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PRESLAUGHTER FEED WITHDRAWAL AND SEX INFLUENCES ON RABBIT PHYSIOLOGICAL RESPONSE AND MEAT QUALITY

Anne-Sophie Larivière-Lajoie*†, Joanie Lemieux*‡, Dany Cinq-Mars*, Frédéric Guay*, Antoni Dalmau§, Linda Saucier*†

*Département des Sciences Animales, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Quebec City, Quebec, Canada, G1V 0A6.
†Institut sur la Nutrition et les Aliments Fonctionnels (INAF), Université Laval, Quebec City, Quebec, Canada, G1V 0A6.
‡Department of Biochemistry, Microbiology and Bioinformatics, Université Laval, Quebec City, Quebec, Canada, G1V 0A6.
§Institut de Recerca I Tecnologia Agroalimentàries (IRTA), Monells, 17121, Girona, Spain.

Abstract: Although preslaughter feed withdrawal is commonly used in the livestock industry to reduce the risk of viscera puncture during evisceration, the optimal feed withdrawal time (FWT) appears to be species dependent and is not yet well defined in rabbits. This study aimed to evaluate the impact of three preslaughter FWTs while considering the effect of sex on physiological response and meat quality of rabbits. Three FWTs (4, 11 and 18 h) were tested using 144 recently weaned Grimaud rabbits fed until they reached an average commercial slaughter live body weight of 2.5±0.2 kg. Rabbits were allocated into 24 cages, with six animals in each cage. Eight cages (four cages for females and four cages for males) were assigned to each of the three FWTs. Blood lactate concentrations measured at exsanguination did not raise concerns related to stress. Female rabbits subjected to 4 h FWT had a higher gastrointestinal tract weight compared to male rabbits and to other females subjected to 11 and 18 h FWT (P=0.04). Stomach content weights were lower after 18 h FWT compared to 4 and 11 h (P=0.002) for both sexes, while caecum weights were not affected by FWT. Caecum pH was higher after 11 and 18 h FWT compared to 4 h (P=0.02). Technological meat quality was not particularly affected by FWT, except for *Longissimus lumborum* drip loss which was lower for rabbits after 18 h FWT compared to 4 h FWT (P=0.005). With respect to microbiological meat quality, the end of shelf life was reached after 10 days for vacuum-packed hind legs from rabbits after 11 h FWT, as *Escherichia coli* cell counts for four out of the eight hind legs analysed were higher than 3 Log_{10} colony-forming units (CFU)/10 g. For the other two FWT groups, the end of shelf life was reached after 15 d, at which point the cell counts for all hind legs were ≥3 Log_{10} CFU/10 g for *E. coli* and ≥7 Log_{10} CFU/g for total aerobic mesophilic and presumptive lactic acid bacteria (the maximum threshold concentrations according to current regulations). Our results demonstrate that when transport (30 min) and lairage (30 min) times are short (1 h in total), an 18-h preslaughter feed withdrawal for both male and female rabbits can allow the gut to empty properly with limited effect on meat quality.

Key Words: fasting, gastrointestinal tract measurements, meat quality, microbiology, rabbits.

INTRODUCTION

Adequate preslaughter management, such as feed withdrawal (FW), transport and lairage at the slaughterhouse can positively influence rabbit meat quality (Bianchi *et al.*, 2008; Jolley, 1990). Preslaughter FW decreases the risk of viscera puncture during evisceration by reducing the size of the gastrointestinal tract (GIT; Martín-Peláez *et al.*, 2008).

Correspondence: L. Saucier, *linda.saucier@fsaa.ulaval.ca.* Received *January 2024 - August 2024.*
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Feed withdrawal also reduces economic losses for the producer by decreasing post-transport mortality (Petracci *et al.*, 2010). Furthermore, fasting the animals before slaughter reduces the amount of feed that is not metabolised into muscle and limits the non-edible waste at the slaughterhouse (Dalle Zotte, 2002; Eikelenboom *et al.*, 1991). Feed withdrawal time (FWT) is the length of time that an animal is deprived of feed. Feed withdrawal time begins the moment the feeders are empty and ends at the point of slaughter. This period therefore includes the time at the farm, transport and lairage at the slaughterhouse (Verga *et al.*, 2009).

Although FWTs have been associated with benefits, FWTs that are too long have been shown to increase the caecum pH to near-neutral levels in rabbits, which can alter the fermentation pattern and produce favourable conditions for *Enterobacteriaceae* growth (Larivière-Lajoie *et al.*, 2023). Long FWTs can also lead to faecal shedding of *Enterobacteriaceae*, creating contamination risk for the carcasses and threatening product safety (Nattress and Murray, 2000; Faucitano *et al.*, 2010a). Extended FWTs are also associated with poor animal welfare conditions, which is evidenced in pigs that showed increased aggressiveness, with longer and more intense fighting (Faucitano *et al.*, 2006). In the first few hours of FW, live body weight loss is due to reduced stomach weight (Frobose *et al.*, 2014). However, when FWTs are longer, live body weight loss also occurs as a result of the loss of nutrients and humidity in rabbit body tissue, leading to lower meat quality and carcass yield (Bianchi *et al.*, 2008; Cornejo-Espinoza *et al.*, 2016). Extended FWTs can reduce the levels of muscular reserves, which would lead to DFD-like meat with undesirably high pH, higher water-holding capacity and darker colour (Bianchi *et al.*, 2008; Verga *et al.*, 2009; Larivière-Lajoie *et al.*, 2021). In hogs, conducting the majority of the FWT at the slaughterhouse prevented the animals from getting proper rest before slaughter due to longer periods of fighting, which increased the occurrence of dark, firm and dry meat (DFD; Dalla Costa *et al.*, 2016). It has therefore been suggested that it is best to conduct most of the FWT in familiar farm environments to reduce stress on the animals.

The optimal FWT appears to be species dependent and is not yet well defined in rabbits. Literature on the impacts of fasting on rabbit meat quality is limited compared to that of other livestock species. Feed withdrawal times that are between 12 and 18 h for rabbits have been suggested for achieving acceptable stomach weights without having major adverse effects on meat quality (Joseph *et al.*, 1994; Bianchi *et al.*, 2008; Xiong *et al.*, 2008). However, FWTs shorter than 18 h are recommended to limit *Enterobacteriaceae* shedding in faeces (Larivière-Lajoie *et al.*, 2023).

Meat quality defects can cause economic losses for both producers and processors, and may lead to a reduction in meat shelf life (Faucitano *et al.*, 2010b). Animals subjected to acute or chronic stress before slaughter can yield either pale, soft and exudative (PSE) meat or DFD meat, respectively (Adzitey and Nurul, 2011). Meat with a pH lower than 6 at 45 min *post-mortem* (pH_{45min}) is likely to end up as PSE meat, whereas an ultimate pH (pH_u) greater than 6 is likely to produce DFD meat. For rabbits, DFD-like meat has been reported in the literature, but PSE-like meat has not (Cavani *et al.*, 2009; Koné *et al.*, 2016; Larivière-Lajoie *et al.*, 2021). The higher pH_u of DFD meat makes it prone to microbial growth, resulting in a shorter shelf life (Faucitano *et al.*, 2010a; Newton and Gill, 1981). Furthermore, because two of the most important variables driving consumer choice are the colour and consistency of raw meat (Dalle Zotte, 2002), DFD meats have lower market value due to their unattractive appearance (Viljoen *et al.*, 2002).

In rabbit production, sex separation is not a common practice, as there is almost no sexual dimorphism (Dalle Zotte, 2000). However, meat quality characteristics may be affected by sex as sex differences become more pronounced as rabbits approach puberty, and if rabbits are slaughtered at a heavy body weight (>2.5 kg; Trocino *et al.*, 2003; Dalle Zotte *et al.*, 2016). There is currently no consensus on the effect of sex on meat quality for rabbits at commercial slaughter age, since they are slaughtered relatively young. Some authors observed significant differences between sexes on carcass traits and meat quality (Lazzaroni *et al.*, 2009; Pla *et al.*, 1998; Pla and Cervera, 1997), but others did not (Piles *et al.*, 2000; Trocino *et al.*, 2003).

To the best of our knowledge, the few studies that evaluated the effect of FWT on meat quality (Joseph *et al.*, 1994; Bianchi *et al.*, 2008; Xiong *et al.*, 2008) selected FWTs that were rather arbitrary with respect to common farming practices used for other farm animals. The objective of our study was to evaluate the effect of three preslaughter FWTs that were selected according to a preliminary study (Larivière-Lajoie *et al.*, 2023), while considering the effect of sex on rabbit physiology and meat quality, including shelf life.

MATERIALS AND METHODS

Animal housing and feeding

All experimental procedures involving live rabbits were approved by Université Laval's Animal Use and Care Committee (2019017-1), which follows the Canadian Council on Animal Care guidelines (CCAC, 2009).

Our research project evaluated the effects of three FWTs (4, 11 and 18 h) on 144 Grimaud rabbits (Commercial hybrids; Groupe Grimaud, La Corbière, Sèvremoine, France). Recently weaned rabbits (1316±126 g; 38-d old; Laprodéo, Saint-Tite, Quebec, Canada) arrived at the animal research facility of Université Laval and were kept in conventional commercial cages, with 0.37 m^2 of space per rabbit. Each rabbit was weighed upon arrival and distributed into one of the 24 cages according to weight to form homogeneous groups of rabbits within each cage. For each of the three FWTs, there were eight cages of six rabbits (four cages of six females and four cages of six males). The cage of six rabbits was the experimental unit. To ensure that each group was not affected by the FWT of another group, they were housed in three different but similar rooms. The temperature was set at 20°C and the humidity level at 37%. A cycle of 12 h of light and 12 h of darkness was maintained throughout the experiment. Since the animals were sent to the abattoir in the same transport unit, lights were synchronised for each experimental FWT group and kept on for the entire fasting period prior to caging, loading and transport. Animals were fed *ad libitum* with a commercial diet (Supplementary material 1). All rabbits were manually weighed every week to determine individual body weight (BW) and average daily gain (ADG; g/d). Feed was manually weighed before being added to each feeder and refusals were measured at the end of each week to determine average daily feed intake per animal (ADFI; g/d) and feed conversion ratio (FCR). Rabbits were fed until they reached an average commercial slaughter live BW of 2.5 ± 0.2 kg (at 63 d old).

Behavioural observations

Rabbit behaviour was evaluated using the scan sampling method with an observation grid, as described in Larivière-Lajoie *et al.* (2023), to assess the level of stress when the FW procedures began. Prior to the observations, the observer stayed in the room with the rabbits for a period of 10 min to minimise any potential influence of observer presence on rabbit behaviour. For all cages, ten scan samplings were conducted at one-minute intervals for a total of 10 min. Briefly, rabbit posture was first noted. These included sitting, lying down or moving. Then, the observer recorded the occurrence and type of activity (resting, drinking, grooming, biting the cage, mating, being at the empty feeder, moving, shaking, stamping feet, sneezing and scratching the cage). Resting behaviours included sitting and lying down when rabbits exhibited no other activities. Interactions were noted as either aggressive or non-aggressive. Aggressive behaviours included chasing other rabbits, triggering escape, leaping, biting another rabbit, bouncing and scratching. All behavioural assessments were performed by the same observer.

Slaughter procedures

Rabbits fasted for 4 (F4), 11 (F11) and 18 h (F18) before slaughter, including transport and lairage time at the abattoir. Transport time to the abattoir was 30 min and animals were allowed a resting period of 30 min before slaughter. Water remained available prior to crating. The rabbits were slaughtered in a provincially inspected establishment (Saint-Henri, Quebec, Canada) according to current regulations for the province of Quebec, Canada (DGSAIA, 2011). The animals were slaughtered by beheading after head electronarcosis. Rabbits from the F11 group were the first to be slaughtered, followed by F4 and F18.

Blood lactate

One rabbit from each cage (eight rabbits per FWT) was randomly selected on the dressing line. Blood lactate was measured in duplicate at the time of exsanguination using hand-held lactate analysers (Lactate scout +, EKF Diagnostics, Cardiff, Wales, UK) according to the manufacturer's instructions. The same rabbits were used for digesta collection, glycolytic potential in the loin muscle and meat quality measurements, as described below.

Digesta collection

Full gastrointestinal tracts were collected on the slaughter line, then identified and stored on ice until they were weighed. Stomachs and caecum were weighed when full and when emptied of their contents. Stomach and caecum pHs were 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). The dry matter (DM) of the stomach and caecum contents were determined by lyophilisation, as described in Larivière-Lajoie *et al.* (2021).

Glycolytic potential

Samples of the *Longissimus lumborum* (LL) muscle were collected from the region between the last third and fourth left ribs after 1 and 24 h *post-mortem* to evaluate muscle glycolytic potential (GP). All samples were transported on dry ice and immediately frozen at –80°C upon arrival at the laboratory until analysis. Glycolytic potential measurements were performed in triplicate using the modified method from Monin and Sellier (1985), as described by Rocha *et al.* (2015). The glycogen was decomposed to glucose and glucose-6-phosphate, and the lactate concentration was evaluated to calculate the GP according to the following formula: GP=2 ([glycogen]+[glucose]+[glucose-6-phosphate]) +[lactate]. The GP is expressed as glucose equivalent per muscle mass (µmol/g).

Meat quality measurement

Meat quality was determined from right and left LL and *Biceps femoris* (BF) muscles, as described in Larivière-Lajoie *et al.* (2021). Briefly, muscular pH of the LL and BF was measured 1 h (pH 1 h) and 24 h (ultimate pH (pH_u)) *post-mortem*. Meat colour of the LL and BF was evaluated 24 h after slaughter according to the reflectance coordinates (L*, a*, b*; CIE, 1976) and after exposing the cut muscle surface to ambient air for 20 min ("blooming time"; Koné *et al.*, 2019) 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. The parameters used to evaluate meat colour were lightness (L*), redness (a*), yellowness (b*), colour intensity (chroma, C*) and 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):

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

$$H^* = Tan^{-1}(\frac{b^*}{a^*}) \quad (2)$$

For samples with a negative a* value, 180° was added to the calculated H* value (McLellan *et al.*, 1995).

Meat drip loss was evaluated using the modified EZ-DripLoss method from Rasmussen and Anderson (1996). The meat cooking loss was measured on a piece of LL muscle that was processed in a water bath at 70°C for 15 min, as described in Larivière-Lajoie *et al.* (2021). Cooked samples were stored overnight at 4°C before the shear force was measured. The Warner Bratzler shear force was measured on the cooked LL muscle using a texturometer (testXpert II, Zwick Roell Group, Ulm, Germany) with a 500 N load cell and a crosshead speed of 200 mm/min. Two rectangular cross sections of 1×1×2 cm were cut along the fibre axis from each cooked portion (Honikel, 1998) before being sheared perpendicular to the direction of the muscle fibre. The peak shear force was recorded.

Meat packaging

All remaining hind legs were vacuum-packed (Sipromac, St-Germain, QC, Canada) in polyethylene bags (2.5 mils; oxygen transmission 142 cc/100 sq. in. per 24 h at 23°C; moisture vapour transmission rate 0.28 g/100 sq. in. per 24 h at 38°C and 90% relative humidity; Emballage LP Aubut Inc, Quebec City, Quebec, Canada) and stored at 4°C for 0, 5, 10, 15 or 20 d before microbial analysis, as described below. One hind leg per cage, for a total of eight hind legs per FWT at each sampling time, were analysed in duplicate for microbial analysis.

Microbial analysis

Bacterial enumeration was performed for the vacuum-packed hind legs using procedures similar to those detailed in Koné *et al.* (2016). First, the hind leg was aseptically placed in a sterile Stomacher bag (Stomacher® 400C, Seward Laboratory Systems Inc., London, UK) and weighed before adding 300 mL of 0.1% (wt/vol) peptone water (Bacto peptone, Difco Laboratories, Inc., Detroit, MI, USA) to the bag. The bag was sealed and placed on a rocking shaker (200 Rocking Platform Shaker Rotator; VWR, Montréal, Quebec, Canada) for 1 min on each side and manually massaged for 30 s to remove microorganisms from the hind leg surface. The first dilution consisted of the hind leg weight divided by the volume of peptone water used (300 mL). Tenfold dilutions were conducted in 0.1% peptone water before enumeration on agar plates (Saucier *et al.*, 2000).

Total Aerobic Mesophilic (TAM) counts were performed on Plate Count Agar medium (PCA; Merck, Darmstadt, Germany) and incubated at 35°C for 48 h. Total Anaerobic Mesophilic (TAnM) counts were conducted by under anaerobic conditions using an envelope generator of H_2 and CO_2 (BD GasPak™; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Presumptive Lactic Acid Bacteria (LAB) were enumerated on de Man, Rogosa and Sharp (MRS) medium (Oxoid Company, Nepean, Ontario, Canada) and incubated at 25°C for 48 h under anaerobic conditions using the same envelope generator as for the TAnM. Presumptive *Pseudomonas* spp. were evaluated on *Pseudomonas* Agar Base (Oxoid Company, Nepean, Ontario, Canada) supplemented with Cetrimide-Fucidin-Cephalosporin (CFC; Oxoid Company, Nepean, Ontario, Canada) and plates were incubated at 25°C for 48 h. Presumptive *Listeria* spp. were evaluated on PALCAM medium (PALCAM *Listeria* Selective Agar; Sigma-Aldrich, Oakville, Ontario, Canada) without supplement and were incubated at 30°C for 48 h. *Enterobacteriaceae* and presumptive *Staphylococcus aureus* counts were obtained using 3M Petrifilm™ according to the manufacturer's instructions (3M Canada, London, Ontario, Canada) and incubated at 37°C for 24-26 h. Coliforms and *E. coli* counts were also obtained using 3M Petrifilm™ incubated at 35°C for 18-24 h. Duplicates were performed for each medium. The bacterial counts in colony-forming units per g were Log_{10} transformed (Log_{10} CFU/g) prior to statistical analysis (Gill, 2000). Counts for coliforms, *E. coli, Enterobacteriaceae* and presumptive *S. aureus* were Log_{10} transformed from colony-forming units per ten grams (Log CFU/10 g). According to the FDA (1999), a Log reduction equal to or above one indicates a meaningful practical value. Shelf life was measured using a three-class sampling plan (MAPAQ, 2019). For the hind leg, the acceptable concentration (m) was established at under 3 Log_{10} CFU/10g for *E. coli* and 6 Log_{10} CFU/g for TAM and LAB. The unacceptable concentration (M), or the value not to be exceeded by any of the samples tested, was 4 Log_{10} CFU/10g for *E. coli* and 7 Log_{10} CFU/g for TAM and LAB, respectively. For each sampling day, eight hind legs were analysed for each FWT. If the microbial concentrations of more than three of these samples exceeded m but did not exceed M, or if at least one of the samples exceeded M, that entire experimental group was classified as unacceptable.

Statistical analysis

A random experimental design was used to perform the animal trial, for which one experimental unit was the cage of six rabbits. Differences in BW, ADG, ADFI and FCR between treatments and for the entire experimental period were assessed using the R software GLM function (R Core Team, 2017; version 4.1.1). The differences in behavioural parameters between rabbits undergoing various FWTs were determined by analysing data using the R software GLM function with a binomial distribution. For this and all following statistical analyses, results were considered to be significantly different when the *P*-value was lower than 0.05 and considered a tendency when the value was lower than 0.10. If significant ($P<0.05$), differences between experimental groups were compared using the Emmeans R package (version 1.7.0) and an adjusted Tukey test.

When analysing meat quality parameters for the right and left LL and BF muscles, the data from both sides were combined when the correlation coefficient (|r|) exceeded 0.50. Pearson correlation coefficients were calculated using R package corrplot (version 0.92). For blood lactate, gut digesta parameters, muscle metabolites and meat quality characteristics, data were checked for normality and homogeneity of variance. When normality and homogeneity of variance could not be assumed, data were transformed. Values for drip loss were transformed using the square root function to normalise the data prior to analysis. In this statistical model, FWT and rabbit sex were considered as fixed effects. Data were assessed with an analysis of variance (ANOVA) using the R software GLM function (R Core

Team, 2017; version 4.1.1). No suitable transformation could be found to achieve homogeneity of variance for GIT weight and stomach pH values. The R software GLS function from the nlme R package (R Core Team, 2017; version 3.1-152) was therefore used to add the term "weights" in order to account for the heterogeneity of variance. When transformed for statistical analysis, data were back-transformed to their original scale for graphical representation and interpretation. A confidence interval of 95% was used as the dispersion parameter instead of the standard error, as this allows for a better understanding of the dispersion (Lee, 2020).

For the microbial analysis, the data were assessed with an ANOVA using the R software GLM function (R Core Team, 2017; version 4.1.1) to determine how FWT, storage duration under anaerobic conditions and the interaction of these variables affect microbial counts. In this statistical model, FWT and storage duration were considered as fixed effects.

Principal component analysis (PCA) was performed to visualise the dispersion of the gastrointestinal and meat quality parameters according to sex and FWT using the FactoMineR and Factoextra R packages (version 2.4 and 1.0.7; Lê *et al.*, 2008; Kassambara and Mundt, 2020).

RESULTS

Growth performance

No significant interaction was observed between FWT and sex for all growth performance parameters. Initial BW did not vary significantly. Similarly, feed intake did not show significant differences between groups during the fattening period (Table 1). The ADG was higher (P=0.002) for rabbits from F4 compared to F11 and F18 (56.2 *vs.* 51.8 and 52.2 g/d, respectively), but the FCR was lower (P=0.049) for rabbits from F4 compared to F18 (2.71 *vs.* 2.94; Table 1). Rabbits from F4 were 5% heavier than rabbits from F11 and F18 at slaughter (2618.3 *vs.* 2498.2 and 2496.7 g, respectively; P=0.014). No significant differences were observed between female and male rabbits across all growth performance parameters.

Behavioural observations

The animals remained calm during the observation periods prior to FW and hardly moved at all (≤0.8%). They mostly remained sitting or lying down and very few rabbits moved (Figure 1). Rabbits from group F11 remained sitting significantly more during the observation period than rabbits from group F18 (P<0.05). Interactions between animals were limited (<5.9%) and no aggressive interactions were observed (Supplementary material 2). Across all FWTs, rabbits displayed similar levels of activity, regardless of the type of activity (Supplementary material 2). The only instances of rabbits sneezing (0.2%) and scratching the cage (0.4%) was observed for groups F11 and F18, respectively. No stretching, shaking, or foot stamping was observed (Supplementary material 2).

Table 1: Growth performance[1] of weaned rabbits[2] during the 24-d fattening period.

	FWT (h)					Sex			
Variables	4	11	18	SEM[3]	P-value[3]	Female	Male	SEM	P-value
Initial BW (g)[4]	1330.0	1314.3	1304.4	18.5	NS	1303.8	1328.6	15.0	NS
Final BW (g)[4]	2618.3[b]	2498.2[a]	2496.7[a]	33.3	0.014	2524.6	2551.2	27.1	NS
ADG (g/d)	56.2[b]	51.8[a]	52.2[a]	0.9	0.002	53.4	53.4	0.2	NS
ADFI (g/d)	155.76	148.91	159.25	3.85	NS	152.64	156.64	3.32	NS
FCR	2.71[a]	2.91[ab]	2.94[b]	0.07	0.049	2.85	2.86	0.05	NS

[1]BW: body weight; ADG: average daily weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio.

[2]Results are expressed as means of eight cages of six rabbits per treatment (four cages with six males and four cages with six females).

[3]SEM: standard error of the mean. Different letters (a, b) within a row indicate significant differences at P<0.05. NS: not significant. Tukey's test was carried out to compare the differences between treatments. No interactions were found between FWT and sex for the variables tested.

[4]Initial BW: body weight at the beginning of the fattening period; Final BW: body weight at the end of the fattening period.

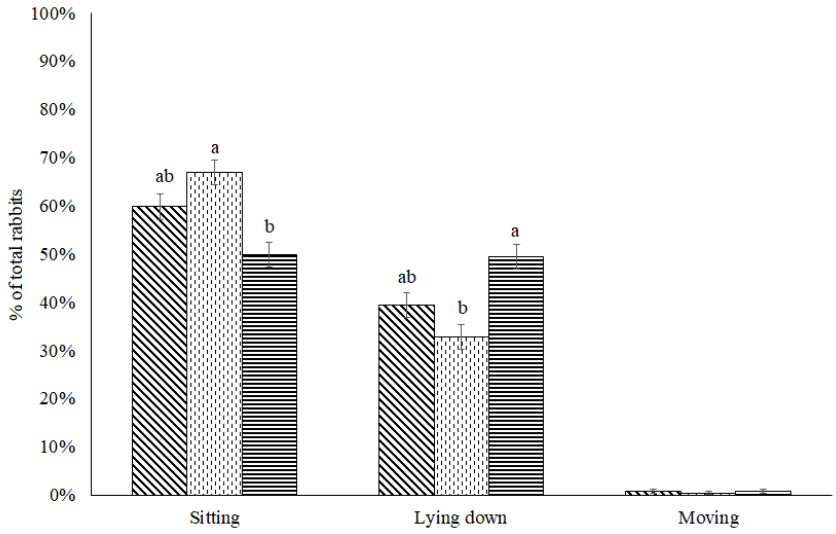

Figure 1: Percentage of rabbits sitting, lying down or moving during the observation periods prior to FW (mean±standard error). FWT*Sex: NS. FWT: $P<0.05$. Sex: NS. For each behaviour type, bars with different letters are significantly different from each other with $P<0.05$. FWT: feed withdrawal time. FWT*Sex: interaction between FWT and sex. NS: not significant. F4, F11 and F18: 4, 11 and 18 h of FWT, respectively. F4, F11, F18.

Blood lactate concentration and gastrointestinal content composition

No interactions between the FWT and sex were observed for blood lactate concentration and gastrointestinal content composition, except whole GIT weight and stomach pH. Even though the average blood lactate concentration for the F4 group was almost twice as high as for F11 and F18, the levels were not significantly different between the three groups. Concentrations for F4 varied from 2.65 to 16.35 mmol/L (variation of 13.70), whereas concentrations varied from 0.75 to 9.40 mmol/L for F11 (variation of 8.65) and 1.10 to 10.15 mmol/L for F18 (variation of 9.05; data not shown).

Significant interactions between FWT and sex were observed for whole GIT weights ($P=0.04$; Figure 2A) and stomach pH ($P=0.03$; Figure 2B). Whole GIT weights for female rabbits that experienced 4 h of FWT were the highest of all the FWTs tested. Female rabbits with 11 h FWT had lower stomach pH than female rabbits with 4 h FWT and male rabbits with 11 h and 18 h of FWT. Stomach pH of male rabbits did not vary significantly between FWTs and was similar to female rabbits with 4 and 18 h of FWT (Figure 2B). Overall, a wide range of stomach pH was recorded, with values ranging from 1.02 to 2.85.

Caecum weight and DM were not affected by the FWTs (Table 2). However, stomach content weight and DM as well as caecum pH were significantly affected by the FWTs (Table 2; all $P<0.020$). Stomach content weight was lower for rabbits with 18 h FWT compared to rabbits with 4 h FWT (27.2 *vs.* 56.3 g; $P=0.002$). Stomach DM significantly decreased with increasing FWTs, ranging from 18.1% after 4 h FWT to 9.7% after 18 h FWT (Table 2; $P<0.001$). Conversely, caecum pH increased significantly from 6.30 after 4 h FWT to 6.55 after 11 h FWT. It then remained relatively stable at 6.61 when FWT was 18 h ($P=0.020$). Sex had no effect on those physiological parameters (Table 2).

Muscle-metabolite concentrations and meat quality characteristics

No interactions were observed between FWT and sex for any of the muscle metabolites and meat quality characteristics measured, except for LL lactate 1 h post-slaughter (Figure 3; $P<0.001$). Concentrations of LL lactate were higher for female rabbits at 4 h compared to 11 and 18 h FWTs. Of the three FWTs tested, the highest LL lactate

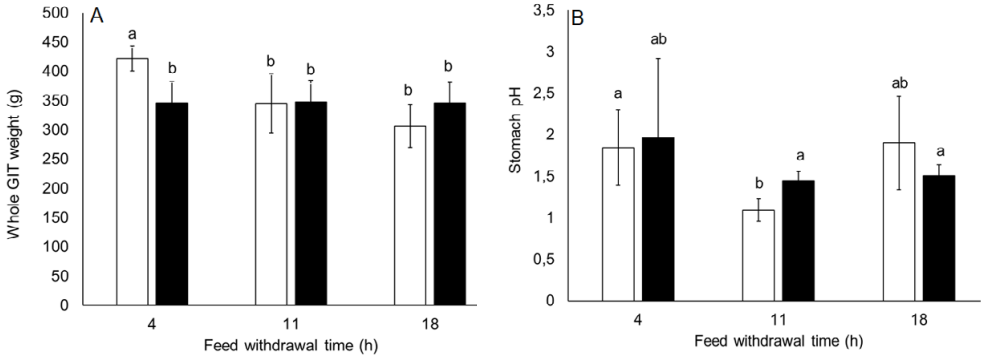

Figure 2: Whole gastrointestinal tract weight (A; FWT*Sex: *P*=0.04, FWT: *P*=0.006, Sex: *P*=0.007) and stomach pH (B; FWT*Sex: *P*=0.03, FWT: *P*=0.001, Sex: *P*=0.001) of rabbits after FWTs of 4, 11 and 18 h. Each value represents the mean for four rabbits with a 95% confidence interval. Means with different letters (a, b) indicate a significant difference at *P*<0.05. GIT: gastrointestinal tract. FWT: feed withdrawal time. FWT*Sex: interaction between FWT and sex. □ Female. ■ Male.

concentration for males was observed at 11 h FWT. After 4 h FWT, concentrations of LL lactate were higher for female rabbits compared to males, but after 11 and 18 h FWT, concentrations for both females and males were similar. No significant differences were found between FWTs or sex for LL lactate at 24 h *post-mortem*, the LL Glucose-6-P or LL GP at 1 or 24 h *post-mortem* (Supplementary material 3). The 1 h *post-mortem* LL GP concentration decreased from 146.50 µmol/g for rabbits with 4 h FWT to 134.53 µmol/g for 18 h FWT, and the 24 h *post-mortem* LL GP decreased from 129.44 µmol/g for rabbits with 4 h FWT to 121.92 µmol/g for 18 h FWT.

Meat quality characteristics (Table 3) showed little variation with FWT and sex. Only meat drip loss decreased significantly with FWT (*P*=0.005), from 1.18% after 4 h FWT to 0.35% after 18 h FWT (Table 3). The LL and BF pH 1 h and the LL pH_u were not affected by the FWTs. *Longissimus lumborum* pH_u was ≤6 for all FWTs and sexes (Table 3). The BF pH_u tended to be higher for F18 compared to F4 (*P*=0.076) and all mean BF pH_u values were higher than 6. Overall, individual BF pH_u measures ranged between 5.92 and 6.27. Female rabbits also tended to have higher BF pH_u compared to males (*P*=0.058), although only a limited pH variation of 0.06 was observed. No significant differences were observed between FWTs for cooking loss or the shear force for both right and left LL muscles (Table 3).

Table 2: Physiological parameters measured after rabbits were slaughtered[1].

	FWT (h)					Sex			
Variables	4	11	18	SEM	*P*-value	Female	Male	SEM	*P*-value
Blood lactate (mmol/L)	7.9	4.6	4.2	1.4	NS	4.9	6.2	1.1	NS
Stomach content weight (g)	56.3^{b}	47.8^{b}	27.2^{a}	5.5	0.002	47.3	40.2	4.5	NS
Stomach DM (%)	18.1^{c}	13.8^{b}	9.7^{a}	1.1	<0.001	15.1	12.6	0.9	NS
Caecum weight (g)	52.8	46.6	53.5	7.5	NS	51.5	50.4	6.1	NS
Caecum pH	6.30^{a}	6.55^{b}	6.61^{b}	0.06	0.020	6.51	6.46	0.05	NS
Caecum DM (%)	22.6	22.9	23.2	0.5	NS	23.1	22.8	0.4	NS

[1]Results are expressed as means of eight rabbits per treatment, one from each cage (four females and four males) for FWT and n=12 for sex.

Different letters (a–c) within a row indicate significant difference at *P*<0.05. NS: not significant. Tukey's test was carried out to compare the differences between treatments. No interactions were found between feed withdrawal time and sex for these variables. FWT: feed withdrawal time. DM: dry matter. SEM: standard error of the mean.

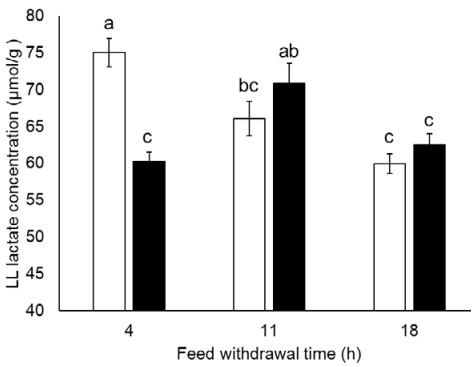

Figure 3: Effect of FWT on *Longissimus lumborum* (LL) muscle lactate concentration 1 h *post-mortem*. Each value represents the mean of four rabbits with the standard error. FWT*Sex: $P<0.001$. FWT: $P=0.003$. Sex: NS. Means with different letters (a–c) indicate significant differences at $P<0.05$. FWT: feed withdrawal time. FWT*Sex: interaction between FWT and sex. ☐ Female. ■ Male.

Overall, the impact of FWT and sex on meat colour was limited. No significant interactions between FWT and sex were found for both LL and BF muscles. Feed withdrawal times did have a significant effect on LL hue angle (H*; Table 4), which was lower for 11 h FWT compared to 4 h FWT ($P=0.040$). For the BF muscle H*, only a tendency was observed ($P=0.056$). The LL muscle tended to be redder (a* higher) for rabbits after 11 h FWT compared to 4 h FWT ($P=0.052$; Table 4). With respect to sex, the LL muscle was yellower for males than for female rabbits (3.50 *vs.* 2.78; $P=0.041$). Female rabbit LL muscles tended to have a lower hue angle than those of male rabbits ($P=0.056$).

Table 3: Rabbit meat quality characteristics according to FWT and sex[1].

	FWT (h)					Sex			
Variables	4	11	18	SEM[2]	*P*-value[2]	Female	Male	SEM	*P*-value
BF muscle									
pH (1 h)[3]	6.56	6.58	6.62	0.07	NS	6.61	6.57	0.05	NS
pH (24 h)[3]	6.05^{x}	6.07^{xy}	6.12^{y}	0.07	0.076	6.11	6.05	0.02	0.058
LL muscle									
pH (1 h)	6.55	6.42	6.52	0.07	NS	6.50	6.49	0.06	NS
pH (24 h)	5.88	5.86	5.91	0.02	NS	5.90	5.87	0.02	NS
Drip loss (%)[4]	1.18^{b}	0.75^{ab}	0.35^{a}	0.22	0.005	0.89	0.63	0.19	NS
Cooking loss right LL muscle (%)[5]	15.9	17.99	15.45	1.08	NS	16.44	16.49	0.89	NS
Cooking loss left LL muscle (%)[5]	15.70	17.89	14.17	1.46	NS	16.34	15.50	1.19	NS
Shear force left LL muscle (N)[5]	34.67	22.27	23.89	4.75	NS	25.66	28.23	3.88	NS
Shear force right LL muscle (N)[5]	23.47	21.73	30.25	2.89	NS	26.22	24.08	2.36	NS

[1]Results are expressed as the mean of eight rabbits per treatment, one from each cage (four females and four males) for feed withdrawal times and n=12 for sex. LL=*Longissimus lumborum* muscle. BF=*Biceps femoris* muscle.

[2]SEM: standard error of the mean. Different letters within a row indicate (a, b) significant differences at $P<0.05$ and (x, y) at $P<0.10$. NS: not significant. Tukey's test was carried out to compare differences between treatments. No interactions were found between FWT and sex for these variables.

[3]pH (1 h) and pH (24 h)=pH measured 1 h and 24 h *post-mortem.*

[4]Drip loss was measured in the LL muscle.

[5]Because weak correlations ($|r|<0.50$) were found between right and left LL muscles for cooking loss and shear force values ($r=0.13$ and 0.23, respectively), both muscles are presented separately.

Table 4: Rabbit meat colour of the *Longissimus lumborum* (LL) and *Biceps femoris* (BF) muscles according to FWT and sex[1].

	FWT (h)					Sex			
	4	11	18	SEM[2]	*P*-value[2]	Female	Male	SEM	*P*-value
BF muscle[3]									
L*	50.36	48.66	49.30	0.70	NS	49.72	49.16	0.57	NS
a*	4.85	5.18	4.91	0.33	NS	4.79	5.16	0.27	NS
b*	2.88	2.20	2.22	0.31	NS	2.30	2.57	0.25	NS
C*	16.46	16.75	15.00	2.33	NS	14.61	17.54	1.90	NS
H*	29.9^{y}	22.7^{x}	24.1^{xy}	2.13	0.063	25.27	25.90	1.74	NS
LL muscle[3]									
L*	51.11	49.15	49.96	0.72	NS	49.80	50.34	0.60	NS
a*	5.50^{x}	6.56^{y}	6.07^{xy}	0.28	0.052	5.92	6.16	0.23	NS
b*	3.23	2.81	3.38	0.28	NS	2.78	3.50	0.23	0.041
C*	21.03	26.18	24.42	2.34	NS	21.77	25.99	1.91	NS
H*	30.29^{b}	22.82^{a}	29.05^{ab}	2.03	0.040	24.99	29.78	1.66	0.056

[1]Results are expressed as means of eight rabbits per treatment, one from each cage (four females and four males) for FWT and n=12 for sex.
[2]SEM: standard error of the mean. Different letters within a row indicate (a, b) significant differences at $P<0.05$ and (x, y) tendencies at $P<0.10$. NS: not significant. NS: not significant. Tukey's test was carried out to compare differences between treatments. No interactions were found between feed withdrawal time and sex for these variables.
[3]BF=*Biceps femoris*, LL=*Longissimus lumborum*, L*=lightness, a*=redness, b*=yellowness, C*=chroma, H*=hue angle.

To visualise the effect of FWT (Figure 4A) and sex (Figure 4B) on gastrointestinal content composition and meat quality, a principal component analysis (PCA) was performed. Rabbits that fasted for 4 or 11 h showed a more heterogeneous distribution in the PCA compared to those that fasted for 18 h (Figure 4A). Values from rabbits that fasted 18 h are thus closer together compared to the other two groups. As for the effect of sex, male rabbits had higher variability than females for gastrointestinal content composition and meat quality (Figure 4B).

Microbial analysis and shelf life of vacuum-packed rabbit hind legs stored at 4°C

Microbial analyses indicated that all bacterial cell counts increased significantly over the storage period (all $P<0.001$; Figure 5). Significant interactions between FWT and storage time were found for all bacterial counts tested (all

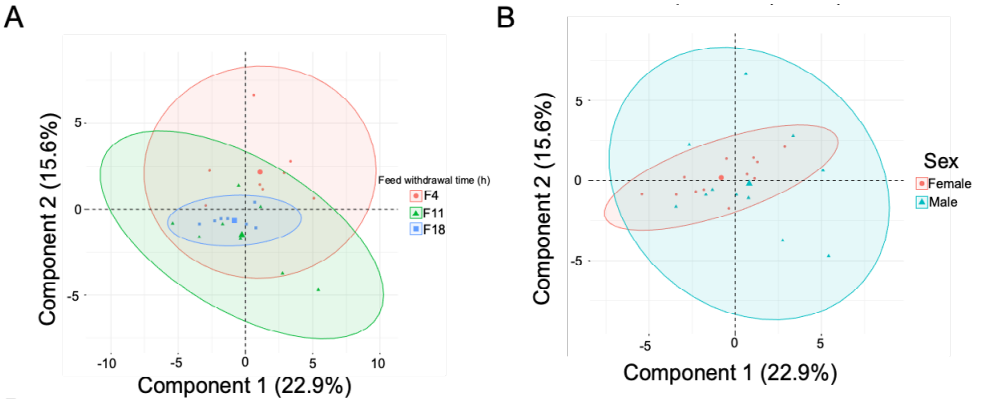

Figure 4: Principal component analysis (PCA) illustrating physiological and rabbit meat quality parameters according to FWT (A) and sex (B). Ellipses represent a 95% confidence level.

P=0.04). The exception was presumptive *S. aureus* (Figure 5l), which remained relatively low (<1.59 Log_{10}/10 g) and was not significantly affected by FWT. The initial contamination levels for TAM and presumptive *Pseudomonas* spp. counts were higher for F11 compared to F4 and F18 groups. After 5 d at 4°C, TAM, TAnM, LAB and *Enterobacteriaceae* counts were significantly higher for F11 compared to F4 and F18. The differences for those cell counts between groups F11 and F4 exceeded 1 Log, while 1 Log was exceeded only for TAM and TAnM between F11 and F18. Presumptive *Pseudomonas* spp. counts were also higher for F11 compared to F4 only, but the difference was just below 1 Log (0.89 Log_{10} CFU/g). The end of shelf life was reached after 10 d for the F11 group according to the three-class sampling plan (MAPAQ, 2019), as cell counts for four out of eight hind legs were higher than 3 Log_{10} CFU/10 g for *E. coli*. Cell counts for TAM and LAB reached 7 Log_{10} CFU/g after 15 d of storage. For F4 and F18, the end of shelf life was reached after 15 d, at which point cell counts from all hind legs were ≥3 Log_{10} CFU/10 g for *E. coli* and ≥7 Log_{10} CFU/g for TAM and LAB, exceeding maximum allowable concentrations according to current regulations. Cell counts were similar for all three FWTs after 20 d of storage, except for *E. coli* and *Listeria* spp. counts which were higher for the F11 group compared to the two other groups (both *P*≤0.002). Overall, the microbial quality of the vacuum-packed hind legs was greater for the F4 and F18 groups.

DISCUSSION

Although rabbits were all obtained from the same farm, at the same age and raised in similar rooms with the same temperature and humidity levels, rabbits from the F4 group exhibited higher ADGs at the end the growing period and before the FWTs compared to F11 and F18. F4 rabbits also had a lower FCR (2.71) compared to F18 rabbits (2.94; Table 1). Water was not available for the F4 rabbits for eight consecutive hours upon arrival due to a technical problem. This might explain, at least in part, the difference in growth performance, but it may have been influenced by other unknown confounding factors. According to Guidenne and Lebas (2005), FCR typically ranges from 2.2 to 3.8 for rabbits aged 5 to 10 wk. Consistent with that range, all three groups had a FCR lower than 3.4 at the end of the 9-wk growth period, despite the significant difference in FCR between F4 and F18. All rabbits were slaughtered at an average live BW of 2.46 to 2.65 kg, which is the commercial weight for slaughter in the province of Quebec (Cliche and Sylvestre, 2009). Hence, the difference observed is likely due to individual variation. According to Dalle Zotte (2002), no relevant differences in technological meat quality is observable when rabbits are slaughtered at the same age but at different slaughter weights.

With respect to animal welfare, rabbit behaviour did not raise any particular concerns prior to FW. Elevated blood lactate concentrations are associated with preslaughter stress, which has an adverse effect on rabbit welfare (Fazio *et al.*, 2015; Nakyinsige *et al.*, 2013; Trocino *et al.*, 2018). In our study, blood lactate levels were not affected by the FWTs or influenced by sex. This lack of significant difference may be attributed to the high variability that was observed for blood lactate concentrations within each FWT group. Nonetheless, the mean values remained within the range reported in Langlois *et al.* (2014) for healthy rabbits (6.9±2.7 mmol/L). This suggests that none of the FWTs, including the longest FWT of 18 h, induced significant levels of stress or modifications to rabbit activity under our experimental conditions. In pigs, it has been reported that when most of the FWT occurs on the farm in a familiar environment, stress is minimised (Dalla Costa *et al.*, 2016). In rabbits, Larivière-Lajoie *et al.* (2023) also reported that fasting alone is not particularly stressful for rabbits when done in a familiar environment.

To the best of our knowledge, this is the first publication evaluating the interactions between FWT and sex for gastrointestinal content composition (stomach and caecum weight, pH and DM) in rabbits that are slaughtered for meat consumption. Female rabbits had a significantly higher GIT after a 4 h FWT compared to males, but not after 11 and 18 h of FWT. It has been reported that female rabbits have a higher feed intake and a higher GIT weight at slaughter when they are not fasted compared to male rabbits (Lazzaroni *et al.*, 2009; Pla, 2008). Even if the female rabbits consumed more feed prior to FW, our results suggest that feed was digested after 11 and 18 h of FW based on the similar GIT weights for both females and males in the F11 and F18 groups. This might explain the absence of sex-related differences for longer FWTs.

Rabbits typically consume their caecotrophs between 8 and 12 h after a meal (Lebas *et al.*, 1997). Caecotrophs are swallowed without mastication and stored in the stomach (Hörnicke, 1981). The bacteria inside the caecotrophs produce amylase, which degrades feed starch into maltose and glucose. These are then converted into mainly volatile

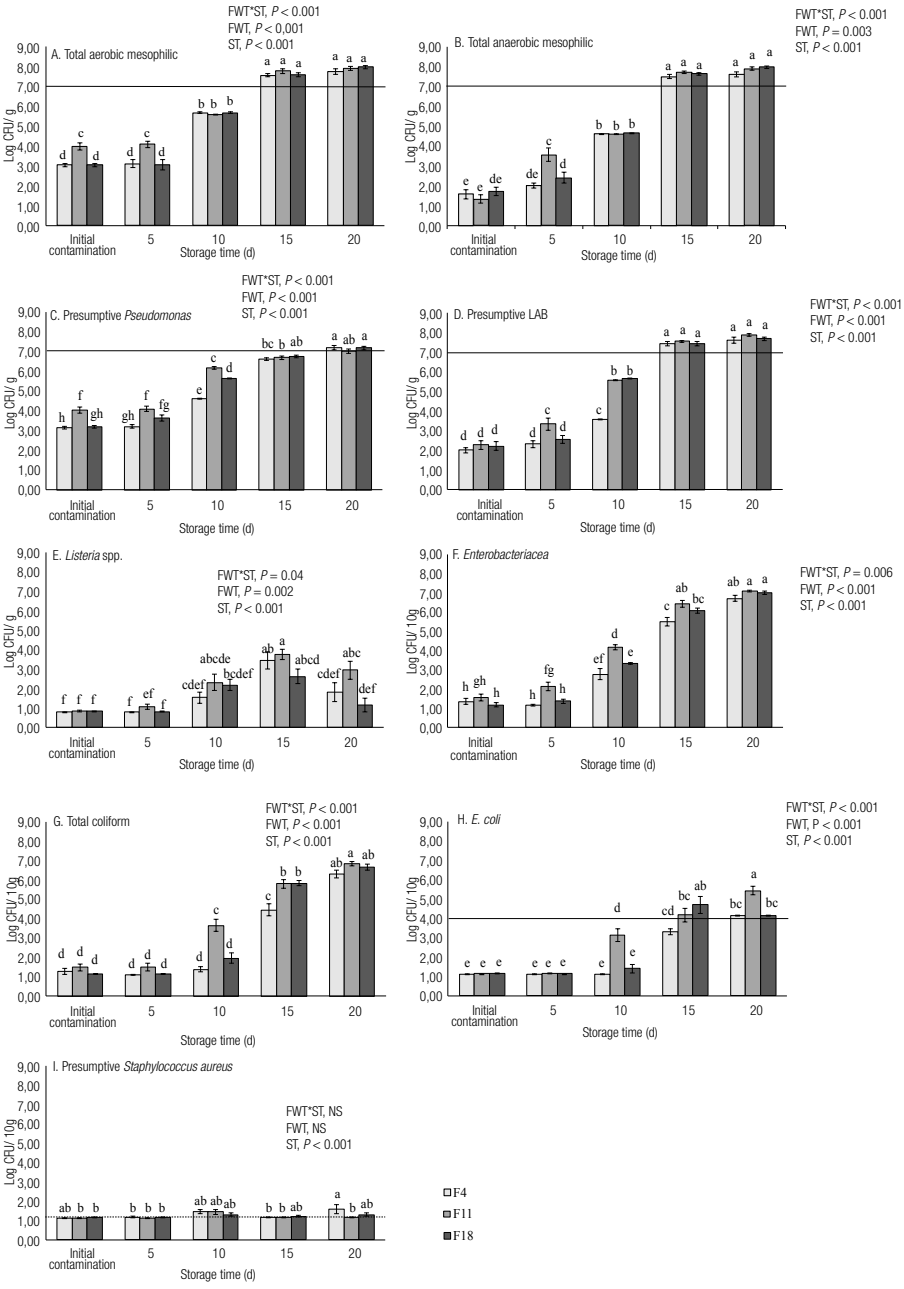

Figure 5: Bacterial cell counts in vacuum-packed rabbit hind legs after 0 to 20 d of storage at 4°C. Total aerobic mesophilic (A), total anaerobic mesophilic (B), presumptive *Pseudomonas* spp. (C), presumptive lactic acid bacteria (LAB; D) and *Listeria* spp. (E) counts were in Log_{10} CFU/g. *Enterobacteriaceae* (F), total coliform (G), *Escherichia coli* (H) and presumptive *Staphylococcus aureus* (I) counts were in Log_{10} CFU/10 g. Each point is a mean of eight rabbit hind legs analysed at each sampling time with the standard error. The horizontal line indicates end of shelf life. The dotted line indicates detection limits. Means with different letters (a–h) indicate significant differences at $P<0.05$. NS: not significant, FWT: feed withdrawal time, ST: storage time and FWT*ST: interaction between FWT and ST. F4, F11 and F18: 4, 11 and 18 h of FWT, respectively.

fatty acids as a result of bacterial metabolism (Hörnicke, 1981). Our data suggest that the production of volatile fatty acids may have contributed to the lower stomach pH that we observed in female rabbits compared to males at 11 h FWT (Figure 2B). This could be attributed to the higher feed intake of females prior to FW as shown by the heavier GIT after a 4 h FWT (Figure 2A).

The lower stomach content weight at 18 h FWT compared to 4 h FWT that we observed was expected, since the 18 h FWT rabbits had gone longer without feeding. These results are consistent with Coppings *et al.* (1989) and Larivière-Lajoie *et al.* (2023), who reported a reduction in stomach weight in rabbits with increasing FWTs of up to 12 h. Our study showed similar stomach content weights at 4 h and 11 h FWT, which might be due to the caecotrophic behaviour of the rabbits during FW. However, stomach content decreased from 4 to 18 h FWT (Table 2). Since soft faeces have a DM content ranging from 18 to 37% (Lebas *et al.*, 1997), the impact of ingesting soft faeces on stomach DM content could be limited. Conversely, Carmichael *et al.* (1945) showed that a 24 h FWT had limited impacts on rabbit stomach DM compared to non-fasted rabbits.

No differences were observed for caecum weights with the FWTs applied (Table 2). Similarly, Coppings *et al.* (1989) and Carmichael *et al.* (1945) reported that a 12 and 24 h FWT, respectively, had a limited impact on rabbit caecum weight. However, when they compared rabbits that fasted with a muzzle for 48 h to prevent caecotrophy, they observed lower caecum dry content weights. This could indicate that the ingestion of caecotrophs contributes to the DM content and weight of the caecum during FW. More studies are needed to confirm this hypothesis. Similar to findings in Ribeiro *et al.* (2021) for rabbits slaughtered at 2.5-2.7 kg, no differences were observed between sexes for stomach and caecum weights or for caecum pH and DM in our study.

An increase in caecum pH was observed with longer FWTs (Table 2). Similarly, Vernay *et al.* (1975) observed an increase from 6.2±0.2 to 6.9±0.1 after a FWT of 48 h. Piattoni *et al.* (1997) reported a caecum pH of 6.7±0.1 after a 16 h FWT compared to 6.2±0.2 for non-fasted rabbits. Martín-Peláez *et al.* (2008) suggested that for longer FWTs in pigs, an increase in caecum pH may be caused by a decrease in available fermentable substrate. This leads to reduced production of short-chain fatty acids and causes changes in the microbiota profile. As caecum pH approaches near-neutral levels, one undesirable effect is an environment that is favourable for the growth of bacteria such as *Salmonella* spp. and *E. coli* (Lebas *et al.*, 1997; Martín-Peláez *et al.*, 2009; Eicher *et al.*, 2017). Further studies are necessary to confirm this hypothesis in rabbits.

Meat quality characteristics

A significant interaction was only found between FWT and sex for LL lactate concentrations at 1 h *post-mortem*. The LL lactate concentration 1 h *post-mortem* was lower for males compared to females after a 4 h FWT. One possible explanation for these results is that male rabbits in the F4 group were more active during their FW period compared to females in the same group, resulting in lower lactate concentrations in the LL muscle in males. However, this difference was not observed for the other two FWT groups. The LL lactate concentrations were lower at 18 h FWT compared to 11 h for males and at 18 h FWT compared to 4 h for females (Figure 3). A decline in LL lactate concentrations is expected with longer FWTs. Since the animals still need energy to maintain their muscle metabolism and posture throughout FW, the glycogen energy reserves in their muscles are used but not replenished due to the lack of feed (Bertol *et al.*, 2005; Leheska *et al.*, 2002).

Depletion of muscle glycogen reserves during preslaughter FW could lead to an increase in muscle pH_u, water-holding capacity and darker meat colour (Bianchi *et al.*, 2008; Lambertini *et al.*, 2006; Xiong *et al.*, 2008). However, there is no consensus in the literature as to the exact effect of FW on meat quality. Some authors have observed a lower pH_u in fasted rabbits (Masoero *et al.*,1992; Cornejo-Espinoza *et al.*, 2016). Muscle GP can be used as an indicator of the muscle's capacity to support glycolysis *post-mortem*. In our study, we found that FWT had no effect on LL GP concentration (Supplementary material 3) and the values observed at 1 h *post-mortem* were similar to those reported by Larivière-Lajoie *et al.* (2023) for rabbits at slaughter (134.53-146.50 µmol/L *vs.* 122.12-151.55 µmol/L). To the best of our knowledge, our study is the first to measure the effects of FWT on LL GP concentration at 24 h *post-mortem* in commercial slaughter conditions.

Except for LL drip loss (Table 3), FWT did not adversely affect technological meat quality including meat colour (Table 4). This result is supported by the limited difference in LL GP between FWT groups, suggesting that the FWTs applied did not deplete the muscle's energy reserves enough to affect meat quality. Our results differ from those of Bianchi *et al.* (2008), who observed a higher LL pH_u, darker meat and lower cooking loss at 9 or 15 h FWT compared to 3 h FWT in rabbits. The literature on pigs suggests that when FW is not combined with other preslaughter practices (e.g., mixing animals not familiar to one another, high ambient temperature, long transport and lairage time, etc.), muscle glycogen does not deplete enough to affect meat quality (Driessen *et al.*, 2020; Faucitano *et al.*, 2006). In our study, LL drip loss decreased with longer FWTs, but LL pH_u did not. Previous studies suggest that even when a correlation between pH_u and drip loss is present (Hulot and Ouhayoun, 1999; Składanowska-Baryza *et al.*, 2018), it is not always strong (Huff-Lonergan *et al.*, 2005; Larivière-Lajoie *et al.*, 2021). Overall, even if some significant differences or tendencies were observed between FWT and meat quality, the effects were limited and should not raise major concerns for commercial practices. Similar results were found by Xiong *et al.* (2008) who reported that withholding feed before slaughter for 16-18 h did not result in a significant deterioration of meat quality. Hence, fasting rabbits for up to 18 h appears acceptable.

The literature on the effects of sex on meat quality in rabbits reports varied results. Trocino *et al.* (2003) found that rabbit meat from females had a darker colour (lower L*) compared to that of males. In another study, Cavani *et al.* (2000) found that rabbit meat from males was redder (higher a*) and more coloured (higher C*) than that of females, but no effect was found for meat water-holding capacity, cooking loss, pH_u LL, pH_u BF, LL L*, LL b* and hue angle. Rabbit sex only had a significant effect on LL muscle colour, where rabbit meat from males was yellower than from females (Table 4). Overall, the impact of rabbit sex on meat quality was limited when rabbits were slaughtered at weights ranging from 2.45 to 2.65 kg.

Microbial analysis and shelf life of vacuum-packed rabbit hind legs stored at 4°C

Preslaughter FW is used to reduce GIT weight which in turn decreases puncture risk during evisceration and helps to reduce carcass contamination (Bianchi *et al.*, 2008). In rabbits, to the best of our knowledge, no study has evaluated the effect of FWT on microbiological meat quality. However, when FW is too long for pigs, faecal shedding of undesirable *Enterobacteriaceae* such as *Salmonella* spp. and *E. coli* increased, creating contamination risk for the carcasses and threatening product hygiene and safety (Nattress and Murray, 2000; Faucitano *et al.*, 2010a). On pig carcasses, Saucier *et al.* (2007) reported that TAM counts were higher after a 14 h FWT compared to a 4 h FWT (2.23 versus 1.99 CFU/cm^2), and to a lesser extent, after a 24 h FWT (2.23 *vs.* 2.08 CFU/cm^2).

The shelf life of vacuum-packed rabbit hind legs stored at 4°C was 15 d for groups F4 and F18, consistent with results from Koné *et al.* (2018). For F11, the end of shelf life was 10 d. Higher bacterial counts for TAM and presumptive *Pseudomonas* spp. were observed at the beginning of the storage period for F11, but counts were relatively similar to F4 and F18 near the end of the storage period (Figure 4). This might have been caused by the slaughter order of rabbits. Rabbits from the F11 group were the first to be slaughtered on a Monday morning after the slaughterhouse had been inactive for two days. There is a possibility that some bacteria that survived the cleaning and sanitising process on the previous Friday formed biofilms during the inactive period (Møretrø *et al.*, 2013). A previous study identified *Pseudomonas* as the dominant genus in a bovine slaughterhouse after cleaning and disinfection (Møretrø *et al.*, 2013), and *Pseudomonas* cell counts were in fact higher in the initial contamination of F11 rabbits. More research is needed to confirm this hypothesis by evaluating the microbial contamination in slaughterhouses before and after a significant period of inactivity. One could also argue that because the rabbits were raised in different rooms, the room could be a factor influencing rabbit microbiota, but this remains to be tested. Overall, rabbits can fast up to 18 h with limited effects on meat microbiological quality.

CONCLUSION

In the current study, the FWTs were chosen based on a preliminary study that examined the rabbit's physiology during preslaughter feed withdrawal. The results obtained demonstrate that when transport and lairage times are short (30 min each phase), an 18 h FWT can allow the rabbit gut to empty with limited effects on meat quality. Feed withdrawal of 4 and 11 h is not recommended, as this led to higher stomach content weights compared to 18-h of

FWT, increasing the risk of contamination at slaughter through stomach puncturing. In our study, an 18 h FWT did not result in more stress for rabbits. The FWT tested had limited impacts on technological meat quality, as all three FWTs produced meat of acceptable commercial quality and the F18 group (18 h FWT) produced meat with lower drip loss. The microbiological meat quality was lower when rabbits fasted for 11 h. The impact of rabbit sex on gastrointestinal parameters and meat quality was limited when rabbits were slaughtered at weights lower than 2.65 kg. However, the lower microbial meat quality found for rabbits that fasted 11 h needs to be investigated further, as well as the meat quality for rabbits that fasted between 11 to 18 h, since it was not measured in the preliminary study.

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SUPPLEMENTARY MATERIALS

Supplementary material 1: Nutritional value of the commercial diet[1,2] fed to rabbits used in this study.

Nutrients	Commercial data
Dry matter (%)	94.00
Digestible Energy (kcal/kg)	2810
Crude protein (%)	17.02
Crude fat (%)	4.90
Crude fibre (%)	19.25
Calcium (%)	1.06
Phosphorous (%)	0.47
Sodium (%)	0.32
Chloride (%)	0.54
Magnesium (%)	0.32
Potassium (%)	1.18
Sulphur (%)	0.27
Iron (mg/kg)	175.77
Zinc (mg/kg)	49.18
Manganese (mg/kg)	26.27
Copper (mg/kg)	125.00
Iodine (mg/kg)	0.20
Vitamin A (UI/kg)	64.19
Vitamin D (UI/kg)	10.83
Vitamin E (UI/kg)	42.55
Total selenium (mg/kg)	0.20
Added selenium (mg/kg)	0.11
All B vitamins (mg/kg)	1512.23

[1]Belisle Solution Nutrition, St-Mathias-sur-Richelieu, Quebec, Canada.

[2]Ground alfalfa, beet pulp, durum wheat, soybean meal, canola meal, soybean shell, soybean oil, molasses, corn gluten feed.

Supplementary material 2: Percentage (%) of rabbits presenting different interactions and activities during the observation period prior to FW[1].

	FWT (h)					Sex			
Variables	4	11	18	SEM[2]	*P*-value[2]	Female	Male	SEM	*P*-value
Interaction, %									
Non-aggressive	4.9	3.6	5.9	1.0	NS	3.9	5.7	1.1	NS
Aggressive	NO[2]	NO	NO			NO	NO		
No interaction	95.1	96.4	94.1	1.3	NS	96.1	94.3	1.1	NS
Type of activity, %									
Resting	80.0	79.4	80.0	1.9	NS	81.6	78.0	1.6	NS
Drinking	1.3	0.6	0.9	0.6	NS	0.5	1.4	0.5	NS
Grooming	14.7	14.6	12.5	2.5	NS	12.8	15.1	2.1	NS
Biting their cage	2.7	2.1	1.6	0.8	NS	2.1	2.1	0.6	NS
Biting a piece of wood	0.4	1.9	3.5	1.2	NS	2.0	1.8	1.1	NS
Mating	0.2	0.0	0.4	0.2	NS	0.0	0.4	0.3	NS
At the feeder	0.0	1.1	0.0	0.5	NS	0.4	0.4	0.3	NS
Moving	0.8	0.4	0.6	0.5	NS	0.5	0.7	0.3	NS
Stretching	NO	NO	NO			NO	NO		
Shaking	NO	NO	NO			NO	NO		
Stamping their feet	NO	NO	NO			NO	NO		
Sneezing	0.0	0.2	0.0	0.2	NS	0.0	0.1	0.1	NS
Scratching the cage	0.0	0.0	0.4	0.2	NS	0.0	0.3	0.2	NS

[1]Results are expressed as the mean of eight cages per treatment (four cage of females and four cage males) for FWTs and n=12 for sex. Observations conducted by scan sampling all rabbits in each group.

[2]SEM: standard error of the mean. NS: not significant. NO: not observed. No interactions were found between FWT and sex for these variables.

Supplementary material 3: Rabbit *Longissimus lumborum* (LL) metabolite concentrations according to FWT and sex[1].

	Feed withdrawal time (h)					Sex			
Variables	4	11	18	SEM[2]	*P*-value[2]	Female	Male	SEM	*P*-value
LL Glucose-6-P (µmol/g)									
T1[3]	14.95	12.53	14.64	0.96	NS	13.17	14.92	0.78	NS
T24	10.01	9.93	8.88	0.65	NS	9.39	9.82	0.53	NS
LL lactate (µmol/g)									
T24	74.66	76.67	74.34	0.77	NS	75.24	75.21	0.77	NS
Glycolytic potential (µmol/g)[4]									
T1	146.50	136.83	134.53	5.22	NS	135.40	143.18	4.26	NS
T24	129.44	130.32	121.92	4.23	NS	125.57	128.89	3.45	NS

[1] Results are expressed as means of eight rabbits per treatment, one from each cage (four females and four males) for FWTs and n=12 for sex.

[2] SEM: Standard error of the mean. NS: not significant. No interactions were found between feed withdrawal time and sex for these variables.

[3] T1=1 h after slaughter, T24=24 h after slaughter.

[4] GP (glycolytic potential)=2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]; measured in µmol lactate/g.