.

The aim of Experiment 3 was to examine whether it was possible to identify sick beef cattle through the continuous monitoring of activity and rumination. Daily rumination time resulted significantly lower on sick animals, while there was an increment of the daily activity time in the same group of animals. In our study it was not possible to register rumination and activity patterns before and after the appearance of clinical signs, because the three sick animals presented the clinical symptoms suddenly after the beginning of the experiment and they all died before the trial end. Thus, we were able to identify RT and AT cut-off in order to distinguish sick and healthy animals using the ROC curves. As regards RT, great sensibility and specificity were obtained at 381 min/day cut-off, whereas worse results were obtained with an AT ROC curve. Indeed at 537 bits/day AT, corresponding to the optimum cut-off designed by the curve, resulted a good sensibility (0.85), but a low specificity (0.48). This low value of specificity is not considered acceptable because of the high number of false positives which had to be taken into account to avoid the unnecessary use of antibiotics. In dairy cows changes in patterns of rumination and activity during *E. Coli* mastitis (Stangaferro et al., 2016) and lameness (Van Hertem et al., 2013) permitted the detection of affected animals before the appearance of clinical symptoms. A recent study of Marchesini et al., (2018) on beef cattle during the early fattening period demonstrated that activity and rumination monitoring allows the detection of bovine respiratory disease and lameness. Conversely to the previous studies, our results showed that activity daily time was higher in sick animals than in healthy ones, suggesting different temperament and probably a

371 more agitation of the sick heifers. In line with the previous studies, RT was significantly  
372 affected by health animal status and the reduced RT in sick animals permitted to define a  
373 valid cut-off (with good sensibility and good specificity) to predict animal disease.

## 375 **Conclusion**

376 In conclusion, this study demonstrated that RT and AT are important welfare indexes  
377 in beef cattle housing. The rumination and the activity patterns of beef animals are modulated  
378 by different parameters like: the housing management, the dietary treatment and the animal  
379 stocking density. In addition, rumination and activity daily time are often affected by health  
380 animal status. These results suggest that the automatic detection of these parameters could be  
381 useful to monitor the animal welfare during the housing period and to study the physiological  
382 rumination and activity patterns of beef cattle. Further studies are needed to correlate RT and  
383 AT patterns to the other welfare indexes such as individual feeding behaviors, rumen activity  
384 parameters and animal performance, in order to evaluate their relationship. In this way, the  
385 routinely use of neck collar system can potentially help the herd management and optimize  
386 the performance.

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392    **Conflicts of interest**

393    The authors declare no conflicts of interest.

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## References

- Adin, G., Solomon, R., Nikbachat, M., Zenou, A., Yosef, E., Brosh, A., Shabtay, A., Mabweesh, S.J., Halachmi, I., Miron, J., 2009. Effect of feeding cows in early lactation with diets differing in roughage-neutral detergent fiber content on intake behavior, rumination, and milk production. *J. Dairy Sci.* 92, 3364–3373.
- AOAC, W.H., 1990. Official methods of analysis. Arlington, VA.
- Barkema, H.W., von Keyserlingk, M.A.G., Kastelic, J.P., Lam, T.J.G.M., Luby, C., Roy, J.-P., LeBlanc, S.J., Keefe, G.P., Kelton, D.F., 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. *J. Dairy Sci.* 98, 7426–7445.
- Beauchemin, K.A., Yang, W.Z., 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. *J. Dairy Sci.* 88, 2117–29.
- Bonfante, E., Palmonari, A., Mammi, L., Canestrari, G., Fustini, M., Formigoni, A., 2016. Effects of a completely pelleted diet on growth performance in Holstein heifers. *J. Dairy Sci.* 99, 9724–9731.
- Borchers, M.R., Chang, Y.M., Proudfoot, K.L., Wadsworth, B.A., Stone, A.E., Bewley, J.M., 2017. Machine-learning-based calving prediction from activity, lying, and ruminating behaviors in dairy cattle. *J. Dairy Sci.* 100, 5664–5674.
- Calamari, L., Soriani, N., Panella, G., Petrera, F., Minuti, A., Trevisi, E., 2014. Rumination time around calving: An early signal to detect cows at greater risk of disease. *J. Dairy Sci.* 97, 3635–3647.
- Cavallini, D., Mammi, L.M.E., Fustini, M., Palmonari, A., Heinrichs, A.J., Formigoni, A., 2018. Effects of ad libitum or restricted access to total mixed ration with supplemental long hay on production, intake, and rumination. *J. Dairy Sci.* 101, 10922–10928.

420 DeVries, T.J., von Keyserlingk, M.A.G., Beauchemin, K.A., 2005. Frequency of Feed  
 421 Delivery Affects the Behavior of Lactating Dairy Cows. *J. Dairy Sci.* 88, 3553–3562.

422 Farmer, E.R., Tucker, H.A., Dann, H.M., Cotanch, K.W., Mooney, C.S., Lock, A.L., Yagi,  
 423 K., Grant, R.J., 2014. Effect of reducing dietary forage in lower starch diets on  
 424 performance, ruminal characteristics, and nutrient digestibility in lactating Holstein  
 425 cows. *J. Dairy Sci.* 97, 5742–5753.

426 Fustini, M., Palmonari, A., Bucchi, E., Heinrichs, A.J., Formigoni, A., 2011. Chewing and  
 427 ruminating with various forage qualities in nonlactating dairy cows. *Prof. Anim. Sci.* 27,  
 428 352–356.

429 Fustini, M., Palmonari, A., Canestrari, G., Bonfante, E., Mammi, L., Pacchioli, M.T., Sniffen,  
 430 G.C.J., Grant, R.J., Cotanch, K.W., Formigoni, A., 2017. Effect of undigested neutral  
 431 detergent fiber content of alfalfa hay on lactating dairy cows: Feeding behavior, fiber  
 432 digestibility, and lactation performance. *J. Dairy Sci.* 100, 4475–4483.

433 Gáspárdy, A., Efrat, G., Bajcsy, Á., Fekete, S., 2014. Electronic monitoring of rumination  
 434 activity as an indicator of health status and production traits in high-yielding dairy cows.  
 435 *Acta Vet. Hung.* 62, 452–462.

436 Gentry, W.W., Weiss, C.P., Meredith, C.M., McCollum, F.T., Cole, N.A., Jennings, J.S.,  
 437 2016. Effects of roughage inclusion and particle size on performance and rumination  
 438 behavior of finishing beef steers. *J. Anim. Sci.* 94, 4759–4770.

439 Gutmann, A.K., Špinka, M., Winckler, C., 2015. Long-term familiarity creates preferred  
 440 social partners in dairy cows. *Appl. Anim. Behav. Sci.* 169, 1–8.

441 Heinrichs, 2013

442 Marchesini, G., Mottaran, D., Contiero, B., Schiavon, E., Segato, S., Garbin, E., Tenti, S.,  
 443 Andrighetto, I., 2018. Use of rumination and activity data as health status and

444 performance indicators in beef cattle during the early fattening period. *Vet. J.* 231, 41–  
445 47.

446 Mertens, D.R., 1997. Creating a system for meeting the fiber requirements of dairy cows. *J.*  
447 *Dairy Sci.* 80, 1463–81.

448 Mertens, D.R., 2002. Gravimetric Determination of Amylase-Treated Neutral Detergent  
449 Fiber in Feeds with Refluxing in Beakers or Crucibles: Collaborative Study [WWW  
450 Document].

451 Mialon, M.M., Martin, C., Garcia, F., Menassol, J.B., Dubroeuq, H., Veissier, I., Micol, D.,  
452 2008. Effects of the forage-to-concentrate ratio of the diet on feeding behaviour in  
453 young Blond d'Aquitaine bulls. *animal* 2, 1682.

454 Miranda-de la Lama, G.C., Mattiello, S., 2010. The importance of social behaviour for goat  
455 welfare in livestock farming. *Small Rumin. Res.* 90, 1–10.

456 Neave, H.W., Weary, D.M., Von Keyserlingk, M.A.G., 2018. Review: Individual variability  
457 in feeding behaviour of domesticated ruminants. *Animal* 1–12.

458 Palmonari, A., Canestrari, G., Bonfante, E., Fustini, M., Mammi, L., Formigoni, A., 2017.  
459 Technical note: In vitro digestibility of amylase-treated, ash-corrected neutral detergent  
460 fiber, with addition of sodium sulfite, at 240 hours with or without rumen fluid  
461 reinoculation. *J. Dairy Sci.* 100, 1200–1202.

462 Plusquellec, P., Bouissou, M.-F., 2001. Behavioural characteristics of two dairy breeds of  
463 cows selected (Hérens) or not (Brune des Alpes) for fighting and dominance ability.  
464 *Appl. Anim. Behav. Sci.* 72, 1–21.

465 Raussi, S., Niskanen, S., Siivonen, J., Hänninen, L., Hepola, H., Jauhiainen, L., Veissier, I.,  
466 2010. The formation of preferential relationships at early age in cattle. *Behav. Processes*  
467 84, 726–731.

468 Rutten, C.J., Velthuis, A.G.J., Steeneveld, W., Hogeveen, H., 2013. Invited review: sensors  
 469 to support health management on dairy farms. *J. Dairy Sci.* 96, 1928–1952.

470 Schirmann, K., von Keyserlingk, M.A.G., Weary, D.M., Veira, D.M., Heuwieser, W., 2009.  
 471 Technical note: Validation of a system for monitoring rumination in dairy cows. *J. Dairy*  
 472 *Sci.* 92, 6052–6055.

473 Schirmann, K., Chapinal, N., Weary, D.M., Vickers, L., von Keyserlingk, M. a G., 2013.  
 474 Short communication: Rumination and feeding behavior before and after calving in  
 475 dairy cows. *J. Dairy Sci.* 96, 7088–92.

476 Stangaferro, M.L., Wijma, R., Caixeta, L.S., Al-Abri, M.A., Giordano, J.O., 2016. Use of  
 477 rumination and activity monitoring for the identification of dairy cows with health  
 478 disorders: Part III. Metritis. *J. Dairy Sci.* 99, 7422–7433.

479 Van Hertem, T., Maltz, E., Antler, A., Romanini, C.E.B., Viazzi, S., Bahr, C., Schlageter-  
 480 Tello, A., Lokhorst, C., Berckmans, D., Halachmi, I., 2013. Lameness detection based  
 481 on multivariate continuous sensing of milk yield, rumination, and neck activity. *J. Dairy*  
 482 *Sci.* 96, 4286–4298.

483 Weary, D.M., Huzzey, J.M., Von Keyserlingk, M.A.G., 2009. Board-invited Review: Using  
 484 behavior to predict and identify ill health in animals. *J. Anim. Sci.* 87, 770–777.

485 Weiss, C.P., Gentry, W.W., Meredith, C.M., Meyer, B.E., Cole, N.A., Tedeschi, L.O.,  
 486 McCollum, F.T., Jennings, J.S., 2017. Effects of roughage inclusion and particle size on  
 487 digestion and ruminal fermentation characteristics of beef steers. *J. Anim. Sci.* 95,  
 488 1707–1714.

489 Zobel, G., Schwartzkopf-Genswein, K.S., Genswein, B.M.A., von Keyserlingk, M.A.G.,  
 490 2011. Impact of agonistic interactions on feeding behaviours when beef heifers are fed  
 491 in a competitive feeding environment. *Livest. Sci.* 137, 1–9.

**Table 1.** Ingredients and chemical composition of corn-silage (CS) and HAY diets fed to beef cattle for a restricted intake; the diets were formulated to be different in forage content (evaluated as physical effectiveness factor and peNDF).

Item, % DM basis	Dietary treatment <sup>1</sup>	
	CS	HAY
Cornsilage	43	
Earlage	25	28.5
Grass hay	-	28.5
Alfalfa hay	-	28.5
Wheat hulls	10	9.5
Corn meal	9	
Wheat straw	7	-
Protein premix <sup>2</sup>	6	5
Forage:Concentrate	50	57
<u>Calculated nutrient values, DM basis</u>		
DM (%)	55.25	72.5
CP (%)	10.67	14.15
Ash (%)	5.49	7.76
aNDFom (%) <sup>3</sup>	36.06	44.85
ADF (%)	22.55	33.69
ADL (%)	5.58	5.81
Starch (%)	35.72	18.6

<sup>1</sup>CS = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and hay (28.5%), earlage (28.5%), wheat hulls (9.5%) and protein premix (5%).

<sup>2</sup>Premix protein of CS diet contained (as fed): crude protein 37.2%, ash 15%, cellulose 9%, crude fat and oils 3%, Ca 2.2%, Na 1.8%, P 0.8%, Mg 0.70%, Methionine 0.5%, Vit A (E 672) 45.000 U.I., Vit D3 (E 671) 14.500 U.I., VIT E 3a700 115mg. Sodium selenite (E 8) 1971 mcg, Zinc sulphate monohydrate (E 6) 1644 mg, Manganous sulphate monohydrate (E 5) 893 mg, Ferrous sulfate monohydrate (E 1) 304 mg, Cupric sulphate pentahydrate (E 4) 275 mg, Potassium iodide (E 232) 32mg, Urea 40000 mg.

<sup>3</sup>aNDFom = amylase- and sodium sulfite treated NDF, corrected for ash residue (Palmonari et al., 2017).



**Table 2.** Physical characteristics and particle size distribution (%) of the corn-silage (CS) and HAY diets fed to beef cattle for a restricted intake; the diets were formulated to be different in forage content (evaluated as physical effectiveness factor and peNDF)

Item	Dietary treatment <sup>1</sup>			P-value
	CS	HAY	SEM	
No. of samples	18	18	-	-
aNDFom (%)	36.06	44.85	2.29	0.04
	Retained screen (%) <sup>2</sup>			
Sieve screen size (mm)				
19.0	9.77	16.50	0.22	0.01
8.0	18.45	23.21	0.32	0.02
4.0	18.94	15.48	0.67	0.01
Pan (particle less than 4mm)	52.83	44.81	0.45	0.01
Physical effectiveness factor <sup>3</sup>	47.17	55.19	5.60	0.01
Estimated peNDF <sup>4</sup> (% DM) <sup>4</sup>	17.01	24.75	3.24	0.01

<sup>1</sup>CS= corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage (28.5%), wheat hulls (9.5%) and protein premix (5%).

<sup>2</sup>Particle size was measured using the PSPS (Heinrichs, 2013).

<sup>3</sup>Physical effectiveness factor: the percentage of sample larger than 4 mm in particle size (top 3 sieves)

<sup>4</sup>Percent peNDF was estimated by multiplying the physical effectiveness factor by the percentage of aNDFom (as a decimal) of the ingredient before separation.

**Table 3**

Effect of two different dietary treatments (CS and HAY diets) on finishing heifer performance.

Item	Dietary treatment <sup>1</sup>		SEM	P- value
	CS	HAY		
n (pens of 10 heifers)	3	3		-
Initial BW (kg)	197.86	203.90	4.84	-
Initial age (day)	214	248	17.43	-
Housing period (day)	285.09	293.93	4.64	0.85
DMI (kg/day)	8.38	8.32	0.16	-
ADG (g /day)	836	731	0.02	0.04
G:F (g/kg)	100.38	87.99	2.80	0.03
Slaughter BW (kg)	442.52	408.96	5.75	0.04
Slaughter age (day)	481.32	489.27	16.80	0.87

<sup>1</sup>CS = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and HAY hay (28.5%), earlage (28.5%), wheat hulls (9.5%) and protein premix (5%).

**Table 4.**

Effect of dietary treatments on rumination time per kg of DM, NDF and physically effective NDF (peNDF) consumed.

Item	Dietary treatment <sup>1</sup>			P-value		
	CS	HAY	SEM	Treatment	Day	Int. <sup>2</sup>
No. of observations (pens of 10 heifers)	3	3		-	-	-
Rumination (min/day)	401.88	481.83	5.73	0.01	<0.01	0.12
Rumination (min/kg)						
DM	47.96	57.91	2.10	<0.01	-	-
NDF	132.99	129.12	6.80	0.16	-	-
peNDF	281.97	233.97	8.37	<0.01	-	-

<sup>1</sup>CS = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage (28.5%), wheat hulls (9.5%) and protein premix (5%).

<sup>2</sup>Int = interaction between Treatment and Day factors

538

539 **Figure 1.**

540 Boxplots representing the daily rumination (min/24h) and activity (bits/24h) time in finishing  
541 beef cattle subjected to two different diet treatments (CS and HAY groups). CS = corn silage  
542 (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and protein  
543 premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage (28.5%), wheat  
544 hulls (9.5%) and protein premix (5%). Notches represent 95% CI. Asterisks mean significant  
545 ( $P < 0.05$ ) differences between the treatment groups.

546 **Figure 2.**

547 Effects of diets (CS and HAY) on rumination behavior (min/2h) in finishing beef feedlot. CS  
548 = corn silage (43%), earlage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%)  
549 and protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage  
550 (28.5%), wheat hulls (9.5%) and protein premix (5%). The arrow represents the feeding at  
551 07.30 h.  $n = 6$  pens (3 for each treatment group). The error bars represent 95% CI. Different  
552 letters mean significant differences between hours, within the same treatment (small letters  
553 for the CS group and capital letters for the HAY one). Treatment ( $P < 0.01$ ; SEM = 1.77).  
554 Hour ( $P < 0.01$ ; SEM = 0.16). Treatment x Hour ( $P < 0.01$ ; SEM = 0.22).

555 **Figure 3.**

556 Effects of diets (CS and HAY) on activity behavior (bits/2h) in finishing beef feedlot. CS =  
557 earlage (43%), corn silage (25%), wheat hulls (10%), corn meal (9%), wheat straw (7%) and  
558 protein premix (6%). HAY = alfalfa hay (28.5%) and grass hay (28.5%), earlage (28.5%),  
559 wheat hulls (9.5%) and protein premix (5%). The arrow represents the feeding at 07:30 h.  $n =$   
560 60 (30 for each treatment group). The error bars represent 95% CI. Different letters mean  
561 significant differences between hours, within the same treatment (small letters for the CS  
562 group and capital letters for the HAY one). Different letters mean significant differences

between hours, within the same treatment (CS or HAY diet). Treatment ( $P = 0.61$ ; SEM= 4.95). Hour ( $P < 0.01$ ; SEM= 0.14). Treatment x Hour ( $P < 0.01$ ; SEM 0.21).

**Figure 4.**

Graphics represents the average daily AT of homogeneous and non-homogeneous pens relative to housing period (t1, t2 and t3). The asterisks indicate significant differences among groups for  $P < 0.05$ ; different superscripts indicate significant differences among periods within the group for  $P < 0.05$ .

**Figure 5.**

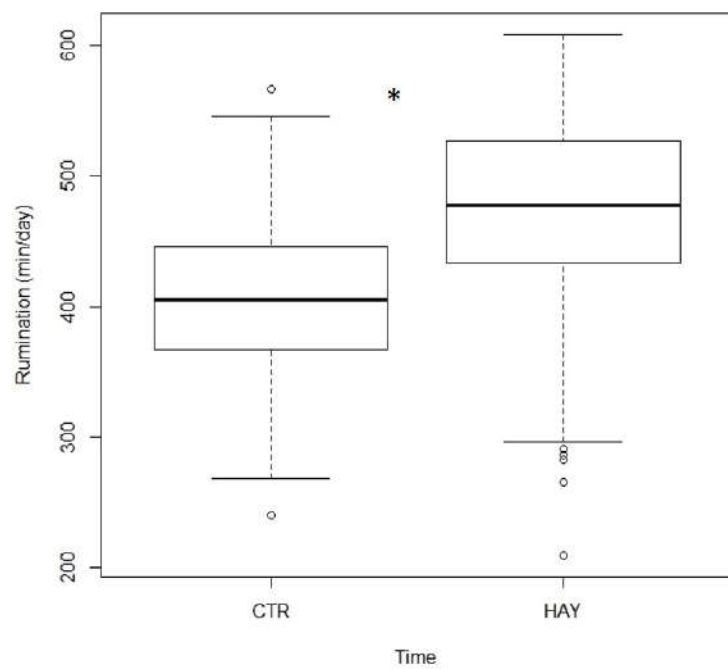
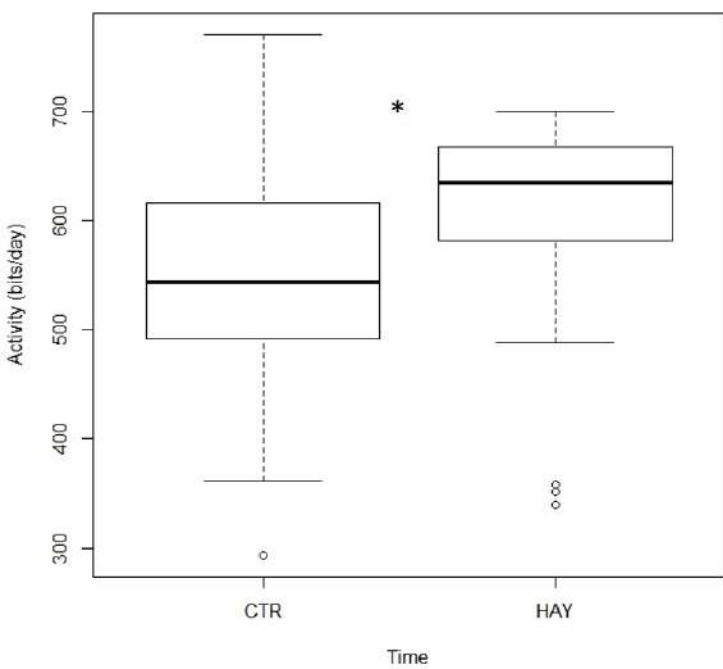
The receiver operator curve showed the best cut-off of AT at 609 bits/min, with a sensitivity of 0.81 and a specificity of 0.91 (area under the curve: 0.92; 95% CI 0.91 to 0.93), to distinguish homogeneous pens from non-homogeneous ones.

**Figure 6.**

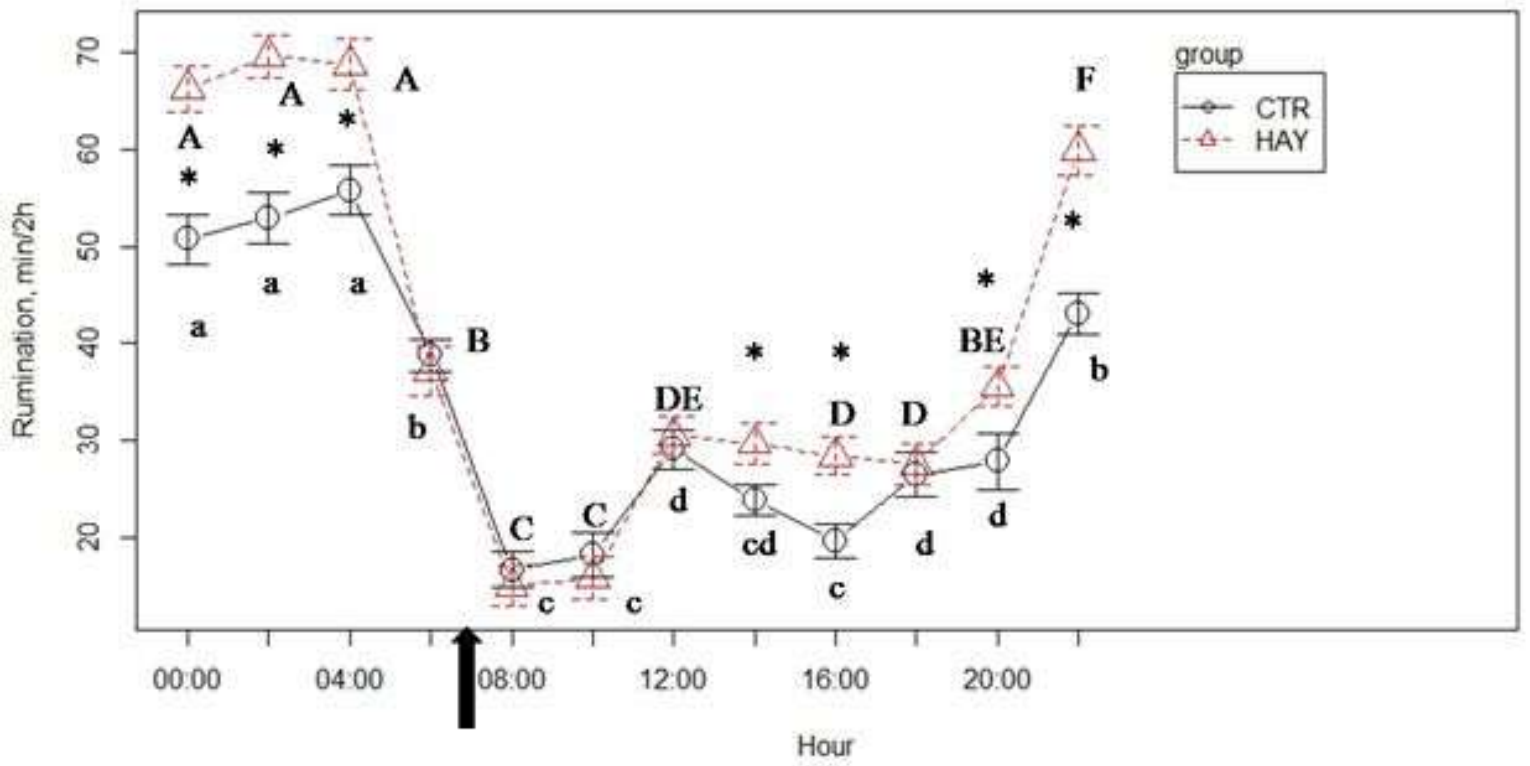
The receiver operator curve showed the best cut-off of AT at 537 bits/min, with a sensitivity of 0.85 and a specificity of 0.48 (area under the curve: 0.70; 95% CI 0.64 to 0.76), to distinguish sick animals from health ones.

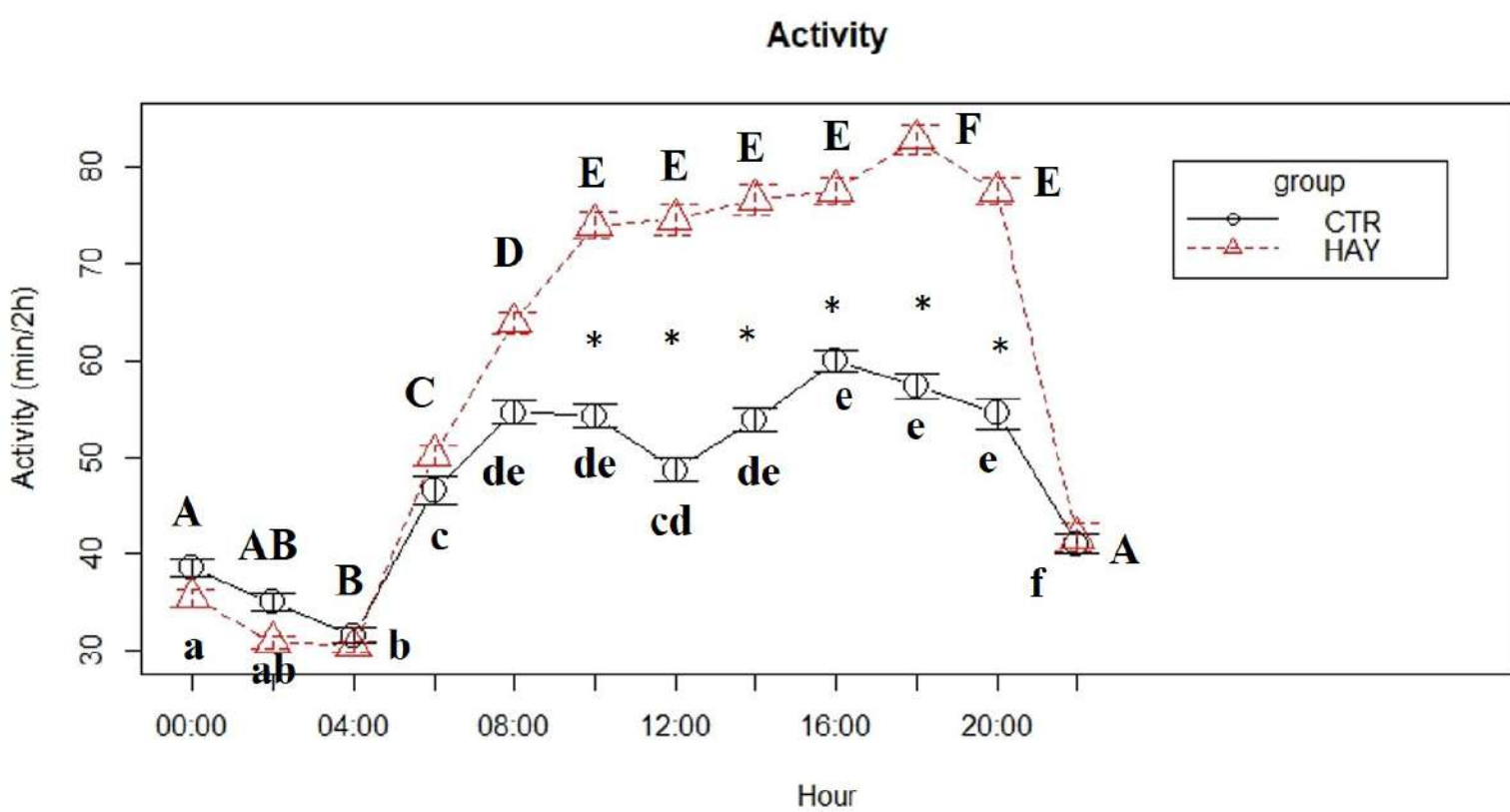
**Figure 7.**

The receiver operator curve showed the best cut-off of RT at 381 bits/min, with a sensitivity of 0.88 and a specificity of 0.83 (area under the curve: 0.90; 95% CI 0.93 to 0.95), to distinguish sick animals from health ones.

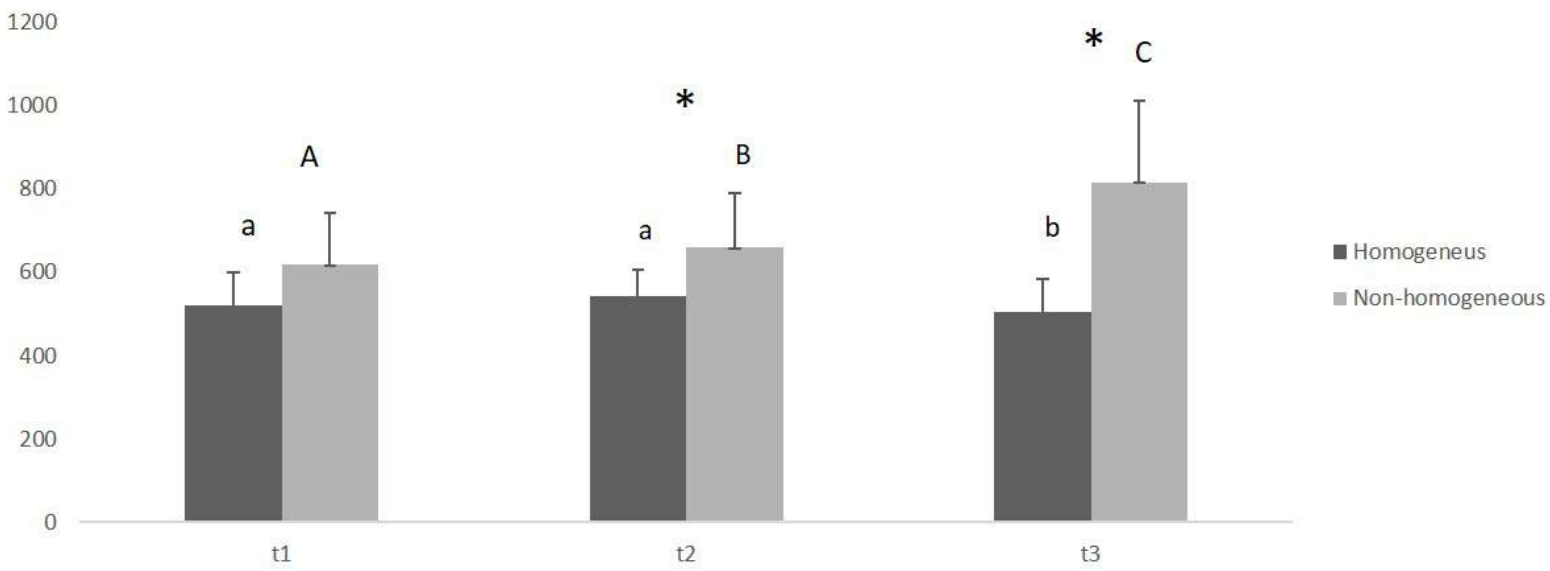


# Rumination

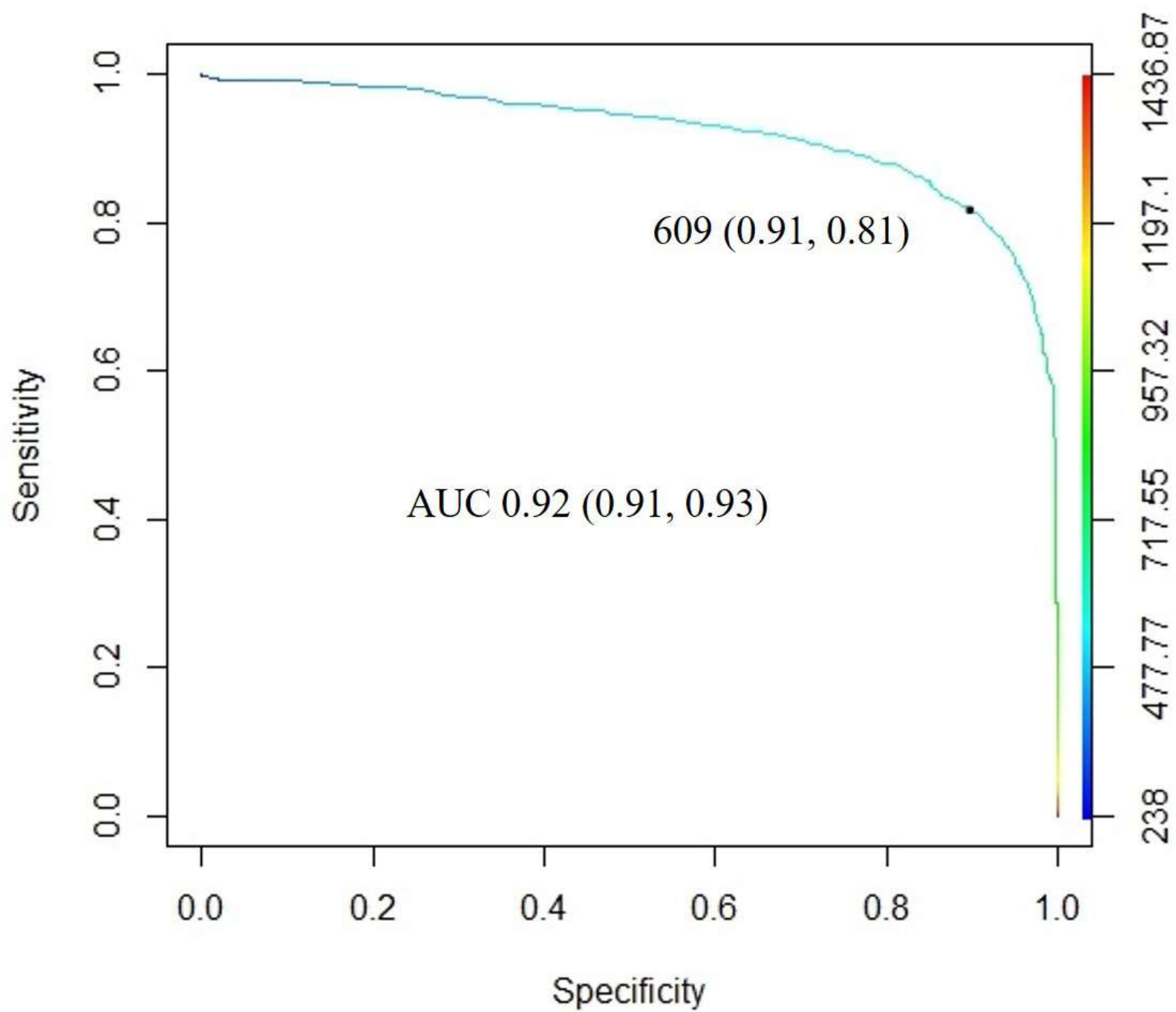




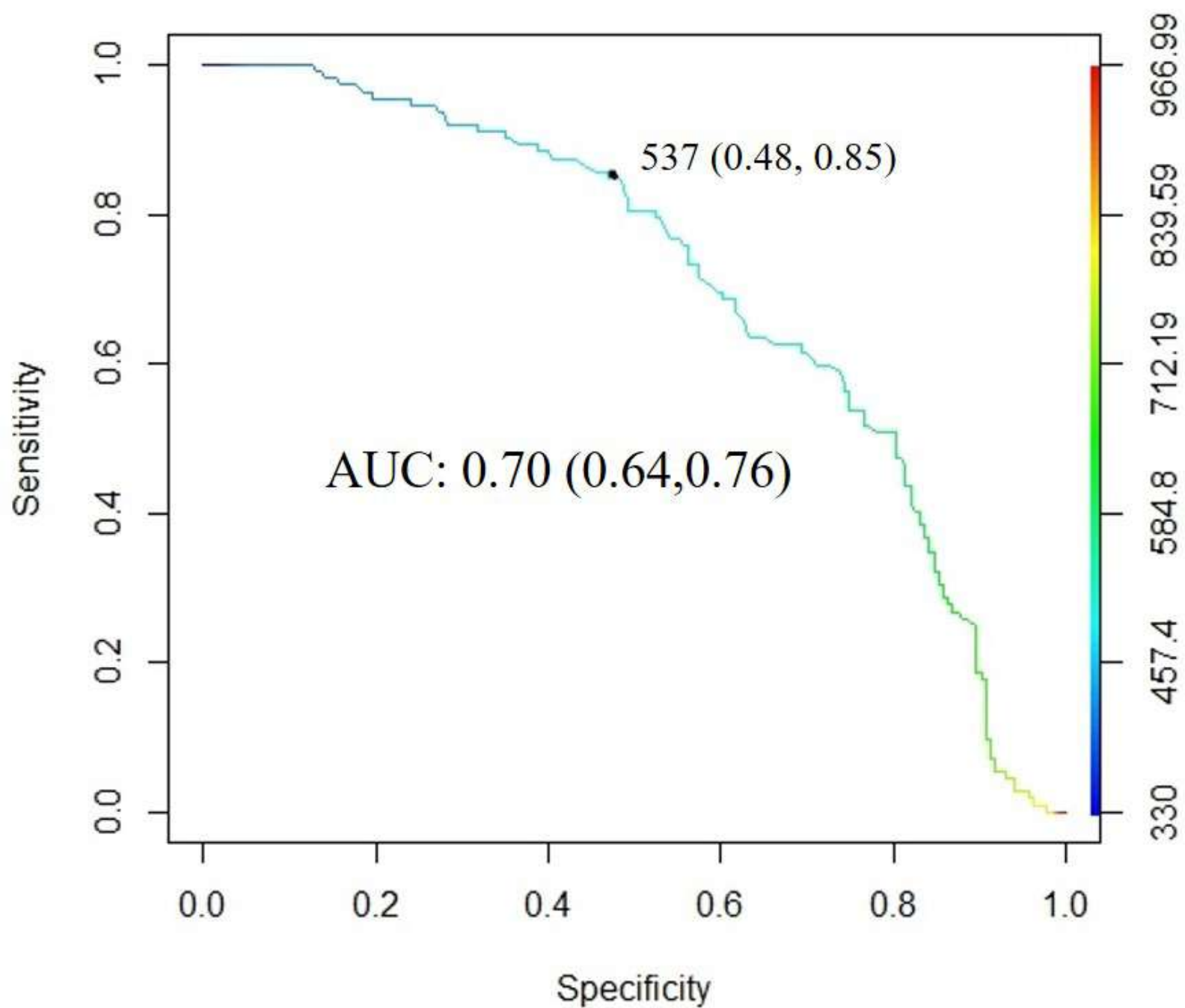




# Activity



## Activity



## Rumination

