

## HIERARCHICAL CLUSTERING AS A TOOL TO DEVELOP A CLASSIFICATION SCHEME FOR RABBIT MEAT QUALITY

LARIVIÈRE-LAJOIE A.-S.\*†, CINQ-MARS D.<sup>ib\*</sup>, GUAY F.<sup>ib\*</sup>, BINGGELI S.<sup>ib\*</sup>, DALMAU A.<sup>ib‡</sup>, SAUCIER L.<sup>ib\*†</sup>

\*Department of Animal Science, Faculty of Agriculture and Food Science, Université Laval, QUEBEC CITY, Quebec, Canada, G1V 0A6.

†Institute of Nutrition and Functional Foods, Université Laval, QUEBEC CITY, Quebec, Canada, G1V 0A6.

‡Institute of Agrifood Research and Technology (IRTA), MONELLS, 17121, Girona, Spain.

**Abstract:** This study aimed to characterise the quality of meat from commercially-raised rabbits. Animals came from five different producers and were slaughtered in three different plants under provincial or federal inspection jurisdiction. Animal behaviour evaluated by scan sampling prior to feed withdrawal (FW) and transport, as well as blood lactate concentration at exsanguination, did not raise concerns with respect to stress. Stomach pH was higher ( $P=0.047$ ) when the FW time was short ( $\leq 13.5$  h), at a mean value of 2.23. All pH values measured 1 h *post-mortem* from the *Biceps femoris* (BF) and almost all (97.6%) from the *Longissimus lumborum* (LL) were higher than 6. Values for ultimate pH measured 24 h *post-mortem* ( $pH_u$ ) ranged from 5.80 to 6.83 and from 5.70 to 6.70 for BF and LL muscles, respectively. The maximum meat drip loss recorded was 2.6%, while cooking loss reached 30%. Meat lightness ( $L^*$ ) and colour intensity ( $C^*$ ) for the long FW times ( $\geq 23$  h) were no different from those with short and intermediate (15.5 to 17.3 h) FW times. However, these colour parameters were higher for the short FW time class compared to the intermediate FW time class ( $P<0.02$ ). A hierarchical cluster analysis based on  $pH_u$ , cooking loss and lightness ( $L^*$ ) from 200 rabbit loins was performed. Of the four clusters created, clusters 1 and 2 had the best and second-best meat quality, respectively. Clusters 3 and 4 had the lowest meat quality and presented DFD-like (dark, firm and dry) characteristics. Meat did not exhibit PSE-like (pale, soft, exudative) characteristics, even for the slaughter lot with the minimum mean  $pH_u$ . Of the eight slaughter lots evaluated, more than 50% of the meat from three of them fell into clusters 3 and 4; all three were in the intermediate FW time class. Overall, the quality of rabbit meat analysed was acceptable for commercial use, but rather variable. This suggests that there are factors within the value chain that are not yet fully controlled and require further investigation.

**Key Words:** cooking loss, DFD meat, feed withdrawal, hierarchical cluster analysis, pre-slaughter management, rabbit.

## INTRODUCTION

According to the FAO (2017), global production of rabbit meat worldwide was about 1.4 million tonnes and came principally from Asia (75.3%), followed by Europe (21.3%), Africa (7.1%) and America (1.2%). Of the 1.2% produced in America, Canada and the US produced 1.03%. In 2016, the provinces of Ontario and Quebec were responsible for 55.32% and 35.49% of Canadian rabbit meat production, as 33.47% and 18.99% of the rabbit farms are located in these provinces, respectively (AAFC, 2019). Since 2011, rabbit production has been relatively stable in Quebec, partly because it is considered to be a specialty meat and is often associated with holiday celebrations. In 2014, consumption was relatively low at 0.0254 kg per capita in Canada (AAFC, 2019) and 0.040 kg in the Quebec province (MAPAQ, 2015). However, from 2010 to 2014, rabbit meat consumption increased to 3% per year in Quebec alone (MAPAQ, 2015). Although the agri-food activity of rabbit production is marginal in Quebec and in Canada in general, it

**Correspondence:** L. Saucier, [linda.saucier@fsaa.ulaval.ca](mailto:linda.saucier@fsaa.ulaval.ca). Received September 2020 - Accepted April 2021.

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contributes to the diversity of the food supply and its development can only be achieved if farmers produce adequate-quality meat.

Meat quality defects can cause economic losses for both producers and processors, and may cause a reduction in meat shelf life (Faucitano *et al.*, 2010; Adzitey and Nurul, 2011). Animals that are exposed to acute or chronic stress just before slaughter can yield either pale, soft and exudative (PSE) or dark, firm and dry (DFD) meat, respectively, which are two well-known meat quality defects (Adzitey and Nurul, 2011). The incidence of PSE meat reduces processing yield, and the high microbial spoilage associated with DFD meat decreases its shelf life (Faucitano *et al.*, 2010; Saucier, 2016; Ponnampalam *et al.*, 2017). Furthermore, because two of the most important variables driving consumer choice are the colour and consistency of raw meat (Dalle Zotte, 2002), PSE and DFD raw meats have lower value compared to normal meats due to their unattractive appearance (Viljoen *et al.*, 2002).

Meat quality is influenced by many factors including pre-slaughter management, such as feed withdrawal (FW), transport and lairage time. Pre-slaughter FW is important for reducing transport-related sickness, incidence of downers and death, as well as microbial contamination during transport (Martín-Peláez *et al.*, 2008). Furthermore, FW reduces the volume of the gastrointestinal tract, in particular the stomach, which also reduces puncture risk during evisceration (Dalle Zotte, 2002). However, previous studies have established that extended FW could result in a reduction in live body weight (Bianchi *et al.*, 2008; Frobose *et al.*, 2014). Within the first hours of FW, stomach weight reduction is observed, while a prolonged FW can cause degradation of body tissues, loss of nutrients and humidity, leading in turn to quality and yield losses (Bianchi *et al.*, 2008). An extended FW period is ascribed to poor animal welfare conditions as expressed by an increase in aggressivity, with longer and more intense fighting (Faucitano *et al.*, 2006), and can reduce the levels of muscular glycogen reserves, which can lead to undesirable meat with a high pH (Faucitano *et al.*, 2006; Verga *et al.*, 2009). Furthermore, it has been reported that longer transport journeys increase bruising and mortality (Petracci *et al.*, 2008).

Pork is classified into five quality categories based on the ultimate pH ( $\text{pH}_u$ ), colour and meat drip loss: PSE, PFN (pale, firm, non-exudative), RSE (reddish-pink, firm, exudative), RFN (reddish-pink, firm, non-exudative; normal pork) and DFD (Faucitano *et al.*, 2010). For beef, the classification method considers carcass yield and texture, but also colour in order to identify DFD meat (Polkinghorne and Thompson, 2010; Ponnampalam *et al.*, 2017). Traditionally, PSE meat was associated with pigs and DFD meat with all species (Adzitey and Nurul, 2011). However, PSE-like meat has now been identified in turkey, chicken and cattle (Adzitey and Nurul, 2011). DFD-like meats have been reported in the literature for rabbits (Jolley, 1990; Koné *et al.*, 2016; Składanowska-Baryza *et al.*, 2018), whereas PSE-like meat has not (Cavani *et al.*, 2009; Blasco *et al.*, 2018).

To our knowledge, no formal and specific meat quality classification has ever been defined for rabbit meat. Despite the implementation of a code of good practices (RMAAQ, 2019), meat quality may vary. Thus, the aim of this study was to characterise meat quality from rabbits commercially raised in Quebec and slaughtered in facilities under federal or provincial jurisdiction. Using a hierarchical cluster analysis based on  $\text{pH}_u$ , cooking loss and lightness ( $L^*$ ), a classification of rabbit meat quality is proposed.

## MATERIALS AND METHODS

All experimental procedures involving live rabbits were approved by Université Laval's Animal Use and Care Committee, which strictly adheres to the Guidelines of the Canadian Council on Animal Care (CCAC, 2009). Rabbits were commercially raised and analyses were performed from January 2018 to August 2019.

### ***Producer selection***

Five rabbit producers were selected in collaboration with the *Syndicat des producteurs de lapins du Québec* to represent the vast majority of procedures in operation within the province of Quebec. Rabbits were slaughtered in three different abattoirs located in either Ontario or Quebec. They operated under provincial or federal inspection. The pre-slaughter management practices are presented in Table 1 for each slaughter lot.

Table 1: Pre-slaughter management according to the producer and the season<sup>1</sup>.

Slaughter lot designation	Producers	Inspection jurisdiction <sup>2</sup>	Season	Rabbits per lot	Feed withdrawal time at the farm (h)	Transport time (h)	Lairage time (h)	Total feed withdrawal time (h) <sup>3</sup>
A-P1-W	A	Provincial 1	Winter	270	6.66	0.17	1.67	8.5
B-F1-W	B	Federal 1	Winter	450	2.5	5	19	26.5
C-F1-W	C	Federal 1	Winter	900	10	5	14	29.0
D-P1-W	D	Provincial 1	Winter	747	9.67	2.25	3.58	15.5
D-P1-S	D	Provincial 1	Summer	800	9.75	2	1.75	13.5
D-P2-S	D	Provincial 2	Summer	805	12.75	2.25	1	16.0
D-F2-S	D	Federal 2	Summer	760	12.75	2.25	2.33	17.3
E-F1-S	E	Federal 1	Summer	320	0	7	16	23.0

<sup>1</sup>Pre-slaughter management varies according to which abattoir the rabbits were delivered.

<sup>2</sup>Indicates the slaughterhouse inspection jurisdiction under which rabbits were slaughtered. Provincial 1 and 2 were located in Quebec; Federal 1 was located in Ontario; Federal 2 is the same slaughterhouse as Provincial 2, but after it received federal accreditation.

<sup>3</sup>Total feed withdrawal time includes all time segments: feed withdrawal time while at the farm, during transport and in lairage at the abattoir.

### Behavioural observations

For each slaughter lot (n=8), animal behaviour was evaluated by visual scan sampling at one minute intervals for a total of 10 min using an observation grid on 10% of the total cages prior to FW. The number of rabbits sitting, lying down or moving was recorded. Observations also included occurrence and types of activities and interactions. Aggressive behaviours relate to chasing and triggering escape, leaping, biting another rabbit, bouncing and paw scraping. All behavioural assessments were performed by the same observer.

### Physiological measures

For each lot slaughtered in the abattoirs located in Quebec, 25 rabbits were randomly selected from the dressing line. Blood samples were collected at exsanguination to measure blood lactate level, in duplicate, using a hand-held lactate analyser (Lactate scout +, EKF Diagnostics, Cardiff, Wales, UK) according to the manufacturer's specifications.

Full gastrointestinal tracts (GIT) were promptly removed and weighed after slaughter. Stomachs and caeca were tied at both ends, removed and weighed when full and then again when empty. The pH of the caecum and stomach contents was measured using a portable pH meter (ROSS, Orion Star A221, Thermo Scientific, Beverly, CA, USA) combined with an Orion Kniphe electrode (ThermoFisher, Nepean, ON, Canada) and a temperature compensation probe (928,007 MD, micro probes ATC, Maryland, USA). Stomach and caecum contents were weighed and kept at -20°C until the percentage of dry matter (DM) was evaluated. DM was calculated after lyophilisation (model 50L Virtual EL-85, VirTis, Los Angeles, CA, USA) at 20°C for 3 d. The water content was determined by calculating the weight difference between the wet and dry contents (Saucier *et al.*, 2007).

### Meat quality measurement

To determine the meat quality, the rabbit carcasses were analysed according to Koné *et al.* (2019). Muscular pH of the *Longissimus lumborum* (LL) and the *Biceps femoris* (BF) muscles were measured 1 h (pH1h) *post-mortem* for animals slaughtered in the abattoirs located in Quebec (since we had access to the processing line), and after 24 h (ultimate pH (pH<sub>u</sub>)) with a portable pH meter (ROSS, Orion 4 Star, Thermo Scientific) combined with an Orion Kniphe electrode (ThermoFisher) and an Orion™ Stainless-Steel Automatic Temperature Compensation (ATC) Probes (#927007MD, Thermo Scientific; Blasco and Ouhayoun, 1996). Meat colour was measured 24 h after slaughter on LL muscle cross sections between the 6<sup>th</sup> and 7<sup>th</sup> lumbar vertebrae (Dalle Zotte *et al.*, 2015) and on the exposed surface overlying the BF (Dalle Zotte *et al.*, 2009). After exposing the cut muscle surface to ambient air for 20 min ("blooming time"; Koné *et al.*, 2019), meat colour was evaluated using a Chroma meter (CR 400, Minolta Ltd., Osaka, Japan) equipped with a conical open port and an 8 mm aperture, a diffuse illumination/0° viewing angle geometry and a D65 light source

according to the reflectance coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ; CIE, 1976). Parameters used to compare meat colour were lightness ( $L^*$ ), redness ( $a^*$ ), yellowness ( $b^*$ ), colour intensity (chroma,  $C^*$ ) and the hue angle ( $h$ ). Equation (1) was used to calculate the chroma, while Equation (2) was used to determine the hue angle (Pathare *et al.*, 2013):

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad (1)$$

$$h = \text{Tan}^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

For samples with a negative  $a^*$  value,  $180^\circ$  was added to the calculated  $h$  value (McLellan *et al.*, 1995). Drip loss was measured by the weight difference of a piece of LL (2 cm thick  $\times$  2.5 cm in diameter) after storage at  $4^\circ\text{C}$  for 48 h using an EZ-Driploss cup (Meat Extract Collector, Sarstedt AG & Co. KG, Nümbrecht, Germany; Rasmussen and Anderson, 1996). The cooking loss was evaluated using a similar piece of LL muscle (Pla, 1999) and is expressed as a percentage of the initial weight loss. Samples were placed individually into a Whirl-Pak bag (S-19793, Nasco Whirl-Pak®, USA), the air was removed from the bag, and it was then submerged in a water bath at  $70^\circ\text{C}$  for 15 min. Samples were then cooled in an ice-water bath, removed from the bag and weighed after removing the excess moisture with filter paper (Vergara *et al.*, 2005).

### Statistical analysis

To determine the differences in the behavioural parameters between slaughter lots, data were assessed using the SAS (Statistical Analysis System, SAS Institute Inc. 2002) GLIMMIX procedure. The LSMEANS statement adjusted by a Tukey's test was used to compare the differences between slaughter lots. In a second analysis, season was used as a fixed effect in order to evaluate its impact; producer was used as a random variable.

For the physiological and meat quality parameters, data were analysed using the SAS GLIMMIX procedure. To measure the effect of FW time, three classes were established according to what was applied by the selected producers (class 1, short  $\leq 13.5$  h; class 2,  $13.5$  h < intermediate <  $23$  h; and class 3, long  $\geq 23$  h; Table 1). FW time, class and season were used as fixed variables, whereas slaughter lot and slaughterhouse were random variables.

Pearson's correlation coefficients were calculated on the residuals of lot analysis to measure the associations between the parameters under study using JMP 15 (SAS Institute Inc. 2002). For the principal component analysis (PCA), only the lots that were slaughtered in the abattoirs located in Quebec were considered. This was because access to the processing line was denied for lots slaughtered in the Ontario abattoir, therefore preventing the collection of physiological data. SAS software was used for this analysis.

A hierarchical cluster analysis was performed using Minitab software (Release 19) to identify groups of rabbit meat with different quality characteristics. Between each pair of observations, the Euclidian distance was used to measure the resemblance between groups and a complete linkage clustering method was used to associate similar samples. Four clusters were formed based on three meat quality variables ( $\text{pH}_v$ , cooking loss and  $L^*$ ). An analysis of variance (ANOVA) using the SAS software MIXED procedure was performed to evaluate differences between quality characteristics of the groups formed by the cluster analysis, and the LSMEANS statement adjusted by a Tukey's test was used to compare the differences between clusters.

## RESULTS

### Behavioural parameters

According to Figure 1, only a small percentage of rabbits were observed to be moving ( $\leq 3.1\%$ ), whereas most were sitting or lying down. Rabbits from A-P1-W were documented as moving more frequently than those from E-F1-S. Rabbits from D-P2-S, A-P1-W and D-P1-W were found to be sitting more often (60.6, 53.5 and 51.9%, respectively) than rabbits from D-P1-S and E-F1-S (35.7 and 32.7%, respectively). Values obtained for the other slaughter lots ranged from 39.8 to 51.5%. Results for rabbits lying down were essentially the opposite of those sitting.

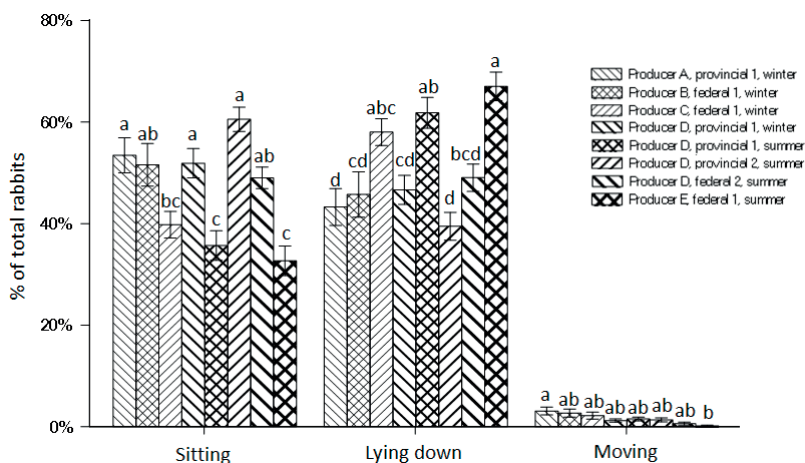


Figure 1: Percentage of rabbits sitting, lying down or moving during observations, prior to feed withdrawal, at each farm visit (mean±standard error). Bars with different letters differ significantly at  $P < 0.05$ .

For all slaughter lots, limited interaction between rabbits prior to FW was observed (Table 2). However, the proportion of rabbits exhibiting nonaggressive interactions was higher ( $P=0.046$ ) for C-F1-W (4.0%) compared to D-P1-S (1.0%; Table 2). A-P1-W was the only slaughter lot where rabbits expressed aggressive behaviour (biting another rabbit; 0.4%).

D-P1-W had more rabbits resting than A-P1-W (89.0 vs. 79.8%;  $P=0.06$ ; Table 2). Slaughter lot D-P2-S (3.8%) had more rabbits that were drinking than B-F1-W (0.8%), C-F1-W (1.2%) and E-F2-S (0.9%; all  $P$ -values $<0.005$ ; Table 2). A-P1-W, E-F2-S, D-P1-S, D-P2-S and D-F2-S had more rabbits that were grooming than D-P1-W (all  $P$ -values $<0.049$ ; Table 2).

B-F1-W (7.0%) had more rabbits that were eating than E-F1-S (1.8%;  $P=0.01$ ). Slaughter lots A-P1-W and C-F1-W had a higher proportion of rabbits that were moving than E-F1-S (both  $P$ -values $<0.08$ ; Table 2). No major difference was observed between slaughter lots for the proportion of rabbits biting or scratching their cage, mating, stretching, shaking, stamping their feet and sneezing. However, E-F1-S was the only one with rabbits that stood up (0.3%).

Of the four rabbit lots from producer D, D-P1-W (51.9%), D-P2-S (60.6%) and D-F2-S (49.0%) had more rabbits that were sitting than D-P1-S (35.7%; all  $P$ -values $<0.04$ ; Figure 1). D-P1-S (62.8%) had more rabbits lying down than D-P1-W (46.9%) and D-P2-S (38.1%; both  $P$ -values $<0.02$ ; Figure 1). With respect to interactions presented in Table 2, D-P1-W (1.9%) had fewer rabbits grooming than D-P1-S (8.0%), D-P2-S (9.5%) and D-F2-S (8.9%; all  $P$ -values $<0.049$ ). No other interactions were different between slaughter lots from producer D.

With respect to season, rabbits were more active in winter than in summer ( $P=0.045$ ). Animals exhibited fewer interactions in summer ( $P=0.009$ ). When the animals interacted in winter, none of these interactions were aggressive ( $P=0.001$ ). Interestingly though, sneezing was more common during the summer ( $P=0.009$ ).

### Physiological parameters

For rabbits slaughtered in Quebec, we had access to the processing line, which enabled us to measure various physiological parameters. The mean, standard deviation, minimum and maximum of different physiological parameters that were measured are presented in Figure 2. Means for blood lactate ranged from  $0.88 \pm 0.19$  mmol/L for D-F2-S to  $8.74 \pm 4.29$  mmol/L for D-P1-S (Figure 2A).

GI tract mean weight ranged from  $344.69 \pm 32.82$  g (D-P2-S) to  $463.85 \pm 58.53$  g (A-P1-W; Figure 2B). Stomach mean weight ranged from  $65.66 \pm 18.25$  g (D-P1-W) to  $111.97 \pm 22.83$  g (A-P1-W; Figure 2C). Average caecum weight was

Table 2: Percentage (%) of rabbits presenting different types of interactions and activities during observations made prior to feed withdrawal at the farm<sup>1</sup>.

Variables	Producer <sup>2</sup>								SEM <sup>3</sup>	P-value
	A Provincial 1 Winter	B Federal 1 Winter	C Federal 1 Winter	D Provincial 1 Winter	D Provincial 1 Summer	D Federal 2 Summer	E Federal 1 Summer			
Interaction, %										
Nonaggressive <sup>4</sup>	3.3±0.6 <sup>ab</sup> (1.60-4.9) <sup>5</sup>	3.0±1.0 <sup>ab</sup> (1.2-4.8)	4.0±0.9 <sup>a</sup> (2.8-5.2)	2.7±0.9 <sup>ab</sup> (1.1-4.2)	1.0±0.3 <sup>b</sup> (0.0-2.5)	2.2±0.6 <sup>ab</sup> (0.0-3.7)	1.3±0.3 <sup>ab</sup> (0.0-2.8)	2.1±0.8 <sup>ab</sup> (0.0-3.8)	0.007	0.05
Aggressive	0.4±0.4 (0.1-0.6)	NO (0.0-0.2)	NO (0.0-0.2)	NO (0.0-0.2)	NO (0.0-0.2)	NO (0.0-0.2)	NO (0.0-0.2)	NO (0.0-0.2)	0.001	ND
No interaction	96.4±0.7 (94.1-98.6)	97.0±1.0 (94.6-99.4)	96.0±0.9 (94.4-97.6)	97.4±0.9 (95.2-99.4)	99.0±0.3 (96.9-100.0)	99.2±1.7 (97.1-100.0)	97.6±1.2 (95.5-99.6)	98.1±0.8 (95.8-100.0)	0.01	ND
Type of activity, %										
Resting	79.8±2.6 <sup>a</sup> (75.3-84.2)	84.9±1.7 <sup>ab</sup> (80.2-89.6)	84.5±1.5 <sup>ab</sup> (81.4-87.6)	89.0±1.6 <sup>ab</sup> (84.9-93.1)	83.1±1.6 <sup>ab</sup> (79.0-87.2)	81.9±2.2 <sup>ab</sup> (77.8-86.0)	82.6±3.0 <sup>ab</sup> (78.5-86.6)	87.3±1.9 <sup>ab</sup> (82.9-91.8)	0.03	0.07
Drinking	1.6±0.6 <sup>ab</sup> (0.6-2.7)	0.8±0.3 <sup>b</sup> (0.0-1.9)	1.2±0.3 <sup>b</sup> (0.5-1.9)	1.9±0.6 <sup>ab</sup> (0.9-2.9)	1.6±0.3 <sup>ab</sup> (0.6-2.6)	3.8±0.5 <sup>a</sup> (2.8-4.7)	2.7±0.8 <sup>ab</sup> (1.7-3.7)	0.9±0.3 <sup>b</sup> (0.0-2.0)	0.008	0.001
Grooming	10.7±2.2 <sup>a</sup> (7.7-13.7)	4.8±0.9 <sup>ab</sup> (1.6-7.9)	7.0±1.3 <sup>ab</sup> (5.0-9.1)	1.9±0.5 <sup>b</sup> (0.0-4.6)	8.0±1.2 <sup>a</sup> (5.3-10.7)	9.5±1.3 <sup>a</sup> (6.8-12.3)	8.9±1.2 <sup>a</sup> (6.2-11.7)	8.2±1.7 <sup>a</sup> (5.3-11.2)	0.01	0.001
Biting their cage	NO	NO	0.4±0.3 (0.1-0.7)	NO	NO	0.2±0.2 (0.0-0.6)	0.2±0.2 (0.0-0.6)	0.6±0.3 (0.2-1.0)	0.002	ND
Eating	5.3±1.1 <sup>ab</sup> (3.3-7.3)	7.0±1.1 <sup>a</sup> (4.9-9.1)	4.0±0.9 <sup>ab</sup> (2.6-5.3)	6.7±1.0 <sup>ab</sup> (4.8-8.5)	5.2±0.5 <sup>ab</sup> (3.4-7.1)	4.2±0.9 <sup>ab</sup> (2.3-6.0)	3.4±0.9 <sup>ab</sup> (1.6-5.2)	1.8±0.6 <sup>b</sup> (0.0-3.8)	0.01	0.006
Mating	NO	0.2±0.2 (0.0-0.6)	0.6±0.3 (0.3-0.9)	0.1±0.1 (0.0-0.5)	NO	0.3±0.2 (0.0-0.7)	0.1±0.1 (0.0-0.5)	NO	0.002	ND
Moving	2.4±0.7 <sup>a</sup> (1.2-3.4)	2.1±0.7 <sup>ab</sup> (0.9-3.2)	2.0±0.6 <sup>a</sup> (1.2-2.7)	0.4±0.3 <sup>b</sup> (0.0-1.4)	1.4±0.4 <sup>ab</sup> (0.4-2.4)	0.8±0.3 <sup>ab</sup> (0.0-1.8)	0.7±0.3 <sup>ab</sup> (0.0-1.7)	NO <sup>y</sup>	0.006	0.01
Stretching	0.4±0.2 (0.0-0.7)	0.2±0.2 (0.0-0.5)	0.3±0.2 (0.0-0.5)	NO	NO	NO	NO	0.2±0.2 (0.0-0.5)	0.002	ND
Shaking	NO	0.2±0.2 (0.0-0.4)	NO	NO	0.2±0.15 (0.0-0.4)	0.1±0.1 (0.0-0.3)	NO	NO	0.001	ND
Stamping their feet	NO	NO	NO	NO	NO	0.1±0.1 (0.0-0.2)	NO	0.2±0.15 (0.0-0.3)	0.001	ND
Sneezing	NO	NO	NO	NO	0.1±0.1 (0.0-0.3)	0.3±0.2 (0.1-0.6)	0.2±0.2 (0.0-0.4)	0.2±0.2 (0.0-0.4)	0.001	ND
Standing up	NO	NO	NO	NO	NO	NO	NO	0.1±0.2 (0.0-0.3)	0.001	ND
Scratching the cage	NO	NO	NO	NO	0.1±0.1 (0.0-0.2)	NO	NO	0.3±0.2 (0.1-0.5)	0.001	ND

<sup>1</sup>Mean±standard error. Observations made by scan sampling on a subsample of 10% of total cages within each slaughter lot.<sup>2</sup>Indicates the slaughterhouse inspection jurisdiction at which rabbits were slaughtered. Provincial 1 and 2 were located in Quebec; Federal 1 was located in Ontario; Federal 2 is the same slaughterhouse as Provincial 2, but after it received federal accreditation.<sup>3</sup>SEM: Standard error of the mean.<sup>4</sup>Different letters (a-d) within rows indicate difference at  $P < 0.05$  and (x-y) at  $P < 0.10$ . ND: not observed. Tukey's test was carried out to compare the differences between producers.<sup>5</sup>Confidence interval (lower limit-upper limit).

RABBIT MEAT QUALITY CLASSIFICATION SCHEME

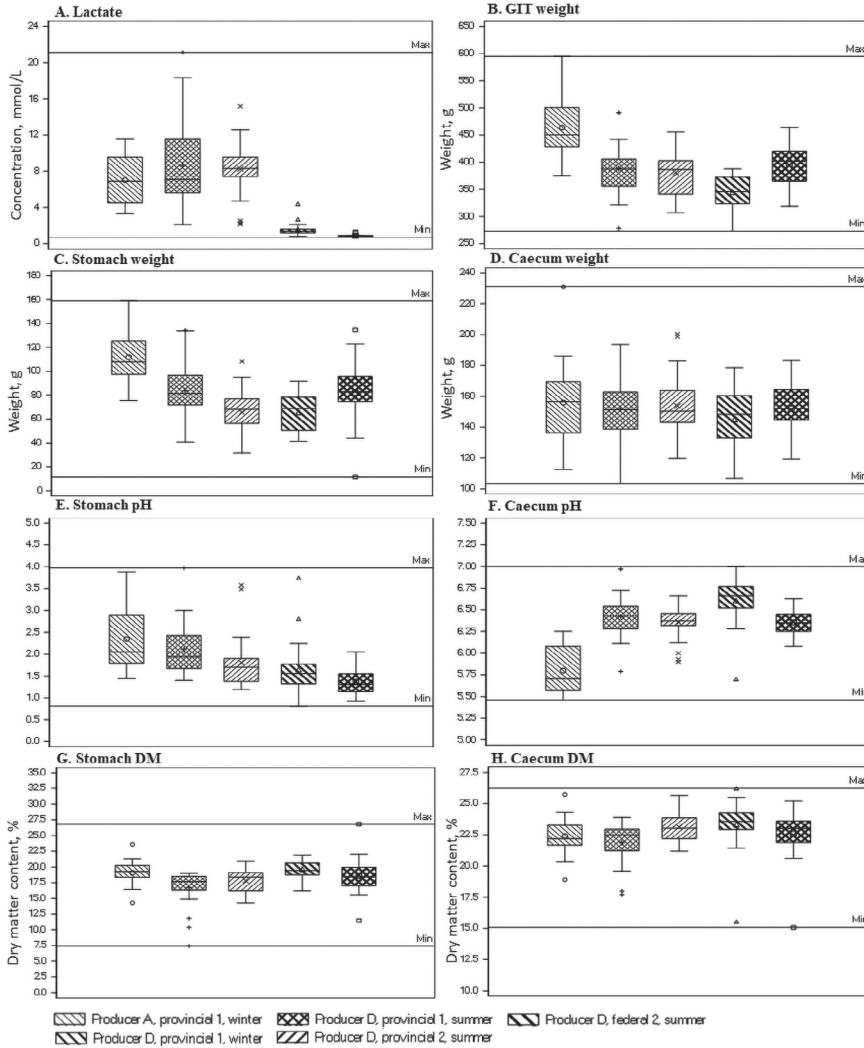


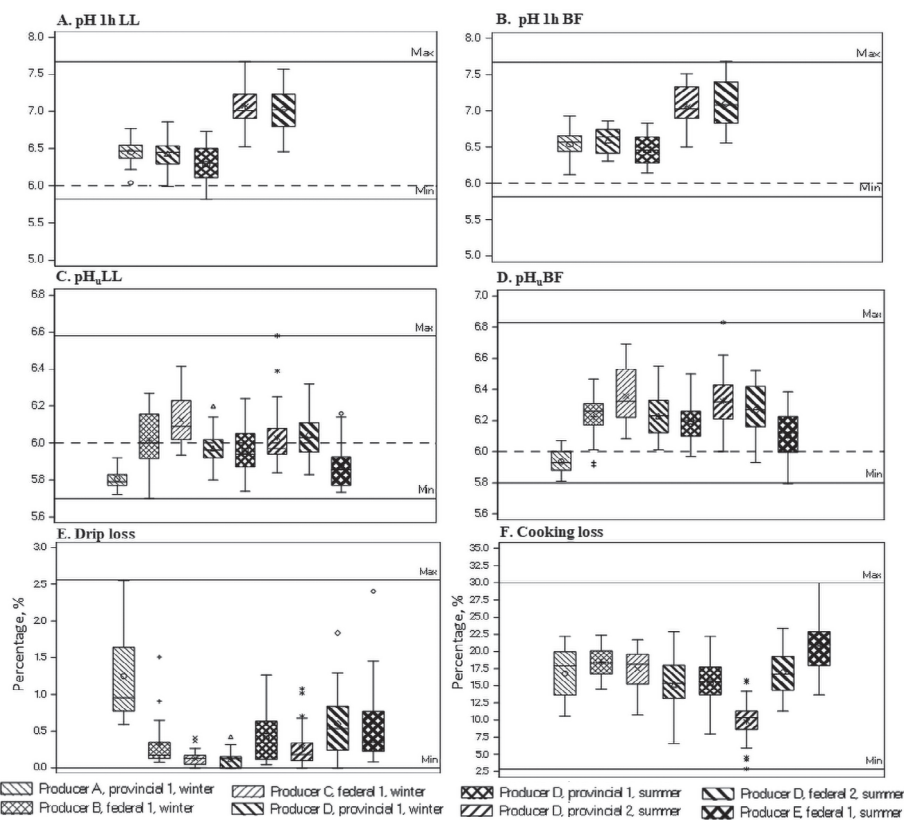
Figure 2: Physiological parameters (means±standard deviations) measured from rabbits (n=120) slaughtered in the province of Quebec; blood lactate concentration (A), gastrointestinal tract (GIT) weight (B), stomach weight (C), caecum weight (D), stomach pH (E), caecum pH (F), stomach DM (G) and caecum DM (H). Max=maximum and Min=minimum. Stomach DM and caecum DM=Stomach and caecum dry matter.

relatively stable for all slaughter lots, with means ranging from 144.92±19.70 g for D-P2-S to 155.90±26.04 g for A-P1-W (Figure 2D).

For stomach pH, averages ranged from 1.37±0.29 for D-F2-S to 2.35±0.73 for A-P1-W (Figure 2E). Except for A-P1-W, caecum pH was relatively similar for all slaughter lots, with means ranging from 6.35±0.14 (D-F2-S) to 6.61±0.25 (D-P2-S; Figure 2F). Slaughter lot A-P1-W was the only one with a mean caecum pH that was below 6 (5.80±0.26), and the minimum value observed (5.46) was also from this lot. The stomach pH and caecum pH levels from A-P1-W were above and below all of the others, respectively.

Stomach DM ranged from  $16.68 \pm 2.81\%$  for D-P1-S to  $19.67 \pm 1.72\%$  for D-P2-S (Figure 2G). Caecum DM also was relatively stable for all slaughter lots, with means ranging from  $21.88 \pm 1.64\%$  (D-P1-S) to  $23.79 \pm 6.35\%$  (A-P1-W; Figure 2H).

FW times observed were categorised into three classes to assess their effect on the parameters measured. Long FW times ( $\geq 23$  h) were observed for rabbits that were slaughtered outside of Quebec in Flinton Ontario where, unfortunately, we did not have access to the processing line. However, statistical analysis revealed a significant interaction between FW time classes and season for blood lactate concentrations ( $P=0.005$ ). The concentrations were lowest for rabbits slaughtered in summer after intermediate FW times. Overall, blood lactate concentrations were higher in winter than in summer ( $P=0.005$ ) and when the FW time was short ( $\leq 13.5$  h;  $P=0.002$ ). Stomach pH was also higher when the FW time was short ( $P=0.047$ ), at  $2.23 \pm 0.66$ .



**Figure 3:** Meat quality characteristics (means $\pm$ standard deviations) measured from rabbits ( $n=200$ ) slaughtered in different abattoirs and raised at different farms; pH1hLL (A), pH1hBF (B), pH<sub>u</sub>LL (C), pH<sub>u</sub>BF (D), drip loss (E) and cooking loss (F). Data for pH1hBF and pH<sub>u</sub>BF=pH after 1 h and 24 h *post-mortem* of the *Biceps femoris* (BF) muscle, respectively; pH1hLL and pH<sub>u</sub>LL=pH after 1 h and 24 h *post-mortem* of the *Longissimus lumborum* (LL) muscle, respectively. Drip loss and cooking loss were from the LL muscle. Dotted lines indicate pH=6.



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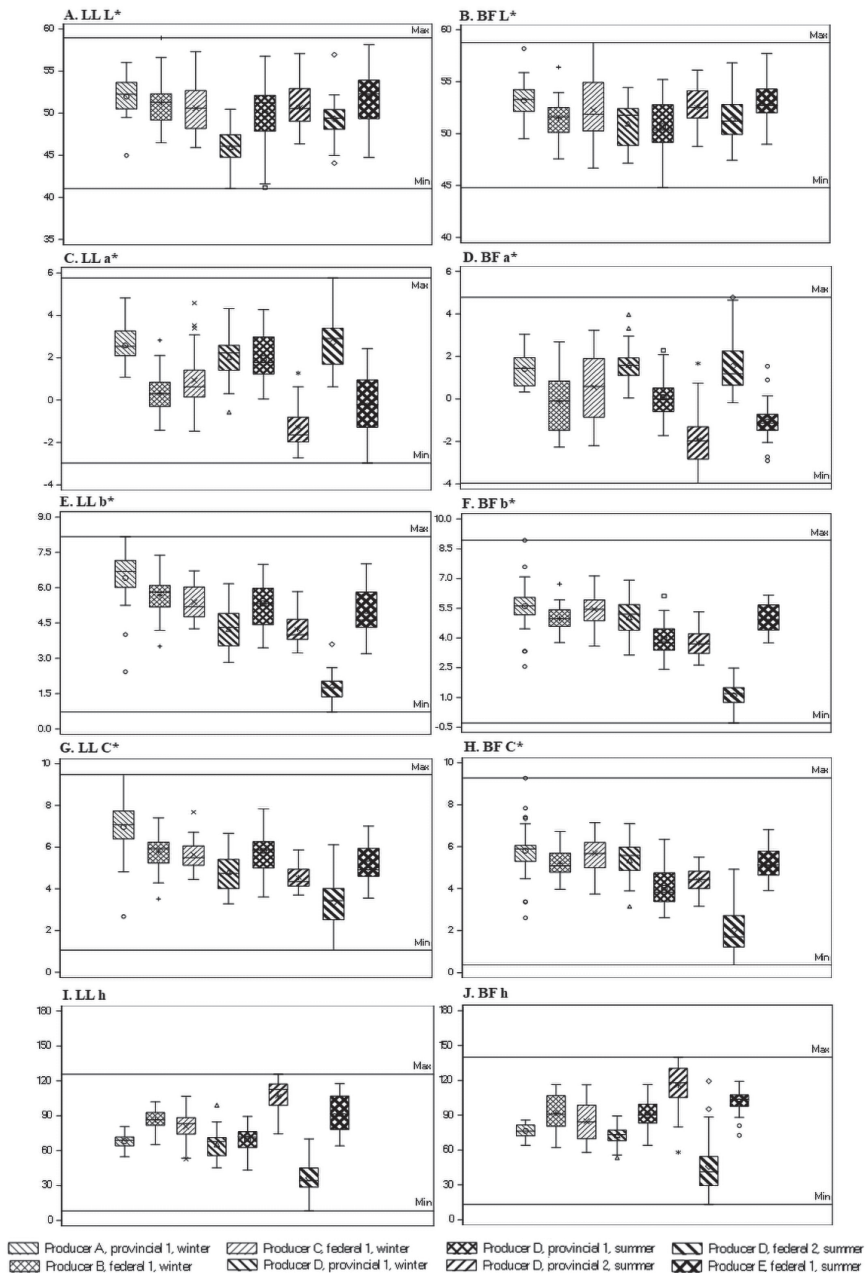


Figure 4: Meat colour parameters (means±standard deviations) measured from rabbits (n=200) slaughtered in different abattoirs and raised at different farms; LL L\* (A), BF L\* (B), LL a\* (C), BF a\* (D), LL b\* (E), BF b\* (F), LL C\* (G), BF C\* (H), LL h (I) and BF h (J). Max=maximum and Min=minimum. LL=*Longissimus lumborum* muscle; BF=*Biceps femoris* muscle; LL C\*, LL h=chroma (C\*) and hue angle (h) of the LL muscle, respectively. BF C\*, BF h=C\* and h of the BF muscle, respectively.

### Meat quality characteristics

The mean, standard deviation, minimum and maximum for each meat quality parameter are presented in Figures 3 and 4. Overall, the pH of both LL and BF muscles 1 h after slaughter was above 6. The pH<sub>1h</sub> of the LL muscle (pH<sub>1h</sub>LL) ranged from 6.29±0.24 (D-P1-S) to 7.07±0.29 (D-P2-S; Figure 3A). Means for the pH<sub>1h</sub> of the BF muscle (pH<sub>1h</sub>BF) varied from 6.47±0.21 (D-P1-S) to 7.10±0.32 (D-F2-S; Figure 3B).

On average, the pH<sub>u</sub> of the LL muscle (pH<sub>u</sub>LL) was below 6 for A-P1-W, D-P1-W, D-P1-S and E-F1-S (Figure 3C). The pH<sub>u</sub> of the BF muscle (pH<sub>u</sub>BF) was below 6, on average, only for A-P1-W (Figure 3D).

For all slaughter lots, the mean drip loss of the LL muscle showed small variations and low values, with means ranging from 0.1±0.1% for D-P1-W to 1.3±0.7% for A-P1-W (Figure 3E). Hence, rabbits from A-P1-W produced meat with a drip loss that was above the others. Compared to drip loss, the cooking loss of the LL muscle exhibited larger variations with means ranging from 9.8±3.7% (D-P2-S) to 21.1±5.2% (E-F1-S; Figure 3F). In this case, rabbit meat from D-P2-S exhibited a cooking loss below the others.

An interaction was observed between FW time classes and seasons for the pH<sub>1h</sub> of the LL and BF muscles (both *P*-values<0.001). The highest pH<sub>1h</sub> (slightly above 7) was observed for rabbits that were slaughtered in the summer following intermediate FW times. Overall, the pH<sub>1h</sub> for LL and BF muscles were higher in the summer (*P*<0.001) and for the intermediate FW time class (*P*<0.001).

For all rabbits, the mean, standard deviation, minimum and maximum of each of the colour parameters are presented in Figure 4. The lightness of the LL muscle (LL L\*) ranged from 45.97±2.47 (D-P1-W) to 51.96±2.41 (A-P1-W; Figure 4A). The BF muscle lightness (BF L\*) was relatively stable for all slaughter lots, with means varying between 50.72±2.39 for D-P1-S and 53.18±1.87 for A-P1-W (Figure 4B).

The redness of the LL muscle (LL a\*) ranged from -1.29±0.97 (D-P2-S) to 2.72±1.34 (D-F2-S; Figure 4C). Means for redness of the BF muscle (BF a\*) varied between 1.88±1.31 (D-P2-S) and 1.61±0.99 (D-P1-W; Figure 4D).

Means for LL muscle yellowness (LL b\*) ranged from 1.78±0.60 for D-F2-S to 6.42±1.20 for A-P1-W (Figure 4E). Means for LL muscle chroma (LL C\*) ranged from 3.34±1.25 for D-F2-S to 6.96±1.29 for A-P1-W (Figure 4G). The yellowness of the BF muscle (BF b\*) ranged from 1.09±0.61 for D-F2-S to 5.60±1.33 for A-P1-W (Figure 4F). The chroma of the BF muscle (BF C\*) ranged from 2.03±1.23 for D-F2-S to 5.81±1.42, for A-P1-W (Figure 4H).

For hue angle (h\*), means ranged from 36.42±13.41 (D-F2-S) to 106.89±13.08 (D-P2-S) and from 46.10±26.43 (D-F2-S) to 115.85±18.98 (D-P2-S) for the LL and BF muscles, respectively (Figures 4I and J). Overall, rabbits from D-F2-S resulted in meat with a distinctive colour compared to the others (Figure 4).

No interactions were observed between FW time classes and seasons for any of the colour parameters tested. However, meat lightness (L\*) and colour intensity (C\*) of the LL muscle were higher for the short FW time class 1 than the intermediate class 2 (both *P*-values<0.02), but were no different from the long FW time class 3. Season appears to have little effect on the colour parameters.

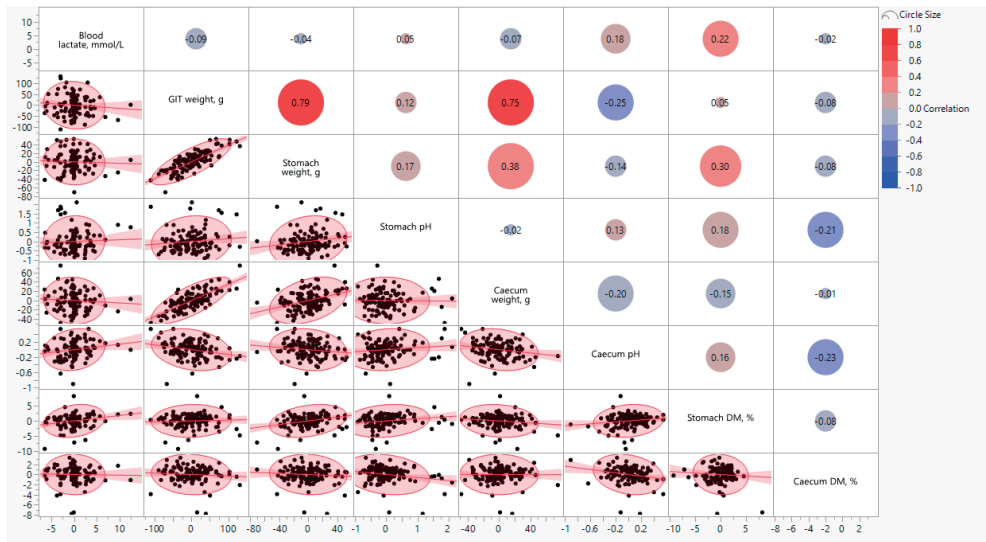
### Correlations among physiological parameters

Figure 5 shows the correlation between the physiological parameters evaluated, which includes blood lactate (mmol/L), GIT, stomach and caecum weight (g), as well as pH and dry matter of the stomach and the caecum. As expected, GIT weight was highly correlated with both stomach (*r*=0.79; *P*<0.0001) and caecum weights (*r*=0.75; *P*<0.0001), but stomach and caecum weights were not (*r*=0.38; *P*<0.0001). Those were the only physiological parameters deemed correlated (*r*>0.50) for the range of commercially-raised rabbit tested.

### Correlations among meat quality characteristics

Figure 6 shows the correlation between the meat quality characteristics evaluated on all the rabbits tested. As expected, the pH values measured after 24 h *post-mortem* in LL and BF muscles were highly correlated (*r*=0.80; *P*<0.0001). The pH<sub>u</sub> and the meat lightness (L\*) were negatively correlated in LL (*r*=-0.62; *P*<0.0001), but not so much in BF muscles (*r*=-0.46; *P*<0.0001). The negative correlation between pH<sub>u</sub> and meat yellowness (b\*) was

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**Figure 5:** Correlations among the physiological parameter measured from rabbits slaughtered in different abattoirs and raised at different farms;  $n=120$ . GIT weight=gastrointestinal tract weight; Lactate=blood lactate concentration; Stomach DM and Caecum DM=Stomach and caecum dry matter, respectively. Ellipses represent a 95% level of confidence.

rather weak for both LL and BF muscles ( $r=-0.52$  and  $-0.32$ , respectively; both  $P$ -values $<0.0001$ ). Surprisingly, drip loss was not correlated with cooking loss ( $r=0.09$ ;  $P=0.195$ ); neither were correlated with any of the parameters tested ( $r < 0.50$ ).

Meat lightness was correlated with yellowness ( $b^*$ ) for both LL and BF muscles ( $r=0.68$  and  $0.59$ , respectively; both  $P$ -values $<0.0001$ ). Chroma between LL and BF muscles were weakly correlated ( $r=0.51$ ;  $P<0.0001$ ), but not so much for redness ( $a^*$ ;  $r=0.49$ ;  $P<0.0001$ ) and yellowness ( $r=0.45$ ;  $P<0.0001$ ). Correlations between  $a^*$  and  $b^*$  values with chroma ( $C^*$ ) and hue angle ( $h$ ) are expected, as the latter requires the former for their calculation (see Equations 1 and 2). Otherwise, correlations were considered weak, given that the correlation coefficients were higher than  $-0.50$  or lower than  $0.50$ .

Although the rabbits were produced within the same province, and therefore were under the same code of practice (NFACC, 2018), to a target slaughter weight of 2.5 kg with the aim of delivering market-quality meat, variations are bound to occur between producers and seasonally. Pooling the results from all 200 rabbits to determine correlation coefficients could lead, for instance, to nonsensical and biased correlations due to sample heterogeneity (Hassler and Thadewald, 2003). Therefore, principal component analyses (PCA) were performed to confirm the correlations that were observed.

### **Relationship between physiological parameters and meat quality characteristics**

The principal component analysis (PCA) plot shows the relationship between meat quality characteristics and physiological parameters for rabbits slaughtered in the province of Quebec (Figure 7). The component 1 (horizontal axis  $x$ ) accounted for 27.17% and the component 2 (vertical axis  $y$ ) explained 17.18% of the total variations. The first component of the PCA was mainly defined by lactate, LL  $b^*$ , BF  $b^*$ , LL  $C^*$  and BF  $C^*$  on the positive side and by pH<sub>1hLL</sub>, pH<sub>1hBF</sub>, pH<sub>LL</sub> and pH<sub>BF</sub> on the negative side. All these parameters fell far from the origin of the first component, showing that they are the main factors defining this component (Figure 7). The second component of the PCA was mostly defined by LL  $h$ , BF  $h$ , LL  $L^*$  and BF  $L^*$  on the positive side and LL  $a^*$  and BF  $a^*$  on the negative side.

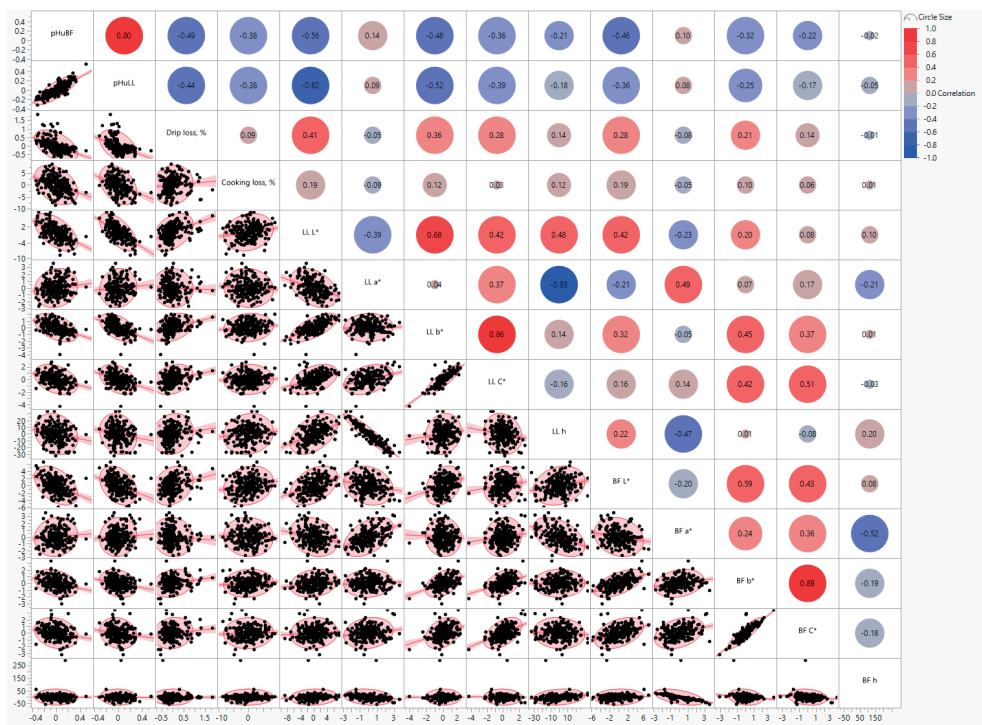


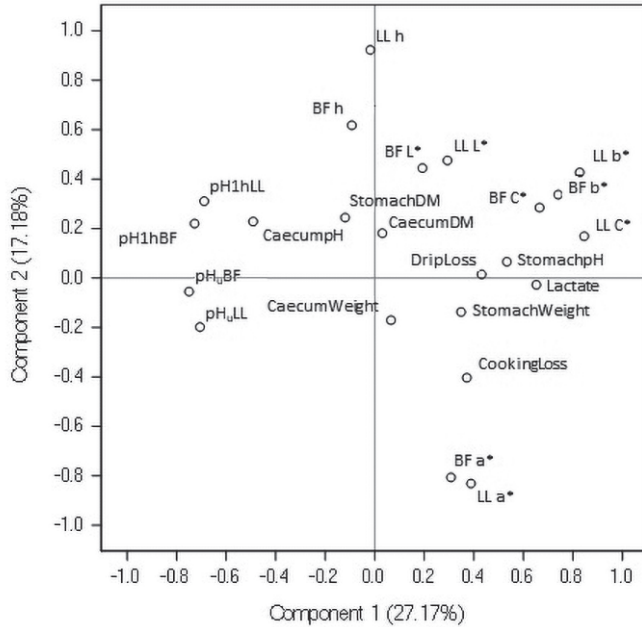
Figure 6: Correlations among the meat quality characteristics measured from rabbits slaughtered in different abattoirs and raised at different farms; n=200. pH<sub>h</sub>BF and pH<sub>h</sub>LL=pH after 24 h *post-mortem* of the *Biceps femoris* (BF) and *Longissimus lumborum* (LL) muscles, respectively. Cooking loss and drip loss were measured in the LL muscle; LL L\*, LL a\*, LL b\*=colour space L\*a\*b\* of the LL surface, respectively; BF L\*, BF a\*, BF b\*=colour space L\*a\*b\* of the BF surface, respectively; LL C\*=chroma (C\*), LL h=hue angle (h) of the LL muscle; BF C\*=chroma (C\*), BF h=hue angle (h) of the BF muscle. Ellipses represent a 95% level of confidence.

Within the range of values observed in this study, a decrease in blood lactate concentration led to an increase in pH<sub>h</sub>LL and pH<sub>h</sub>BF, as indicated by the 180° separation between variables on the PCA plot. The plot also illustrates that pH<sub>h</sub>LL and pH<sub>h</sub>BF are positively correlated, given their close proximity. Similarly, pH<sub>h</sub>LL and pH<sub>h</sub>BF were also positively correlated. Furthermore, an increase in pH<sub>h</sub>LL resulted in a decrease in meat cooking loss, but drip loss was not affected, as the two variables are separated by a 90° angle on the PCA plot. However, meat with an increased in pH<sub>h</sub>LL saw a decrease in drip loss. An increase in pH<sub>h</sub>LL and pH<sub>h</sub>BF decreased the lightness (L\*), yellowness (b\*) and colour intensity (C\*) of these meats. Physiological parameters, except for blood lactate, are located near the origin, indicating the lack of importance of these parameters in defining the two components compared to meat quality.

### Meat quality clustering

Meat quality was distributed into four groups using a hierarchical cluster analysis based on pH<sub>h</sub>LL, cooking loss and the L value (Table 3). Of the 200 rabbits sampled, 32 were grouped into cluster 1, 89 in cluster 2, 19 in cluster 3 and 60 in cluster 4. The pH<sub>h</sub> of LL was below 6 in clusters 1 and 2 and different from clusters 3 and 4, which had a pH<sub>h</sub>LL slightly above 6 (all *P*-value<0.0001). Cooking loss was the highest in cluster 2, reaching an average of 20.3±0.2%, followed by clusters 4 (15.3±0.3%; *P*<0.0001), 1 (12.6±0.4%; *P*<0.0001) and 3 (8.2±0.5%; *P*<0.0001). Meat from cluster 1 had a lighter colour, as the LL L\* value was the highest (53.33±0.44) compared to

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**Figure 7:** Principal component analysis: relationship between physiological parameters and meat quality characteristics for rabbits (n=120) slaughtered in the province of Quebec. StomachDM and CaecumDM=stomach and caecum dry matter, respectively; StomachpH and CaecumpH=pH of the stomach and caecum, respectively; StomachWeight and CaecumWeight=weight of the stomach and caecum, respectively; lactate=blood lactate concentration; pH<sub>1h</sub>BF and pH<sub>u</sub>BF=pH of the *Biceps femoris* (BF) muscle after 1 h and 24 h *post-mortem*, respectively; pH<sub>1h</sub>LL and pH<sub>u</sub>LL=pH of the *Longissimus lumborum* (LL) muscle after 1 h and 24 h *post-mortem*, respectively; DripLoss and CookingLoss=drip loss and cooking loss of the LL muscle; LL L\*, LL a\*, LL b\*=colour space L\*a\*b\* of the LL surface, respectively; BF L\*, BF a\*, BF b\*=colour space L\*a\*b\* of the BF surface, respectively; LL C\*, LL h=chroma (C\*) and hue angle (h) of the LL surface, respectively; BF C\*, BF h=C\* and h of the BF surface, respectively.

cluster 2 (51.66±0.26; *P*=0.007) and also compared to clusters 3 and 4, which had darker colours (47.58±0.57 and 47.21±0.32, respectively; both *P*-values<0.0001).

The LL muscles grouped in clusters 1 and 2 are distinguished from those in clusters 3 and 4 based on their pH<sub>u</sub> mean values, which are below 6 (Table 3; Figure 8A). Although LL L\* from cluster 1 was higher than LL L\* from cluster 2, the difference was small (<2 units; Table 3; Figure 8B). Cluster 1 is distinguished from cluster 2 mostly by its cooking loss

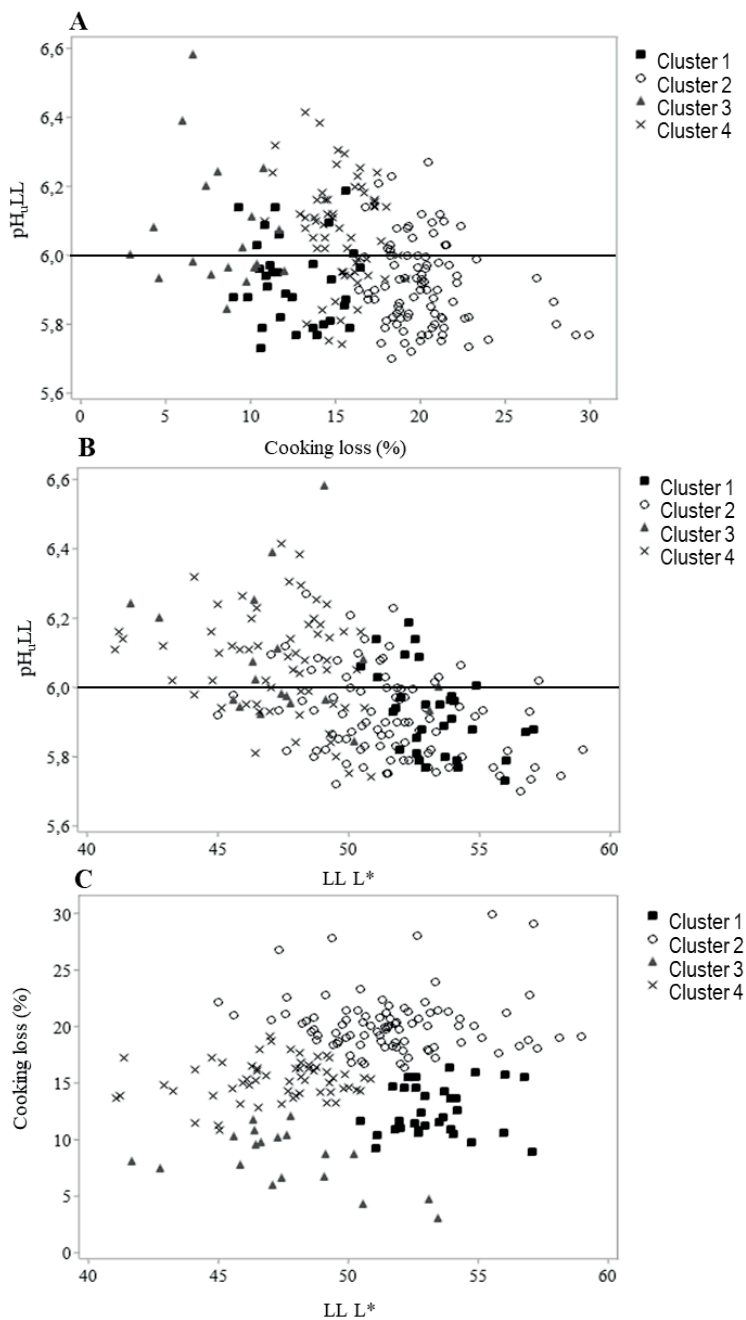
**Table 3:** Meat quality characteristics of rabbit loin (*Longissimus lumborum* muscle; LL) grouped by a hierarchical cluster analysis based on pH<sub>u</sub>, cooking loss and L\*<sup>1</sup>.

Quality class	n	pH <sub>u</sub> LL	Cooking loss,%	LL L*
Cluster 1	32	5.93±0.02 <sup>b</sup> (5.88-5.97)	12.6±0.4 <sup>c</sup> (11.79-13.39)	53.33±0.44 <sup>a</sup> (52.46-54.19)
Cluster 2	89	5.91±0.01 <sup>b</sup> (5.88-5.94)	20.3±0.2 <sup>a</sup> (19.79-20.75)	51.66±0.26 <sup>b</sup> (51.14-52.18)
Cluster 3	19	6.07±0.03 <sup>a</sup> (6.01-6.13)	8.2±0.5 <sup>d</sup> (7.13-9.20)	47.58±0.57 <sup>c</sup> (46.45-48.70)
Cluster 4	60	6.07±0.02 <sup>a</sup> (6.04-6.10)	15.3±0.3 <sup>b</sup> (14.70-15.86)	47.21±0.32 <sup>c</sup> (46.58-47.84)
SEM <sup>2</sup>		0.04	0.50	0.51
<i>P</i> -value		< 0.0001	< 0.0001	< 0.0001

<sup>1</sup> Mean±standard error; n=200; confidence interval (lower limit-upper limit). L\*=lightness.

<sup>2</sup> SEM: standard error of the mean.

<sup>abc</sup>Means with a column not sharing superscript differ at *P*<0.05 based on a Tukey's test.



**Figure 8:** Distribution of the 200 rabbit *Longissimus lumbarum* (LL) samples, assigned to one of the four clusters that were formed using a hierarchical analysis, based on (A) pH<sub>u</sub>LL and cooking loss (%), (B) pH<sub>u</sub>LL and LL L\* and (C) cooking loss (%) and meat lightness (LL L\*). The horizontal line on (A) and (B) indicates pH<sub>u</sub>=6.

**Table 4:** Proportion (%) of rabbit loins (*Longissimus lumborum* muscle) from each slaughter lot distributed among the four quality clusters.

	n	Producer <sup>1</sup>							
		A Provincial 1	B Federal 1	C Federal 1	D Provincial 1	D Provincial 1	D Provincial 2	D Federal 2	E Federal 1
		Winter	Winter	Winter	Winter	Summer	Summer	Summer	Summer
Feed withdrawal time, h		8.5	26.5	29.0	15.5	13.5	16.0	17.3	23.0
		Class 1 <sup>2</sup>	Class 3	Class 3	Class 2	Class 1	Class 2	Class 2	Class 3
Cluster 1 <sup>3</sup>	32	36	12	8	0	24	40	0	8
Cluster 2	89	56	68	52	20	36	0	44	80
Cluster 3	19	0	0	0	20	8	48	0	0
Cluster 4	60	8	20	40	60	32	12	56	12

<sup>1</sup>Indicates the slaughterhouse inspection jurisdiction in which rabbits were slaughtered. Provincial 1 and 2 were located in Quebec; Federal 1 was located in Ontario; Federal 2 is the same slaughterhouse as Provincial 2, but after it received federal accreditation.

<sup>2</sup>Three classes were established for feed withdrawal time: class 1, short $\leq$ 13.5 h; class 2, intermediate (15.5 to 17.3 h); and class 3, long $\geq$ 23 h.

<sup>3</sup>Rabbit loins (n=200) were grouped using a hierarchical cluster analysis based on pH<sub>u</sub>, cooking loss and L of the *Longissimus lumborum* muscle resulting in four quality clusters.

(Table 3; Figure 8C), and the difference between the two groups reached 8% (Table 3). The pH<sub>uLL</sub> and the lightness (LL L\*) of clusters 3 and 4 were similar ( $P=1.0$  and  $P=0.94$ , respectively; Table 3). They can be distinguished based on cooking loss (Figure 8C), as the cooking loss of cluster 4 was higher than that of cluster 3 (Table 3).

Table 4 shows the proportion (%) of rabbits per producer distributed among the four quality clusters. For A-P1-W, B-F1-W, C-F1-W, D-P1-S, D-P2-S and E-F1-S, 36, 12, 8, 24, 40 and 8% of their rabbits, respectively, were grouped into cluster 1. Most rabbits from slaughter lots A-P1-W, B-F1-W, C-F1-W and E-F1-S were grouped into cluster 2 with 56% from A-P1-W, 68% from B-F1-W, 52% from C-F1-W and 80% from E-F1-S. However, unlike the other producers, less than 50% of rabbits from all producer D lots (20% of D-P1-W; 36% of D-P1-S; 0% of D-P2-S; 44% of D-F2-S) were grouped into cluster 2. Rabbit loins grouped into cluster 3 came from D-P1-W, D-P1-S and D-P2-S. The proportion of rabbits in the lowest meat quality cluster (cluster 4) was less than 50% for almost all slaughter lots, with the exception of D-P1-W (60%) and D-F2-S (56%). For all slaughter lots in the intermediate FW time class 2, 50% or more of their rabbits fell in the lower quality clusters 3 and 4. However, more than 50% of the rabbits from lots in the other two FW time classes (short and long) were in the high-quality clusters 1 and 2 (Table 4).

## DISCUSSION

### Rabbit behaviour

Behaviour was assessed by scan sampling to detect any welfare issues before the pre-slaughter FW requested by the Code of Practice for the Care and Handling of Rabbits (NFACC, 2018). Apart from A-P1-W, where aggressive interactions were observed to a limited extent (0.4%; Table 2), behavioural observations prior to FW did not raise particular concerns with respect to welfare for any of the rearing conditions encountered. Rabbits raised in enriched cages with a platform were, however, able to express a greater variety of behaviours (E-F1-S). Hansen and Berthelsen (2000) reported that rabbits kept in cages with access to a shelter and raised height at the back of the cage used the former as a lookout point and the latter to stand upright, which was not, obviously, observed for rabbits kept in conventional cages.

### Physiological parameters

Blood lactate at exsanguination was used to measure animal short-term stress levels (e.g., lack of lairage) before slaughter (Nakyinsige *et al.*, 2013; Rocha *et al.*, 2015; Trocino *et al.*, 2018), and high lactate levels are associated with pre-slaughter stress in rabbits (Fazio *et al.*, 2015; Nakyinsige *et al.*, 2013; Trocino *et al.*, 2018). Mean blood

lactate concentrations were lower or within the range of reported basal values for rabbits ( $6.9 \pm 2.7$  mmol/L; Langlois *et al.*, 2014), suggesting that in general, rabbits were not particularly stressed shortly before slaughter.

Reduction of both GIT and stomach weight with total FW time is consistent with Ouhayoun and Lebas (1994) and Bianchi *et al.* (2008), who reported more GIT weight loss and stomach weight loss with longer FW times for rabbits. The target slaughter weight for rabbits in Quebec is 2.5 kg. At this weight, the GIT tract weight, as a percentage of slaughter weight, ranged from 13.8% for D-P1-S to 18.6% for A-P1-W (data not shown). The values obtained in the present study were within and higher than the range reported by Ouhayoun and Lebas (1994) for 11 wk-old New Zealand rabbits that fasted for 0, 17 and 24 h (15.4, 14.0 and 13.7%, respectively). Our results were in the lower range of those reported by Bianchi *et al.* (2008) for 11 wk-old rabbits that fasted for 3, 9 and 15 h (21.2, 19.8 and 18.6%, respectively). Overall, our results suggest that FW time was efficient for reducing GIT weight.

The caecum weight remained relatively stable regardless of the total FW times (Figure 2) which is consistent with Piattoni *et al.* (1997) for FW times of 0 or 16 h. Similarly, Coppings *et al.* (1989) did not find a difference in caecum weight when rabbits fasted for 12 h, but these authors found lower caecum weights after 24 and 36 h of FW compared to non-fasted rabbits.

Stomach and caecum DM were not influenced by total FW time, which is consistent with Carmichael *et al.* (1945), who reported a limited impact of a 24 h FW time on rabbit stomach and caecum DM. As rabbits are caecotrophs, these authors suggested that the limited impact of a 24 h FW period on stomach and caecum DM was due to the circulation of faeces within the digestive tract. This could also explain the lack of correlation between stomach weight and DM in this study (Figure 5).

Contrary to what was observed by Lang *et al.* (1998) and Friendship *et al.* (2000) in pigs, lower stomach weight was not correlated with a decrease in the stomach pH. The decrease in the stomach pH in pigs was attributed to gastric content mixing and increased fluidity. In this study, caecum pH did not increase with low GIT weight, in contrast to what was observed by Piva *et al.* (1996) and Martín-Peláez *et al.* (2009), who found higher caecum pH when GIT weight was low for pigs. This suggests that coprophagic rabbits react quite differently from monogastric pigs with respect to FW time.

### **Meat quality characteristics**

The effect of FW on rabbit meat quality reported in the literature is varied. Some authors found a lower  $pH_u$  in fasted rabbits (Masoero *et al.*, 1992; Cornejo-Espinoza *et al.*, 2016), whereas others found that the meat of fasted rabbits had a higher  $pH_u$ , darker colour, lower  $a^*$  and lower drip and cooking losses (Ouhayoun and Lebas, 1994; Bianchi *et al.*, 2008; Xiong *et al.*, 2008). In this study, higher  $pH_u$  and lower drip loss have been associated with longer FW at the farm, transport and total FW times. Meat lightness was variable with total FW times, but lower redness ( $a^*$  value) was associated with longer total FW times (Figure 4). Meat colour is also known to be influenced by many other factors including rearing technique, season, *ante-mortem* stress, transport procedures and slaughterhouse conditions (Calnan *et al.*, 2016; Neethling *et al.*, 2017).

The relationships between  $pH_u$ , drip loss and lightness in rabbit meat are well documented (Hulot and Ouhayoun, 1999; Bianchi *et al.*, 2008; Edwards *et al.*, 2010a). When the  $pH_u$  is high, there is an increase in the meat's water-holding capacity (lower drip loss) and a decrease in brightness due to a meat surface that is less reflective (lower L; Hulot and Ouhayoun, 1999; Składanowska-Baryza *et al.*, 2018). In this study, a high  $pH_u$  was also associated with a darker meat colour especially in the LL muscle (Figure 6). The weak correlation between high-pH meat with lower yellowness ( $b^*$  value) supports the results found by Allen *et al.* (1997) in broiler breast meat and Edwards *et al.* (2010a) in pork. However, Edwards *et al.* (2010a) reported a lower  $a^*$  value with a higher  $pH_u$ , which was not observed here in rabbit meat. Gagaoua *et al.* (2018) reported that an increase in  $pH_u$  decreased the colour intensity in beef. These authors also observed a relationship between  $pH_u$  and hue angle (h). This was not observed in the present study for rabbits.

Pre-slaughter stress has been reported to influence meat quality in several domestic animals (Jolley, 1990; María *et al.*, 2006; Rocha *et al.*, 2015). High levels of blood lactate concentration have been associated with a  $pH_{1h} < 6$ , leading to PSE pork (Edward *et al.*, 2010b; Choe and Kim, 2014; Qu *et al.*, 2017). High blood lactate concentration



is a sign of pre-slaughter stress and has been shown to be associated with a low pH 1 h and a low pH 24 h in pigs (Choe *et al.*, 2015; Rocha *et al.*, 2015) and with a low pH 24 h in cattle (Gruber *et al.*, 2010). Unlike in pigs and cattle, higher blood lactate concentration was not associated with lower LL and BF pH<sub>1h</sub> and pH<sub>24h</sub> in the rabbit meat analysed in this study (Figure 6). As indicated above, blood lactate concentrations obtained were not particularly high.

Results for all pH<sub>1h</sub>BF and 97.6% of the pH<sub>1h</sub>LL were higher than 6 (Figure 3). Furthermore, variations in the pH<sub>u</sub> of LL (5.70-6.58) and BF (5.80-6.83) muscles can be as high as one pH unit. These values were within or higher than the reported range for normal rabbit meat (LL: 5.7-5.9, BF: 5.8-6.3; Cullere and Dalle Zotte, 2018). BF pH<sub>u</sub> was higher than LL pH<sub>u</sub>, which is a result of the higher proportion of white fibres present in the LL muscle (Gondret and Bonneau, 1998; Lefaucheur, 2010; Cullere and Dalle Zotte, 2018). For rabbit meat drip loss, low variations (0.0-2.6%) were observed and values were in the lower range of those reported in the literature (0.0-4.2%; Cullere and Dalle Zotte, 2019; Koné *et al.*, 2018, 2019, Składanowska-Baryza *et al.*, 2018). Interestingly, rabbit meat drip loss values were closer to values reported for beef (0.5-5.3%; Holdstock *et al.*, 2014; Hopkins *et al.*, 2014; Puente *et al.*, 2019) than those reported for pork loin (0.0-15.6%; Purslow *et al.*, 2008; Choe and Kim, 2014; Dokmanovic *et al.*, 2015), suggesting that rabbit meat produced in Quebec is not particularly exudative. The rabbit meat tested did not exhibit the characteristics of PSE-like meat. The pH closer to 6 confers the meat a lower drip loss and meat characteristics closer to beef than pork, even though rabbit is viewed as a white meat.

### **Rabbit meat classification**

A hierarchical cluster analysis was performed to define different meat quality groups for rabbits raised in Quebec. Initially, rabbit loins were clustered based on the same parameters used to cluster pork (pH<sub>u</sub>, drip loss and L\*; Correa *et al.*, 2007). As rabbit meat drip loss variation was low (0.00-2.56%), it did not exhibit a notable distinction between the formed clusters, contrary to what has been observed with pork. Cooking loss was used instead, as there was wider variation among the samples (2.9-30.0%), and this variable exhibited a greater distinction between the clusters. Drip loss was not correlated with cooking loss, but both variables evaluated muscle water-holding capacity (Figure 6). Furthermore, a pH<sub>u</sub> higher than 6 is known to be the threshold pH value for DFD meat and is considered undesirable, as it favours microbial growth (Faucitano *et al.*, 2010; Ponnampalam *et al.*, 2017). A low cooking loss is favourable in order to offer a cost effective processing yield (Interbev, 2006). Raw rabbit meat with a bright, pearly pink colour (USDA, 2015) is most favourable for appealing to consumers (Dalle Zotte, 2002; Viljoen *et al.*, 2002).

The pH<sub>u</sub>LL values distinguished clusters 1 and 2, which had pHs lower than 6, from clusters 3 and 4, which had pHs higher than 6. All four groups created with the hierarchical cluster analysis had lower cooking loss and LL lightness (L\*) values than the reported range for normal rabbit loins (cooking loss: 22.5-28.5%; L: 56-60; Table 3; Dalle Zotte, 2000; Cullere and Dalle Zotte, 2018).

Of the four clusters produced in the analysis, clusters 3 and 4 had the lowest meat quality. Indeed, they had a high ultimate pH (pH<sub>u</sub>LL>6) and a darker colour, both of which are characteristics for DFD-like meat (Faucitano *et al.* 2010; Ponnampalam *et al.* 2017). In the current study, high-pH meat represented around 40% of the loins that were analysed and DFD-like meat has been previously reported in the literature for rabbit meat (Jolley, 1990; Koné *et al.*, 2016). The second-best meat quality was found in cluster 2 because it had a pH<sub>u</sub>LL that was lower than 6, the second-highest L value, but also the highest cooking loss compared to the other clusters (Table 3). The best meat quality among the four clusters was found for cluster 1, as the pH<sub>u</sub>LL was lower than 6, its lightness was the closest to those commonly reported for rabbit meat, and its cooking loss is the second lowest of the four clusters. Unfortunately, this cluster only represents 16% of the 200 loins analysed, although when combined, clusters 1 and 2 represent 60% of all the loins tested.

The creation of the four meat quality clusters indicates that there is variability in the meat quality from rabbits raised in Quebec. Rabbits slaughtered in Ontario had a long transport time of 5-7 h compared to 0.17-2.25 h for those slaughtered in Quebec. Despite the long journey, most of the rabbits from E-F1-S were grouped into clusters 1 and 2 (8 and 80%, respectively) compared to B-F1-W (12 and 68%, respectively) and C-F1-W (8 and 52%, respectively; Table 4). Interestingly, E-F1-S has different pre-slaughter management practices before the long transport. Unlike B-F1-W, who turned the lights on in the room and removed the feeders at the same time 2.5 h before crating, and

C-F1-W, who did the same 10 h before crating, E-F1-S turned the lights on at 4 a.m. and began crating 8 h later, during which time the feeders were not removed and the animals had access to feed. Even if domestic rabbits are more active during the day than wild rabbits, they remain nocturnal animals that prefer to eat during the night as opposed to during the day (Lebas, 1997; Trocino and Xiccato, 2006). Thus, the dominant animals were likely to occupy the feeders while the lights were off, leaving the feeders available for subordinate rabbits after the lights were turned on. Therefore, it is possible that by leaving the feeders accessible, rabbits were able to maximise their muscle reserves before the long (7 h) transport journey to the slaughterhouse without access to feed and water (Table 1). This resulted in an ultimate mean pH for the LL muscle of below 6, even when those rabbits underwent a long transport time, whereas the two other lots in the long FW time class 3 were above 6 (Figure 3C).

For rabbits slaughtered in Quebec, A-P1-W had the shortest transport time and more of those rabbit loins were grouped into clusters 1 and 2 (92%) compared to D-P1-W (20%), D-P1-S (60%) and D-P2-S (40%; Table 4). In pigs, it has been reported that shorter transport times (<1 h) may not allow enough time during transport for animals to recover from the stress incurred from loading (Sutherland *et al.*, 2009). A short transport has been reported to cause stress in rabbits, which could accelerate muscle glycogen depletion resulting in more acidic meat when the lairage time before slaughter is also too short (Trocino *et al.*, 2018). These authors reported that rabbits transported for one hour and laired for 30 min before slaughter had a lower pH<sub>LL</sub> (5.57) than rabbits transported for one hour and laired for 3 h (5.71) or transported for 3 h and laired for either 30 min (5.70) or 3 h (5.77). This indicates that rabbits subjected to shorter transport times needed longer lairage times to recover before slaughter. The shorter transport time associated with A-P1-W might explain the low meat pH, notably in the BF muscles (Figure 3D). However, the pH was not so low that it resulted in PSE-like meat.

Another factor that must be considered is that A-P1-W raises different rabbit lines than the other producers. The other producers all raise Grimaud rabbits (Californian×New Zealand White), whereas A-P1-W raises Chinchilla and CLP (Californian×(New Zealand White×Flemish Giant rabbit)). In the literature, differences in meat quality have been reported between different rabbit lines and breeds (Blasco *et al.*, 2018; Hulot and Ouhayoun, 1999).

Rabbit meat quality was also variable within the four lots from producer D and even within the three lots slaughtered in Quebec (Table 4). As meat quality is influenced by many factors, the variability could be due to differences in season (winter vs. summer), the slaughterhouses, the slaughterhouse jurisdiction (provincial vs. federal; Table 1), or may be related to the new staff members that were hired just as the experiments began.

Surprisingly, it was the intermediate FW time class 2 (15.5 to 17.3 h) and not class 3 (≥23 h total FW time) that yielded the lowest meat quality, with more than 50% of the samples belonging to meat quality clusters 3 and 4. Bianchi *et al.* (2008) observed higher pH<sub>u</sub> values, higher water-holding capacity and darker coloured meat with increased total fasting times of up to 15 h, but they concluded that the differences in meat quality were not large enough to rank the product as being of poor or defective quality. However, losses of moisture and nutrients have been shown to affect carcass yield after 6h of FW (Trocino *et al.*, 2003; Bianchi *et al.*, 2008). Furthermore, it has been reported that extending FW times beyond 24 h increases *Enterobacteriaceae* and *Salmonella* shedding in pigs (Martín-Peláez *et al.* 2008, 2009). Such changes in microbial shedding associated with increasing FW times have yet to be established for rabbits.

## CONCLUSION

Overall, rabbit meat quality is variable among the examined sector. The 200 rabbit loins (LL and BF muscles) tested in this study did not exhibit the characteristics of PSE-like meat. When analysed by a hierarchical cluster analysis, 40% of the loins were grouped into clusters 3 and 4, which do exhibit DFD-like characteristics. That said, the majority (60%) of the analysed loins were grouped into the higher quality clusters 1 and 2. The observed variability suggests that many factors are not yet fully controlled or understood within the value chain. In this study, FW, transport and lairage times were evaluated, but other factors that are known to influence meat quality such as transport conditions, loading and unloading procedures, slaughter conditions and *post-mortem* refrigeration rate remain to be investigated.

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