

## Using sensors to detect individual responses of lambs during transport and pre-slaughter handling and their relationship with meat quality

S Ogun<sup>†</sup>, I Viola<sup>†</sup>, M Obertino<sup>†</sup>, I Manenti<sup>†</sup>, U Ala<sup>†</sup>, A Brugiapaglia<sup>‡</sup>, L Battaglini<sup>‡</sup>, G Perona<sup>†</sup> and M Baratta<sup>\*†§</sup>

<sup>†</sup> Department of Veterinary Science, University of Turin, Grugliasco (TO), Italy

<sup>‡</sup> DISAFA, University of Turin, Grugliasco (TO), Italy

<sup>§</sup> Department of Chemistry, Life Science and Environmental Sustainability, SCVSA, University of Parma, Italy

\* Contact for correspondence: mario.baratta@unipr.it

### Abstract

Rapid analysis of animal welfare is a crucial component of the assessment of the meat quality supply chain, ensuring management procedures confer optimum standards of welfare. Further, there is increasing interest in monitoring the welfare state of each individual animal. This study looked at transport and pre-slaughter management in terms of meat quality evaluated in two breeds (Biellesse and Sambucana) across two different farming systems. Precision Livestock Farming (PLF) technologies were implemented, including accelerometer and rumination activity ear-tag sensors, as potential welfare indicators during transportation and pre-slaughter. Significant correlations were found between sensors' parameters, such as total activity and rumination and physical and chemical meat quality characteristics such as drip loss. Lambs with lower rumination and/or lower total activity were found to have lower drip loss indicating reduced meat quality. Sensors have the potential to help detect those animals particularly sensitive to stressors during transport and pre-slaughter handling and may allow real-time measurement of the impact of transport and handling in abattoirs, enabling better animal management via specific customised strategies.

**Keywords:** animal welfare, cortisol, lamb, meat quality, sensors, transportation

### Introduction

There is increasing demand from consumers of products of animal origin for the animals in question to not only be healthy and sustained organically but also to be produced, transported and slaughtered to a high standard of welfare. In lambs, stress during growth has been shown to have an effect both on the palatability and cooking characteristics of the resultant meat (Bramblett *et al* 1963). Livestock transportation is an integral step in the meat production process and all forms of mechanical transport are potentially hazardous, irrespective of distances travelled, putting animals at risk of stress responses due to fasting, exercise or emotional trauma (Knowles *et al* 1995). However, more careful consideration for animals' transport conditions has been shown to reduce such responses (Broom 2005). Transport to slaughter includes the rounding-up of animals and their loading onto approved vehicles, followed by careful carriage and lairage for recuperation, in accordance with international welfare regulation (EC Council Regulation No 1/2005 2005b) prior to slaughter. It is important to handle animals carefully, with the welfare of the animals taken into account throughout the entire chain of events (Cockram 2019).

Knowles (1998) formulated a review of the factors affecting the welfare of sheep during transport and recommended best practice techniques to minimise the effect. While more recently, Messouri *et al* (2015) developed an assessment tool using animal-, resource- and management-based measures for sheep welfare at transport and the European Union (Consortium of Animal Transport Guides Project 2017) produced a good practice guide devoted to the transport of sheep. Llonch *et al* (2015) carried out a review of the animal-based indicators of sheep welfare and, despite noting many valid indicators of disease and injury and sick animals, stressed the need for novel indicators to assess the onset of short-term hunger and thirst. In recent years, many studies have examined the potential for Precision Livestock Farming (PLF) to both monitor and improve the welfare assessment of animals on-farm (Schillings *et al* 2021). PLF uses sensors which can provide real-time information to aid the evaluation of supply chain procedures and possible impacts on the quality of the meat (Caja *et al* 2020). Advances in engineering, along with decreased costs of new technologies have enabled many sensor-based systems to be developed for the livestock industry (Caja *et al* 2016; Halachmi *et al* 2019). These sensors are capable of automat-

ically collecting data in real-time, allowing the early detection of specific problems (eg production losses, poor health, and threats to well-being) at group or individual level (Caja *et al* 2016; Krueger *et al* 2020; Maltz 2020). Sensor-based solutions are implemented in PLF systems at the level of the smallest manageable production unit, ie the ‘sensor-based individual animal’ approach (Halachmi *et al* 2019). Animal well-being increases the added value for all stakeholders of the animal production supply chain (in particular for consumers and farmers) (Alonso *et al* 2020). Further, there is a desire for sensor technologies to elevate welfare assessment and management on livestock farms from the level of the farm (Krueger *et al* 2020) to automated or semi-automated continuous monitoring at the level of the individual animal (Maroto-Molina *et al* 2019). PLF systems are generally seen as a clear opportunity to improve the profitability and sustainability of livestock farms, including those of small ruminants under extensive conditions (Rutter 2017; Morgan-Davies *et al* 2018).

An analysis of certain parameters allows rapid identification of an altered state in the well-being of sheep, ie a sudden cessation or reduction in rumination over a period of time may be an indication that welfare has been compromised. Immobilisation can occur in response to stressors but may indicate docility and absence or fear or it may be reflecting a high degree of disturbance and nervousness (Romyer & Boissou 1992). Studies in sheep have shown that exposure to several aversive stimuli (eg such as social isolation, separation of a lamb from ewe, hot temperatures) can lead to a reduction in rumination (Cockram 2004). In recent years, biosensors and various biochemical parameter indicators have been developed, especially in beef and pork meat assessment (Sionek *et al* 2020).

Drip loss is deemed significant in terms of pork meat’s overall palatability and has thus been a problem for the meat industry for many years (Forrest *et al* 2000). Excessive drip loss from fresh meat not only sees financial losses but also losses in valuable vitamins, minerals, flavour compounds, and water (Walshe *et al* 2006).

In the ovine pre-slaughter supply chain, lambs may be exposed to a number of stressors, such as feed and water deprivation while poor handling and transport procedures can negatively impact welfare which may increase ultimate pH reducing meat product quality and consistency (Sutherland *et al* 2016). Another study (Hemsworth *et al* 2019) highlighted the potential benefits of training-dedicated personnel to reduce fear and stress in sheep at abattoirs.

Animals may show differing responses to the same environmental conditions even when these are routine and regulated. The use of biosensors can quickly monitor the animal’s reaction to potential aversive factors, enabling effective management action to be taken to improve welfare and product quality during supply chain processes. Here, the aim was to determine whether alterations in transportation and lairage would be reflected by significant changes in lambs’ rumination and motion activity prior to slaughter and whether these stress indicators had any impact on meat quality.

## Materials and methods

### Breeding systems

Two breeding systems were monitored: 12 Biellese lambs (four females and eight males) were raised in the Teaching Animal Farm of the Veterinary Science Department, University of Turin, and ten Sambucana lambs (three females and seven males) bred in pasture at 1,800–2,000 m above sea level in Val Maira (CN), Cuneo, Italy. The Biellese lambs were reared semi-extensively, ie they consumed approximately 500 g of milk per day, after weaning (day 60), they were fed ~120 g of concentrate per day and hay *ad libitum* until slaughter and flocks were permitted to partake in supplementary grazing in the autumn-winter season (period of investigation). Sambucana lambs, on the other hand, were reared extensively, leaving at dawn and grazing until evening, relying solely on suckling and grazing natural pasture. In both systems animal health and behaviour were monitored monthly as described previously (Bodas *et al* 2021). Briefly, welfare assessment was applied as per the AWIN protocol for sheep (AWIN 2015): (i) qualitative behaviour assessment (QBA), social withdrawal, stereotypy, excessive itching, panting; (ii) fleece cleanliness, fleece quality, tail length, faecal soiling, lameness; (iii) familiar human approach; (iv) stocking density, access to shade/shelter, water availability; (v) lamb mortality. These indicators were deemed appropriate (Bodas *et al* 2021), addressing the main welfare concerns.

The study procedures were authorised by the Ethics and Animal Welfare Commission (n 1865/2017) of the University of Turin.

### Wool sample collection

Cortisol levels of study animals were checked every 30 days via collection of wool samples from lambs’ first month of life to the age of four months. The procedures were authorised by the Ethics and Animal Welfare Commission of the University of Turin.

Wool samples were collected using a shave and re-shave technique as previously reported (Geß *et al* 2020) from the posterior vertex region of the neck between the cisterna magna and scapular bones. Approximately 10 cm<sup>2</sup> was shaved in order to obtain sufficient wool for laboratory analysis.

### Hormone analysis

Cortisol was extracted as per Geß *et al* (2020) and extracted samples were reconstituted in duplicate with 250 µL of ImmunoAssay Buffer (IAB) before the quantification of cortisol in the wool. Wool cortisol levels were determined using a commercial AlphaLISA Assay Kit (Cortisol AlphaLISA Kit-PerkinElmer, USA) according to the manufacturer’s specifications. The intra- and inter-assay coefficients of variation were 3 and 4%, respectively. The analytical sensitivity (Lower Detection Limit; LDL) of the method was 177 pg ml<sup>-1</sup> and showed the following cross-reactivity: 21-deoxycortisol 9%, prednisolone 5%, cortisone, and corticosterone 1%.

### Sensor monitoring

The commercial ear-tag sensor (eSense Flex, Allflex, Dallas, USA) had been previously tested for use in sheep (Caja *et al* 2020) and were active PLF devices containing a 3-axial accelerometer designed for measuring rumination and motion activity in cattle (calves and adult). Data were calculated according to manufacturer's instructions. The sensors were powered by a small battery with an expected lifespan of three years and communicated with a reading unit via UHF over an area of approximately 200 Å~ 500 m. Their material (waterproof plastic case), shape (keychain medal) and dimensions (68 Å~ 38 Å~ 15-mm; weight, 28 g), meant it was possible for eSense devices to be readily inserted into the ears of large-sized sheep. Lambs from both breeds were tagged at two months of age and monitored every 15 min for rumination and motion activities right up until slaughter. After becoming adapted to the sensors, the lambs were recorded every day for a period of approximately 2 months, including the critical 48-h period pre-slaughter, ie during transportation and lairage. No ear or sensor issues (loss of tag, tearing of ear, breakage, failure) were reported during the experimental period.

The eSense accelerometer showed base line and maximum comparable values for rumination and motion. Moreover, standard errors were low (mean variation coefficients ranged between 35 and 48% for rumination, and between 22 and 33% for motion activity). We used this analysis to detect real-time changes within these parameters as induced by potential stressful events related to transport, such as the containment space linked to the transport time and sudden unexpected movements during transport.

### Animal transportation

The Biellese lambs were picked up from the stable at 0600h and transported (transport truck area:  $3.12 \times 2$  m [length  $\times$  width] = 6.24 m<sup>2</sup>) for 2 h, before being unloaded at the slaughterhouse and slaughtered within 30 min. The Sambucana lambs were loaded onto the same size van the day prior to slaughter and transported for 5 h before being unloaded and left to rest for 6 h in a pre-slaughter barn (sheep stable area:  $3 \times 2.5$  m [length  $\times$  width] = 7.5 m<sup>2</sup>) and subsequently slaughtered.

### Lamb meat analysis

All lambs were aged four and a half months at slaughter. The analyses were carried out blindly and different researchers managed the results of the analyses on the animal and on the animal product via numerical codes. Slaughter took place in the Department of Veterinary Science slaughterhouse in accordance with EU Council Regulation No 1009/2005 (2005a) on the Protection of Animals at Time of Killing. Carcasses were processed as previously reported (Gonzales-Barron *et al* 2021).

### Water-holding capacity

Water-holding capacity was measured by drip loss (DL) and cooking loss (CL) according to the methods described by

Honikel (1998). For DL determination, meat samples stored in a plastic box provided with a double bottom at 4°C for 24 h were weighed before and after storage. DL was defined as % losses during storage, calculated as  $100 \times (1 - \text{weight after storage/weight before storage})$ . To determine CL, meat samples were weighed, vacuum-sealed in a polyethylene bag and cooked in a water-bath, set at 75°C until an internal core temperature of 70°C was attained (monitored with a thermocouple). After cooking, samples were cooled using cold water, while still in bags before being removed, blotted and re-weighed. WBL was defined as % losses during cooking, calculated as  $100 \times (1 - \text{weight after cooking/weight before cooking})$ .

Physicochemical analyses elucidated pH, moisture and dry matter, fat content, protein content ash content, drip loss, cooking loss — which were carried out on day 1 after slaughter. The intrinsic properties of meat were assayed at the beginning of the cold maturation.

### Statistical analysis

In accordance with ARRIVE guidelines (Percie du Sert *et al* 2020), sample size estimation was carried out and obtained using a retrospective study (Caja *et al* 2020). From that study, with an alpha = 0.05 and a statistical power = 0.80, the sample size estimate was between nine and twelve subjects.

Statistical analysis for hormone evaluation was carried out using SPSS® for Windows, version 23.0. The Kolmogorov-Smirnov test for normality was employed to check whether the data followed a Gaussian distribution. As the normality was not verified for all results, different sets of parametric and non-parametric tests were used. To compare mean wool cortisol concentrations in the two different productive systems, the parametric unpaired and paired *t*-test and the non-parametric Mann-Whitney *U* test were performed. Statistical differences were considered significant at  $P < 0.05$ .

The sensor parameters were analysed separately: 'Activity', which indicates the amount of motion made by the animal, 'Rumination', which evaluates the number of minutes of rumination of the subject. These indicators were correlated with data relating to certain carcass characteristics, in particular: Drip Loss at 3, 7 and 14 days (DL d3%, DL d7%, DL d14%) and pH 24 h post-slaughter (pH 24 h). Data from the sensors, recorded within the time-frame of the 'transport' event, calculated from the time of loading and unloading in the pre-slaughter area, were processed according to the median for each animal and group. The correlation between these parameters and the characteristics of the meat was analysed using the two-tailed Pearson correlation test. The *P*-value and the correlation coefficient obtained were evaluated. RCommander software (a platform-independent basic-statistics GUI [graphical user interface] for R, based on the tcltk package, see <https://www.r-project.org>) was used for the execution of the two-tailed Pearson correlation test.



## Results

### Assessment of lambs' welfare

The results of the first level welfare assessment on farms showed, in general, a high degree of compliance with the needs of the animals (Tables 1 and 2) for Biellese and Sambucana lambs, respectively. In terms of productivity, both farms showed average offspring survival values of greater than one lamb per ewe. Neither stereotypy nor excessive itching were observed in any of the animals assessed in the farms — these indicators being characteristic in closely confined animals (EFSA Panel on Animal Health and Welfare [AHAW] 2014). Social withdrawal was also not observed in any of the farms. No panting behaviour was observed and very few animals had dirt in their fleeces. In fact, following the AWIN protocol recommendations, the second level welfare analysis was not considered necessary in any of the animals or farms. Laminitis, which is a common problem in dairy herds or adult animals, was not a problem in the farms evaluated. Likewise, water was always available in quantity and quality. The familiar human approach test yielded a flight distance of approximately 2 m, the highest score corresponding to the shepherded farm. It is worth mentioning that most of the animals eventually approached the assessing person. It should also be noted that almost half of the sheep were ruminating during the observation period, an activity that tends to be associated with animals feeling relaxed and free from anxiety). QBA relies on the ability of humans to integrate perceived details of behaviour into descriptors with emotional connotation that can be scaled and added to other quantitative indicators (Figure 1). The two breeding systems can be considered well-organised in terms of management of animal breeding. The data reported are specifically those individual animals monitored prior to slaughter however they are generally representative of the entire flock as a whole. QBA relies on the ability of humans to integrate perceived details of behaviour into descriptors with emotional connotation that can be scaled and added to other quantitative indicators. Following the AWIN protocol (AWIN 2015), we assessed the emotional state of animals by observing and scoring a series of 17 descriptors on a scale (see Tables 1 and 2 for Biellese and Sambucana breeds, respectively), which may have negative (aggressive, agitated, apathetic, defensive, fearful, frustrated, listless, physically uncomfortable, subdued, tense, wary) or positive (active, alert, assertive, bright, calm, content, inquisitive, relaxed, sociable, vigorous) connotations. We observed a convergent validity between QBA, behaviour and physiology has been previously demonstrated (Mialon *et al* 2021). Animals from both rearing systems seemed to express differing degrees of positive emotional state.

### Chronic cortisol detection

The mean wool cortisol concentration in both breeding systems ranged from 10.2 ( $\pm$  3.5) to 6.5 ( $\pm$  2.3) and from 9.2 ( $\pm$  4.0) to 5.9 ( $\pm$  2.4) pg mg<sup>-1</sup>, respectively, for in

pasture and in semi-extensive systems. Within each system the comparison between the four-monthly times of the wool collection showed high levels of cortisol in the first month of life (T1) with a constant decrease in values over successive monthly periods of wool collection (T2, T3, and T4) in both systems (Figure 2[c]). These absolute levels of cortisol are comparable with previous studies (Stubsj oen *et al* 2015, 2018). Age, in fact, is a factor that influences the levels of cortisol in the hair, as at birth it is higher and decreases with the passage of time, something that is also observed in other farm animals.

### Lamb production in the two systems

The production profile for the two breeds (Biellese and Sambucana) were kept fairly uniform at the slaughterhouse (ie birth weight, weight gain, live weight at slaughter, hot carcass weight, cold carcass weight, yield at slaughter to ensure homogeneity (Table 3). No other correlation was detected with the physical and chemical meat characteristic analysis carried out on the two breeds (data are summarised in Tables S4 and S5 in the Supplementary material online).

### Rumination and total activity monitored by sensors

The study found no significant impact of production system on the motion nor reduction in rumination as measured by the sensors (see Figure S3[a]–[d] in Supplementary material) and therefore focused on the latter part of the experiment, ie transport and lairage stage regarding stress factors impacting meat quality as we previously reported. Results showed the absence of any significant correlation between total activity and pH values at 24 h post-slaughter and similar results were also obtained for ruminal activity (data not shown). However, sensors recorded a significant correlation between total motor activity, ruminal activity and drip loss (DL) seven days after slaughter (Figure 4[a] and [b]). The correlation analysed according to Pearson two-tailed was  $P < 0.00009$ . The study also identified a significant correlation between the motor and ruminal activity recorded by the sensors and the drip loss value after 14 days of slaughter (Figure 2[a], [b]). The correlation analysed according to Pearson two-tailed was  $P < 0.00009$ . Analogous result with the drip loss values measured seven and 14 days after slaughter ( $P < 0.0002$  and  $P < 0.00001$ , respectively).

## Discussion

Bio-sensors may be used to improve meat production including testing for safety and quality. In this study we found lower levels of rumination and motor activity to be associated with increased drip loss at seven and 14 days, post slaughter. This not only indicates that meat would be of lower quality, incurring significant economic costs but also that animals with these lower levels may have lower health and fitness levels and/or cope less well with stressors experienced during transport, lairage and slaughter. Previous articles, such as Caja *et al* (2020), reviewed the potential for sensor technologies to identify changes in activity and rumination which may be useful in monitoring the welfare of the sheep.

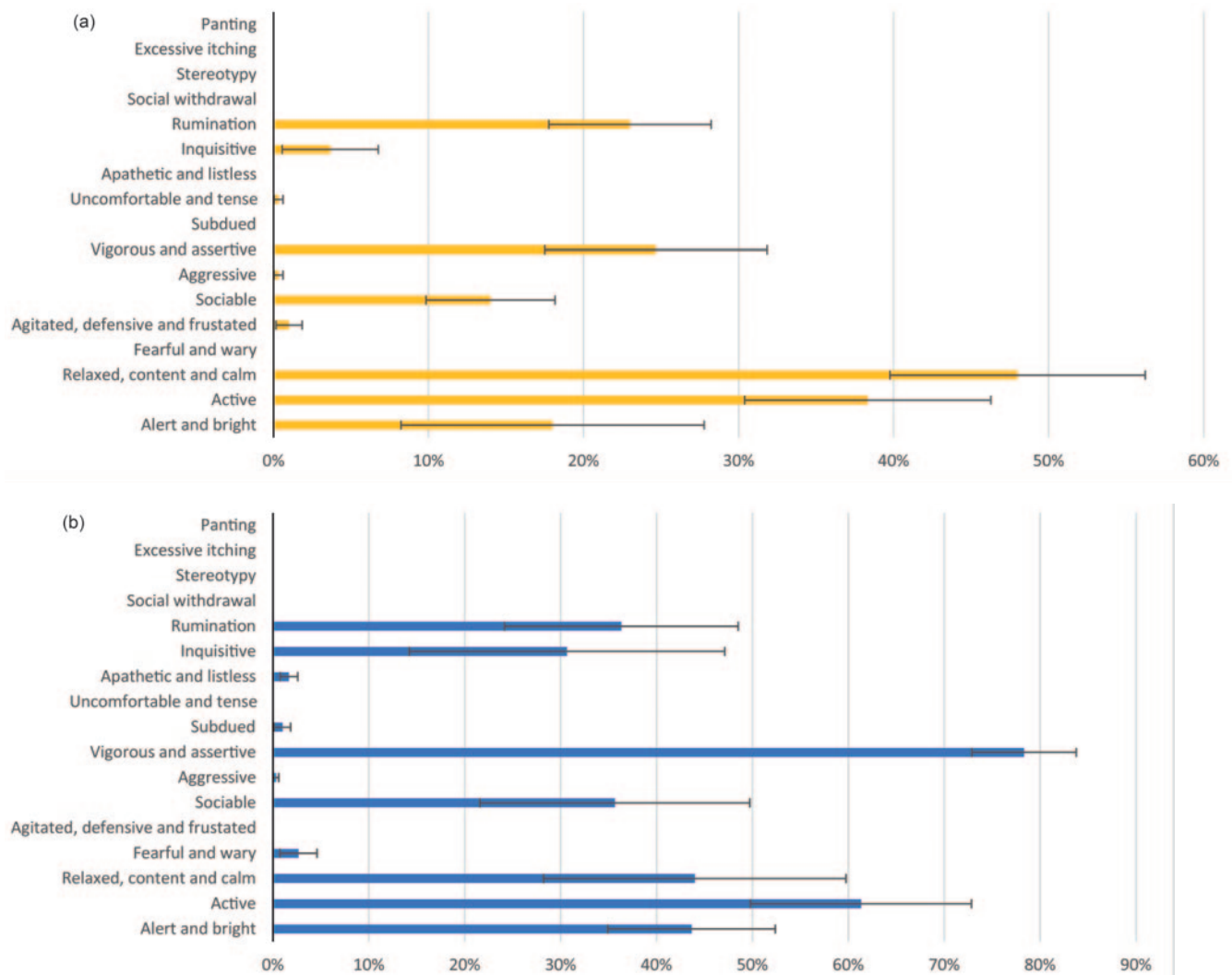
**Table 1** Welfare assessment of Biellese lambs during the entire breeding period. Indirect welfare indicators of Biellese lambs (n = 12) were individually evaluated monthly from 1 month of age to slaughter (parameters expressed as percentages).

Welfare assessment		1 month	2 month	3 month	4 month
Body Condition Score	Emaciated	0	0	0	0
	Thin	0	20	20	20
	Good	80	50	80	70
	Fat	20	30	0	10
Lesions to head/neck region	Presence	0	0	0	0
	Absence	100	100	100	100
Lesions to body and legs	Presence	0	0	0	0
	Absence	100	100	100	100
Ocular discharge	Presence	0	0	0	0
	Absence	100	100	100	100
Myiasis	Presence	0	0	0	0
	Absence	100	100	100	100
Mucosa score	Not anaemic	90	80	100	90
	Borderline anaemic	10	20	0	10
	Anaemic	0	0	0	0
	Severely anaemic	0	0	0	0
Respiratory problems	Presence	0	0	0	0
	Absence	100	100	100	100
Fleece cleanliness	Clean and dry	90	80	100	100
	Dry or damp, light soiling	10	20	0	0
	Wet, soiled with mud or faeces	0	0	0	0
	Very wet, heavily soiled	0	0	0	0
	Filthy	0	0	0	0
Fleece quality	Good	100	100	100	100
	Some loss	0	0	0	0
	Significant loss	0	0	0	0
Hoof overgrowth	Clean and dry	100	100	100	100
	Absence	0	0	0	0
Faecal soiling	None	90	100	70	60
	Very light soiling	10	0	30	40
	Light soiling and dags	0	0	0	0
	Soiling and dags	0	0	0	0
	Extensive soiling and dags	0	0	0	0
Tail length	Undocked	100	100	100	100
	Docked	0	0	0	0
	Short docked	0	0	0	0
Lameness	Not lame	100	100	100	100
	Minor lameness	0	0	0	0
	Lame	0	0	0	0
	Severely lame	0	0	0	0

**Table 2 Welfare assessment of Sambucana lambs during the entire breeding period. Indirect welfare indicators of Sambucana lambs (n = 12) were individually evaluated monthly from 1 month of age to slaughter (parameters expressed as percentages).**

Welfare assessment		1 month	2 month	3 month	4 month
Body Condition Score	Emaciated	0	0	0	0
	Thin	25	25	16.7	16.7
	Good	66.7	66.7	40	25
	Fat	16.7	8.3	33.3	58.3
Lesions to head/neck region	Presence	16.7	16.7	8.3	8.3
	Absence	83.3	83.3	91.7	91.7
Lesions to body and legs	Presence	0	8.3	8.3	0
	Absence	100	91.7	91.7	100
Ocular discharge	Presence	8.3	0	8.3	0
	Absence	91.7	100	91.7	100
Myiasis	Presence	0	16.7	0	8.3
	Absence	100	83.3	100	91.7
Mucosa score	Not anaemic	100	100	91.7	100
	Borderline anaemic	0	0	8.3	0
	Anaemic	0	0	0	0
	Severely anaemic	0	0	0	0
Respiratory problems	Presence	0	0	0	0
	Absence	100	100	100	100
Fleece cleanliness	Clean and dry	100	100	100	100
	Dry or damp, light soiling	0	0	0	0
	Wet, soiled with mud or faeces	0	0	0	0
	Very wet, heavily soiled	0	0	0	0
	Filthy	0	0	0	0
Fleece quality	Good	100	100	100	100
	Some loss	0	0	0	0
	Significant loss	0	0	0	0
Hoof overgrowth	Clean and dry	100	100	100	100
	Absence	0	0	0	0
Faecal soiling	None	100	83.3	100	100
	Very light soiling	0	16.7	0	0
	Light soiling and dags	0	0	0	0
	Soiling and dags	0	0	0	0
	Extensive soiling and dags	0	0	0	0
Tail length	Undocked	100	100	100	100
	Docked	0	0	0	0
	Short docked	0	0	0	0
Lameness	Not lame	100	100	100	100
	Minor lameness	0	0	0	0
	Lame	0	0	0	0
	Severely lame	0	0	0	0

Figure 1

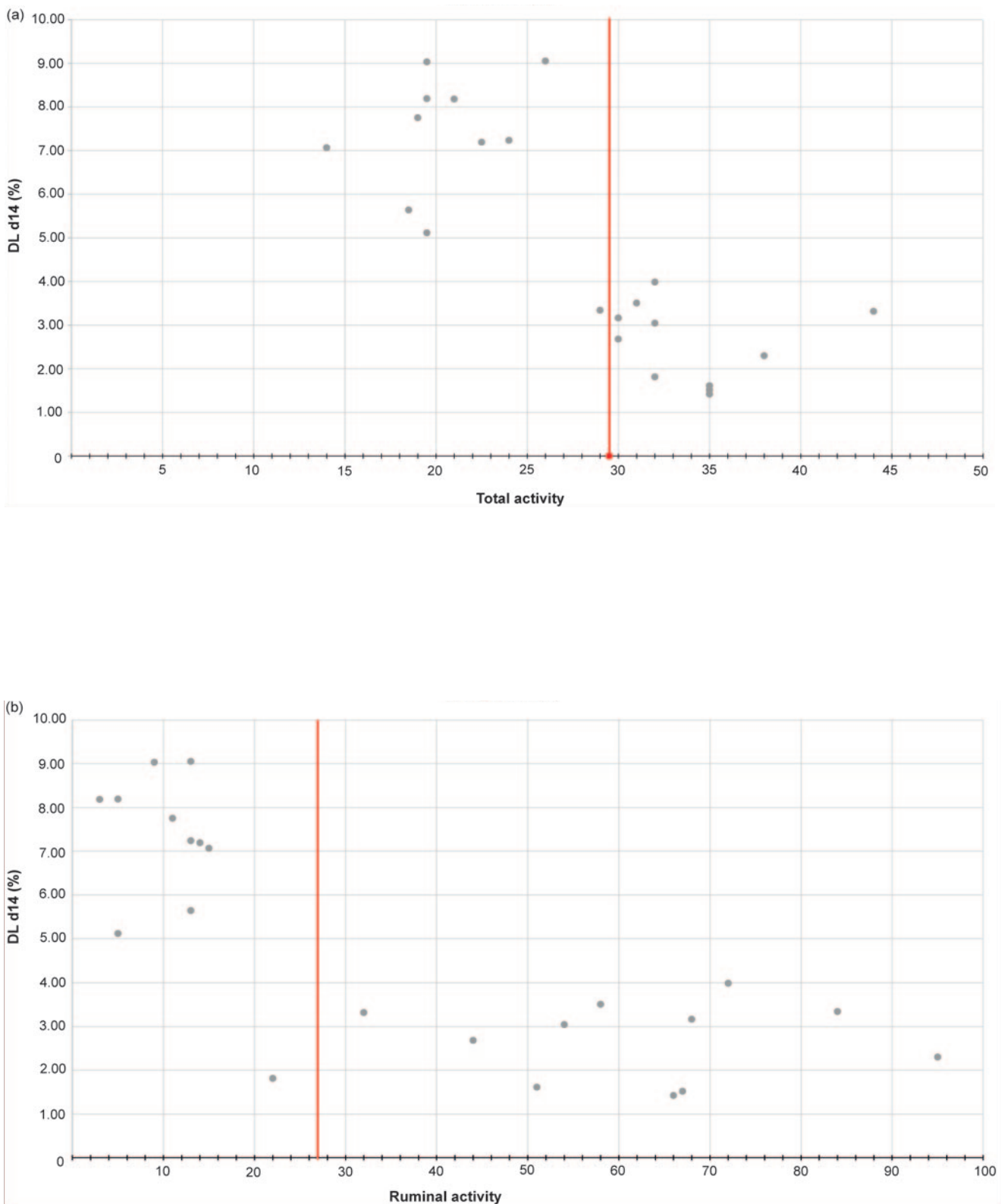


Qualitative Behaviour Assessment (QBA) of (a) Biellese and (b) Sambucana breeds. Behavioural expression patterns of the flocks were recorded in accordance with the QBA of AWIN Welfare Assessment Protocol for Sheep. One hundred randomised animals were observed for 1 h each session six times during the entire breeding period. All parameters are reported as percentages ( $\pm$  SEM).

As we reported, our observations in the welfare assessment of both breeding systems did not report major and persistent changes in animal welfare. The assessment of animal welfare in these farms supports the notion that lambs were reared under conditions that more than fulfil the requirements for adequate welfare, providing good feeding, good housing, good health and appropriate behaviour. In fact, following the AWIN protocol recommendations, the second level welfare analysis was not considered necessary during the entire rearing period. However, our assessment during the final hours of the animals' lives has improved the effective monitoring and identification of systemic welfare failures and the active enhancement of opportunities for positive welfare experiences. We think that PLF technology would also be useful in correctly addressing specific and persistent welfare problems, especially those not detectable by clinical observation. Rumination and total activity may

indicate that welfare has perhaps been compromised since immobilisation can occur in response to environmental stressors as previously shown (Caja *et al* 2020). That said, it may also merely reflect docility and an absence of fear (Romyer & Boissou 1992). Since neither supply chain showed evidence of stress at a hormonal (cortisol) or clinical level during rearing, we propose that animals perceived the transport and the pre-slaughter phase differently. Indeed, both systems (analysed for four months) showed animals to generally be able to adapt to their surroundings, showing a progressive reduction in accumulation of cortisol, an indication that the supply chain in the grazing and semi-extensive conditions managed to maintain a positive sense of well-being (Geß *et al* 2020). A degree of deterioration in the sense of well-being has been associated with the hours immediately preceding slaughter. As animal responses were analysed individually it was possible to distinguish total

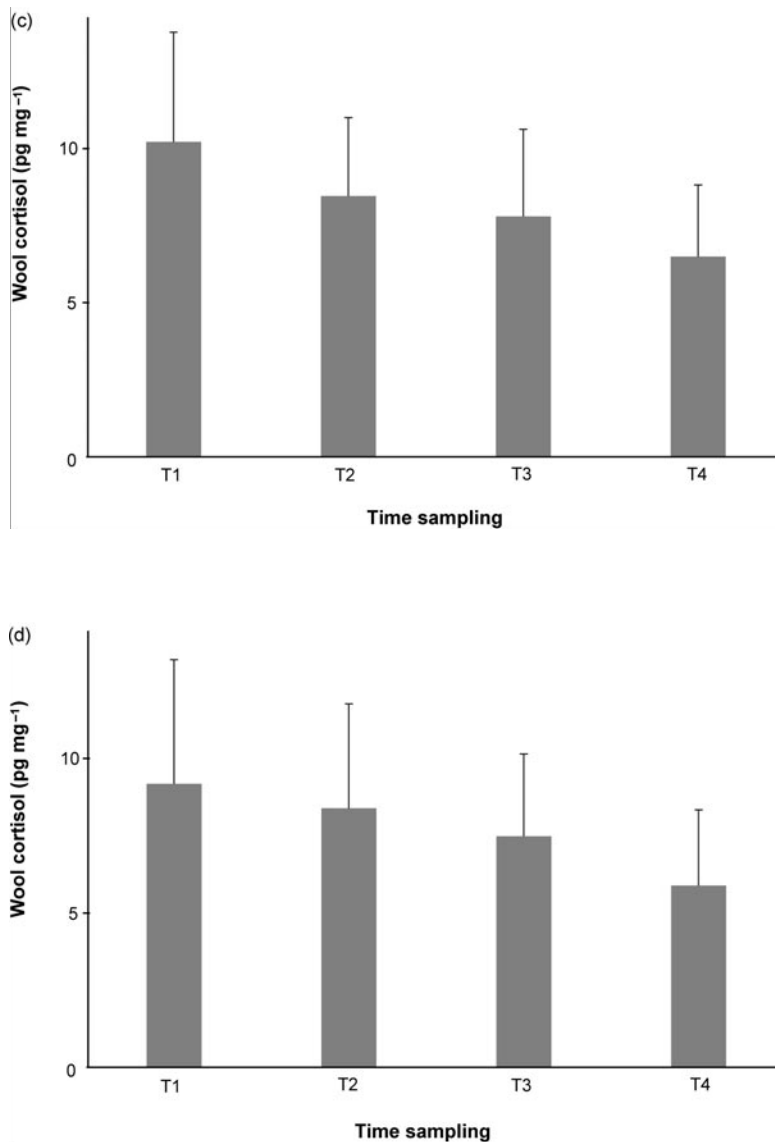
Figure 2



Mean drip loss levels of 12 Sambucana and ten Biellese lamb carcasses 14 days post-slaughter at various levels of (a) total activity and (b) ruminal activity recorded by the sensors from during the transport period from farm to point of slaughter until the completed unloading period. The vertical red line indicates the median. The correlation analysed according to Pearson's two-tailed is (a)  $-0.7862837$  with  $P < 0.00009$  and (b)  $0.7896369$  with  $P < 0.00001$  while (c) represents mean levels of wool cortisol concentration ( $\text{pg mg}^{-1}$ ) measured in a group of 12 lambs of Sambucana and (d) a group of ten lambs of Biellese breeds sampled monthly from 1 to 4 months of age. Sampling: T1 = first month of life; T2 = second month of life; T3 = third month of life; T4 = fourth month.



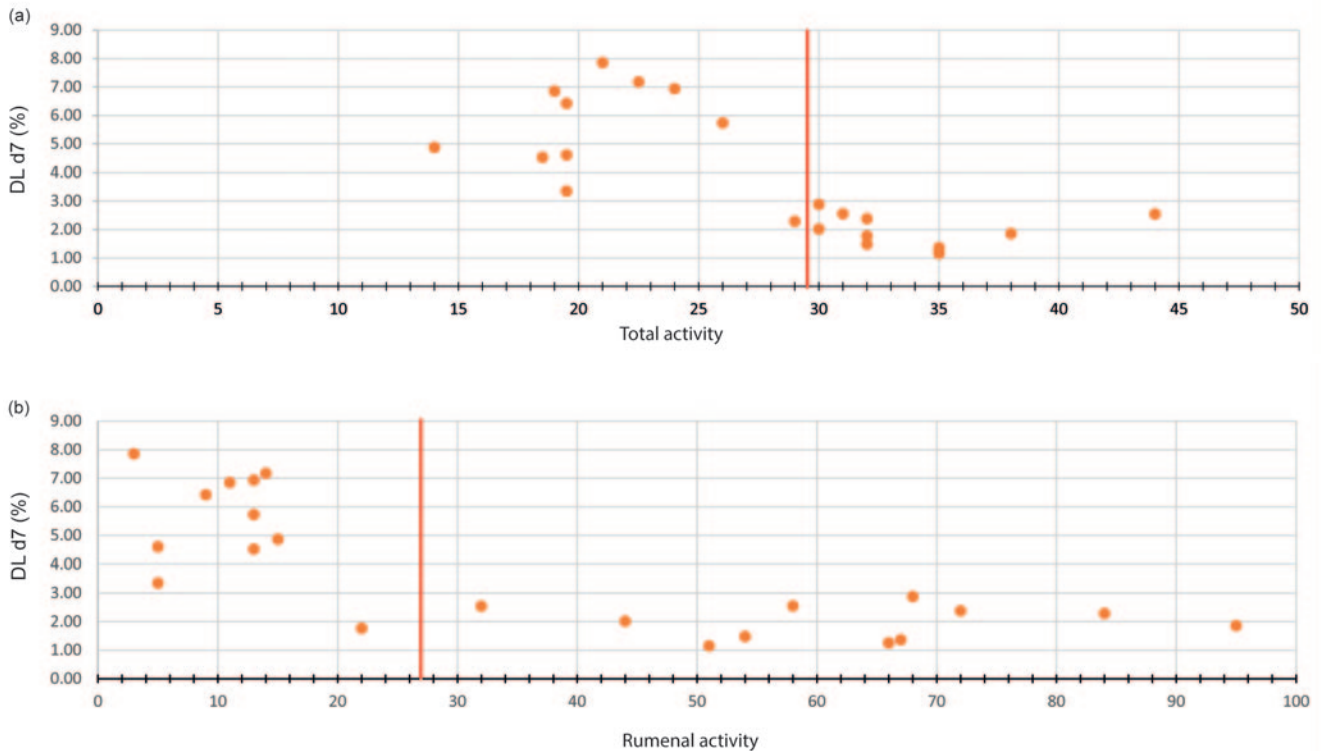
Figure 2 (cont)

Table 3 Mean ( $\pm$  SD) production profile of animals at the slaughterhouse.

Breed	Age of the lambs at slaughter (days)	BW (kg)	WG (kg)	LWS (kg)	HCW (kg)	CCW (kg)	YS (%)
Biellese	123.50 ( $\pm$ 13.4)	4.83 ( $\pm$ 13.4)	0.23 ( $\pm$ 0.02)	33.50 ( $\pm$ 1.41)	17.30 ( $\pm$ 0.42)	16.90	50.47 ( $\pm$ 0.86)
Sambucana	135.50 ( $\pm$ 9.2)	6.10 ( $\pm$ 9.2)	0.16 ( $\pm$ 0.04)	27.55 ( $\pm$ 4.60)	15.25 ( $\pm$ 3.18)	14.75	53.32 ( $\pm$ 2.65)

BW = birth weight;  
 WG = weight gain;  
 LWS = live weight at slaughter;  
 HCW = hot carcass weight;  
 CCW = cold carcass weight;  
 YS = yield at slaughter.

Figure 4



Mean drip loss levels of 12 Sambucana and ten Biellese lamb carcasses seven days post-slaughter at various levels of the (a) total motor activity and (b) ruminal activity recorded by the motion sensors during the transport period from farm to point of slaughter. The vertical red line indicates the median. The correlation analysed according to Pearson two-tailed is (a)  $-0.7361697$  with  $P < 0.00009$  and (b)  $0.7517323$  with  $P < 0.00005$ .

activity and rumination responses according to typology quite effectively, which could be useful for further studies. Other reports have shown that biosensors can be used effectively for drip loss analysis and glycolytic potential evaluation; by employing glucose and lactate measured in drip loss as potential markers of muscle glycolytic potential and meat quality traits (Przybylski *et al* 2016). For pigs, it has been shown that there is a link between fast-glycolysing muscles or muscles rich in fast-twitch fibres and rapid glycogen metabolism, post mortem, as well as a rapid pH fall, resulting in higher drip loss from the meat (Mephram 2000). Whilst our research did not detect any such relation with pH, we did however see a significant correlation between ruminal activity, total motor activity and drip loss.

The lack of correlation with pH change could be related to the sampling times, having data available at 24 h or possible slight variations were not identified by the number of observations available. However, these experiences may have been detected in real time by accelerometers and then correlated individually to quality parameters of the meat. Excessive drip loss linked to different handling conditions of animals prior to slaughter significantly affects fresh meat parameters that signify not only financial losses associated with such meats but losses in valuable vitamins, minerals, flavour compounds and water in different species (Danso *et al* 2017; Carrasco-García *et al* 2020; Składanowska-Baryza *et al* 2020; Zheng *et al* 2020).

### Animal welfare implications

The use of biosensors measuring activity and rumination have the potential to help monitor and improve animal welfare. They may be adopted for monitoring the quality of transport and handling pre-slaughter and could allow for the prediction of certain meat quality parameters.

### Conclusion

Real-time individual sensors allowed exploration of the possible connection between levels of total activity and rumination (used as potential indicators of deterioration of animal welfare in sheep during transport and pre-slaughter) and a wide range of meat quality indicators. Differences in two production systems were demonstrated as was a significant correlation with drip loss. Animals underwent veterinary inspection during the breeding period through until slaughter without any health problems being detected; but sensors were able to determine varying levels of activity and rumination during both transports.

Statistically significant direct correlations were found between both activity and rumination levels and drip loss at days seven and 14. Lower activity and rumination levels were associated with greater drip loss indicating lower meat quality.

### Declaration of interest

None.

## Acknowledgements

The authors are grateful to the EU ERA-NET programme and Italian Ministry of Agriculture, MIPAAF for funding the project EcoLamb–Holistic Production to Reduce the Ecological Footprint of Meat (SusAn/0002/2016) and CRC Foundation to grant Smartsheep project. This work has benefited from the equipment and framework of the COMP-HUB Initiative (UniPR) and of the Department Veterinary Science (UNITO), funded by the ‘Departments of Excellence’ programme of the Italian Ministry for Education, University and Research (MIUR, 2018-2022).

## References

- Alonso ME, González-Montaña JR and Lomillos JM** 2020 Consumers' concerns and perceptions of farm animal welfare. *Animals* 10: 385-391. <https://doi.org/10.3390/ani10030385>
- AWIN** 2015 AWIN Welfare Assessment Protocol for Sheep. [https://doi.org/10.13130/AWIN\\_sheep\\_2015](https://doi.org/10.13130/AWIN_sheep_2015)
- Bodas R, García-García JJ, Montanes M, Benito A, Peric T, Baratta M, Viola I, Geß A, Ko N, Cadavez V, Gonzales-Barron U, Domínguez E and Olmedo S** 2021 On farm welfare assessment of European fattening lambs. *Small Ruminant Research* 204: 106533. <https://doi.org/10.1016/j.smallrumres.2021.106533>
- Bramlet VD, Judge MD and Vail GE** 1963 Stress during growth 2. Effect on palatability and cooking characteristics of lamb meat. *Journal of Animal Science* 22: 1064-1067. <https://doi.org/10.2527/jas1963.2241064x>
- Broom DM** 2005 The effect of land transport on animal welfare. *Revue scientifique et technique-Office international des epizooties* 24: 683-687
- Caja G, Castro-Costa A and Knight CH** 2016 Engineering to support wellbeing of dairy animals. *Journal of Dairy Research* 83(2): 136-147. <https://doi.org/10.1017/S0022029916000261>
- Caja G, Castro-Costa A, Salama AAK, Oliver J, Baratta M, Ferrer C and Knight CH** 2020 Sensing solutions for improving the performance, health and wellbeing of small ruminants. *Journal of Dairy Research* 87(S1): 34-46. <https://doi.org/10.1017/s0022029920000667>
- Carrasco-García AA, Pardío-Sedas VT, León-Banda GG, Ahuja-Aguirre C, Paredes Ramos P, Hernández-Cruz BC and Murillo VV** 2020 Effect of stress during slaughter on carcass characteristics and meat quality in tropical beef cattle. *Asian-Australasian Journal of Animal Science* 33(10): 1656-1665. <https://doi.org/10.5713/ajas.19.0804>
- Cockram MS** 2004 A review of behavioural and physiological responses of sheep to stressors to identify potential behavioural signs of distress. *Animal Welfare* 13: 283-291
- Cockram MS** 2019 Sheep transport. In: Grandin (ed) *Livestock Handling and Transport, Third Edition*. CABI International: Wallingford, UK
- Danso AS, Richardson RI and Khalid R** 2017 Assessment of the meat quality of lamb *M. longissimus thoracis et lumborum* and *M. triceps brachii* following three different Halal slaughter procedures. *Meat Science* 127: 6-12. <https://doi.org/10.1016/j.meatsci.2016.12.014>
- EFSA Panel on Animal Health and Welfare (AHAW)** 2014 Scientific Opinion on the welfare risks related to the farming of sheep for wool, meat and milk production. *EFSA Journal* 12: 3933. <https://doi.org/10.2903/j.efsa.2014.3933>
- EU** 2017 Consortium of the Animal Transport Guides Project 2017. Guide to good practices for the transport of sheep. <http://animaltransportguides.eu/wp-content/uploads/2017/03/Animal-Transport-Guides-Sheep-2017-2.pdf>
- EU Council Regulation No 1099/2005** 2005a The protection of animals at time of killing. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L2009:303:0001:0030:EN:PDF>
- EU Council Regulation No 1/2005** 2005b The protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97. EU: Brussels, Belgium
- Forrest JC, Morgan MT, Borgard C, Rasmussen AJ, Jespersen BL and Andersen JR** 2000 Development of technology for the early post mortem prediction of water holding capacity and drip loss in fresh pork. *Meat Science* 55: 115-122. [https://doi.org/10.1016/S03091740\(99\)00133-3](https://doi.org/10.1016/S03091740(99)00133-3)
- Geß A, Viola I, Miretti S, Macchi E, Perona G, Battaglini L and Baratta M** 2020 A new approach to LCA evaluation of lamb meat production in two different breeding systems in Northern Italy. *Frontiers in Veterinary Science* 7: 651. <https://doi.org/10.3389/fvets.2020.00651>
- Gonzales-Barron U, Popova T, Bermúdez Piedra R, Tolsdorf A, Geß A, Pateiro M, Chiesa F, Brugiapaglia A, Viola I, Battaglini L, Baratta M, Lorenzo JM and Cadavez V** 2021 Fatty acid composition of lamb meat from Italian and German local breeds. *Small Ruminant Research* 200: 106384. <https://doi.org/10.1016/j.smallrumres.2021.106384>
- Halachmi I, Guarino M, Bewley J and Pastell** 2019 Smart animal agriculture: Application of real-time sensors to improve animal well-being and production. *Annual Review of Animal Biosciences* 7: 403-425. <https://doi.org/10.1146/annurev-animal-020518-114851>
- Hemsworth PH, Rice M, Borg S, Edwards LE, Ponnampalam EN and Coleman GJ** 2019 Relationships between handling, behaviour and stress in lambs at abattoirs. *Animal* 13: 1287-1296. <https://doi.org/10.1017/S1751731118002744>
- Honikel KO** 1998 Reference methods for the assessment of physical characteristics of meat. *Meat Science* 49: 447-457. [https://doi.org/10.1016/S0309-1740\(98\)00034-5](https://doi.org/10.1016/S0309-1740(98)00034-5)
- Knowles TG** 1995 Effect of sheep transport by road for up to 24 hours. *Veterinary Records* 136: 431-438. <https://doi.org/10.1136/vr.136.17.431>
- Knowles TG** 1998 A review of the road transport of slaughter sheep. *Veterinary Record* 143: 212-219. <https://doi.org/10.1136/vr.143.8.212>
- Krueger A, Cruickshank J, Trevisi E and Bionaz M** 2020 Systems for evaluation of welfare on dairy farms. *Journal of Dairy Research* 87(S1): 13-19. <https://doi.org/10.1017/s0022029920000461>
- Llonch P, King EM, Clarke KA and Downes JM** 2015 A systematic review of animal-based indicators of sheep welfare on farm, at market and during transport, and qualitative appraisal of their validity and feasibility for use in UK abattoirs. *The Veterinary Journal* 206: 289-297. <http://dx.doi.org/10.1016/j.tvjl.2015.10.019>
- Maltz E** 2020 Individual dairy cow management: Achievements, obstacles and prospects. *Journal of Dairy Research* 87(S1) 2: 145-157. <https://doi.org/10.1017/S0022029920000382>

- Maroto-Molina F, Navarro-García J, Príncipe-Aguirre K, Gómez-Maqueda I, Guerrero Ginel JE, Garrido-Varo A and Pérez-Marín DC** 2019 A low-cost IOT-based system to monitor the location of a whole herd. *Sensors* 19(10): 2298-2313. <https://doi.org/10.3390/s19102298>
- Mepham B** 2000 Animal welfare and meat science. *Meat Science* 56: 101. [https://doi.org/10.1016/s0309-1740\(00\)00009-7](https://doi.org/10.1016/s0309-1740(00)00009-7)
- Messouri S, Sossidou E, Buonanno M, Mounaix B, Barnard S, Vousdouka V, Dalla Villa P, de Roest K and Spooler H** 2015 A pilot study to develop an assessment tool for sheep welfare after long journey transport. *Animal Welfare* 24: 407-416. <https://doi.org/10.7120/09627286.24.4.407>
- Mialon MM, Boivin X, Durand D, Boissy A, Delval E, Bage AS, Clanet C, Cornilleau F, Parias C, Foury A, Moisan MP, Fassier T, Marcon D, Guilloteau LA and Nowak R** 2021 Short- and mid-term effects on performance, health and qualitative behavioural assessment of Romane lambs in different milk feeding conditions. *Animal*: 100157. <https://doi.org/10.1016/j.animal.2020.100157>
- Morgan-Davies C, Lambe N, Wishart H, Waterhouse T, Kenyon F, McBean D and McCracken D** 2018 Impacts of using a precision livestock system targeted approach in mountain sheep flocks. *Livestock Science* 208: 67-76. <https://doi.org/10.1016/j.livsci.2017.12.002>
- Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lalic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen O, Rawle F, Peynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T and Wurbel H** 2020 The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol.* <https://doi.org/10.1371/journal.pbio.3000410>
- Przybylski W, Sionek B, Jaworska D and Santé-Lhoutellier V** 2016 The application of biosensors for drip loss analysis and glycolytic potential evaluation. *Meat Science* 117: 7-11. <https://doi.org/10.1016/j.meatsci.2016.02.025>
- Romyer A and Bouissou MF** 1992 Assessment of fear reactions in domestic sheep and influence of breed and rearing conditions. *Applied Animal Behaviour Science* 34: 93-119. [https://doi.org/10.1016/S0168-1591\(05\)80060-7](https://doi.org/10.1016/S0168-1591(05)80060-7)
- Rutter SM** 2017 Advanced livestock management solutions. *Advances in Sheep Welfare* 13: 245-261. <https://doi.org/10.1016/B978-0-08-100718-1.00013-3>
- Schilling J, Bennet R and Rose CR** 2021 Exploring the potential of precision livestock farming technologies to help address farm animal welfare. *Frontiers of Animal Science* 2: 639678. <https://doi.org/10.3389/fanim.2021.639678>
- Sionek B, Przybylski W and Tambor K** 2020 Biosensors in evaluation of quality of meat and meat products-a review. *Annals of Animal Science* 20: 1151-1168 <https://doi.org/10.2478/aoas-20200057>
- Składanowska-Baryza J, Ludwiczak A, Pruszyńska-Oszmałek E, Kołodziejski P and Stanisław M** 2020 Effect of two different stunning methods on the quality traits of rabbit meat. *Animals* 10: 700-714. <https://doi.org/10.3390/ani10040700>
- Stubsjøen SM, Bohlin J, Dahl E, Knappe-Poindecker M, Fjeldaas and Lepsch M** 2015 Assessment of chronic stress in sheep (part i): the use of cortisol and cortisone in hair as non-invasive biological markers. *Small Ruminant Research* 132: 25-31. <https://doi.org/10.1016/j.smallrumres.2015.09.015>
- Stubsjøen SM, Sørheim K, Chincarini M, Bohlin J, Brunberg E and Fuchs B** 2018 Exploring hair cortisone concentration as a novel tool to assess chronic stress in sheep with tick-borne fever. *Small Ruminant Research* 164: 110-119. <https://doi.org/10.1016/j.smallrumres.2018.05.009>
- Sutherland MA, Worth GM, Stuart AD, Dobbie PM and Clerens S** 2016 Effect of pre-slaughter handling, exercise and the presence of a dog on lamb welfare and meat quality. *Animal* 10: 1360-1367. <https://doi.org/10.1017/S1751731116000197>
- Walshe BE, Sheehan EM, Delahunty CM, Morrissey PA and Kerry JP** 2006 Composition, sensory and shelf life stability analyses of *Longissimus dorsi* muscle from steers reared under organic and conventional production systems. *Meat Science* 73: 319-325. <https://doi.org/10.1016/j.meatsci.2005.12.008>
- Zheng A, Lin S, Pirzado SA, Chen Z, Chang W, Cai H and Liu G** 2020 Stress associated with simulated transport, changes serum biochemistry, postmortem muscle metabolism, and meat quality of broilers. *Animals* 10: 1-12. <https://doi.org/10.3390/ani10081442>