

CODE OF PRACTICE FOR THE CARE AND HANDLING OF RABBITS: REVIEW OF SCIENTIFIC RESEARCH ON PRIORITY ISSUES

January 2017

Rabbit Code of Practice Scientific Committee

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ACKNOWLEDGEMENTS

This report was a significant undertaking that benefited from the help and guidance of a number of people. The Scientific Committee wishes to thank Dr. Stephanie Torrey for her significant contributions and efforts throughout the development of this report. We also wish to acknowledge Dr. Renée Bergeron, who kindly coordinated the peer review. The report also benefited from the thoughtful comments brought forward by two anonymous peer reviewers. Thank you also to Caroline Ramsay for her invaluable support throughout the process.

Funding for this project has been provided through the AgriMarketing Program under Growing Forward 2, a federal–provincial–territorial initiative.

Excerpt from Scientific Committee Terms of Reference

Background

It is widely accepted that animal welfare codes, guidelines, standards or legislation should take advantage of the best available knowledge. This knowledge is often generated from the scientific literature.

In re-establishing a Code of Practice development process, NFACC recognized the need for a more formal means of integrating scientific input into the Code of Practice process. A Scientific Committee review of priority animal welfare issues for the species being addressed will provide valuable information to the Code Development Committee in developing or revising a Code of Practice. As the Scientific Committee report is publicly available, the transparency and credibility of the Code is enhanced.

For each Code of Practice being developed or revised, NFACC will identify a Scientific Committee. This committee will consist of a target number of 6 scientists familiar with research on the care and management of the animals under consideration. NFACC will request nominations from 1) Canadian Veterinary Medical Association, 2) Canadian Society of Animal Science, and 3) Canadian Chapter of the International Society for Applied Ethology. At least one representative from each of these professional scientific bodies will be named to the Scientific Committee. Other professional scientific organizations as appropriate may also serve on the Scientific Committee.

Purpose & Goals

The Scientific Committee will develop a report synthesizing the results of research relating to key animal welfare issues, as identified by the Scientific Committee and the Code Development Committee. The report will be used by the Code Development Committee in drafting a Code of Practice for the species in question.

The Scientific Committee report will not contain recommendations following from any research results. Its purpose is to present a compilation of the scientific findings without bias.

The full Terms of Reference for the Scientific Committee can be found within the NFACC Development Process for Codes of Practice for the Care and Handling of Farm Animals, available at www.nfacc.ca/code-development-process#appendixc.

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Introduction: Approaches to Defining and Evaluating Animal Welfare

The scientific evaluation of animal welfare involves the use of empirical methods to obtain information about animals that can be used to inform ethical decision-making regarding their quality of life. One major challenge is that people have diverse views about what constitutes a good quality of life and therefore express a variety of ethical concerns and use different criteria for defining animal welfare. These have been grouped into three general categories: 1) biological functioning; 2) affective states; and 3) natural living, and form the bases for different approaches to animal welfare research (Fraser et al., 1997). The biological functioning approach emphasizes basic health and normal function and includes measures having to do with health and productivity, stress response and normal (or lack of abnormal) behaviour (Broom, 1991). Animal welfare defined in terms of affective states, often referred to as the feelings-based approach, concerns the subjective experiences of animals with an emphasis on states of suffering (pain, fear, frustration), states of pleasure (comfort, contentment) and the notion that animals should be housed and handled in ways that minimize suffering and promote positive experiences (Duncan, 1993). The concept of natural living emphasizes the naturalness of the circumstances that the animal experiences and the ability of the animal to live according to its nature (Fraser, 2008). While the natural living approach provides another viewpoint for what constitutes a good quality of life for animals, it is more difficult to derive specific measures from it that can be used to evaluate welfare (Fraser, 2008).

The mandate of the Scientific Committee was to address the implications for rabbit welfare within the topics identified. Few, if any, references are made to economic considerations or human health and welfare concerns as these were beyond the scope of the committee's mandate and were rarely addressed in the papers reviewed. The Code Development Committee, for which this report was prepared, represents considerable expertise in these areas, and is tasked with considering such factors in its discussions.

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1. Housing

Conclusions

- 1. Plastic or coated slatted flooring or provision of a slatted resting mat can reduce foot injuries in adult rabbits compared with housing on wire flooring alone.**
- 2. Space allowance affects social and locomotory behaviour. For growing rabbits, increased space per rabbit results in improved bone quality due to increased weight-bearing activity. Overcrowding can result in increased agonistic behaviour and injuries.**
- 3. Rabbits perform hopping behaviours and stand fully upright when provided with sufficient cage height. They tend to seek enclosed areas for resting.**
- 4. Provision of in-cage platforms permits rabbits to express natural behaviours, such as jumping, and also provides them with a shelter under which they may rest. Providing does with a platform allows them to withdraw from the kits. However, as soon as the kits can reach the platform, use of the platform by the doe decreases.**
- 5. Group housing of does leads to increased kit mortality. Waiting for approximately two weeks after kindling before group housing may reduce kit mortality; however, aggression and injury among does are still significant issues.**
- 6. Provision of wooden gnawing objects, such as sticks, reduces the incidence of ear lesions in growing rabbits.**

1.1 Flooring Type

Conventionally, growing rabbits, does, and bucks are housed in cages with wire mesh or slatted floors to control parasitism and maintain uniform growth rates. However, there is increased interest in using more natural conditions for housing rabbits, especially as wire flooring is directly linked to pododermatitis and related degenerative conditions of the feet. Using measures of production, physiology, behaviour, preference, mortality, bone quality, and carcass quality, researchers have examined the influence of alternative flooring on the welfare of rabbits (see *Table 1*).

When compared to rabbits housed on wire flooring, growing rabbits had similar rates of growth, feed intake, and mortality when housed on plastic mesh or slats (Trocino et al., 2008; Gerencsér et al., 2014). For growing rabbits, there were no differences in femur dimensions or bone strength when comparing wire to plastic flooring (Trocino et al., 2008). However, when compared to plastic, wire flooring for adult breeding does resulted in a significantly increased onset of early pododermatitis when used over several reproductive cycles (wire: 65–68% prevalence; plastic: 5% prevalence; Buijs et al., 2014). No differences were observed in the behavioural time budgets of rabbits housed on either plastic or wire flooring (Princz et al., 2008). Additionally, growing rabbits displayed strong preferences for plastic over wire flooring (Princz et al., 2008), although this preference was lost as the rabbits aged (Princz et al., 2008) and when they were housed at higher ambient temperatures (Gerencsér et al., 2014).

Researchers have also studied the welfare impact of housing rabbits on litter. Compared to other flooring types (wire, plastic, or stainless steel), straw litter that was replaced on a weekly basis impaired growing rabbit growth rates (Dal Bosco et al., 2002; Trocino et al., 2008; Gerencsér et al., 2014) and increased overall mortality due to an increased prevalence of enteric disorders (Dal Bosco et al., 2002). Two studies observed increased levels of locomotion in rabbits housed on straw rather than wire (Dal Bosco et al., 2002; Siloto et al., 2008), leading Dal Bosco et al. (2008) to hypothesize that straw bedding was more comfortable for rabbits. However, when given the choice between wire mesh flooring and litter that was replaced every 3 weeks, rabbits spent between 77% and 89% of their time on the wire flooring (Morisse et al., 1999). This finding was repeated in other studies examining preferences among plastic and wire flooring and deep litter (Orova et al., 2004; Gerencsér et al., 2014). Deep straw litter that was replaced weekly was the least preferred flooring, regardless of whether rabbits were housed in low, moderate, or high ambient temperatures (Gerencsér et al., 2014). Rabbits preferred to crowd together on wire flooring, resulting in a stocking density of 27.5 rabbits/m², rather than be on litter flooring, with the possibility of 4.5 rabbits/m² (Orova et al., 2004). Siloto and colleagues (2008) also found that rabbits preferred wire mesh flooring over a wooden board covered with straw in naturally ventilated barns in a warm climate, although the preference disappeared in a mechanically controlled environment at 20°C and 71% relative humidity. Morisse and colleagues (1999) hypothesized that rabbits chose wire mesh flooring over straw litter changed once every 3 weeks as they preferred a cooler resting place or because the litter was perceived as dirty and unsuitable for anything but elimination. Orova et al. (2004) found that while rabbits overall preferred to spend time on wire mesh flooring rather than straw litter, there was an increase in the proportion of rabbits on litter up to 3 hours immediately after top-dressing, suggesting that the condition of the straw is integral to its value.

1.2 Resting Mats

While wire mesh is the most common flooring type for both growing rabbits and does, the use of wire long term significantly increases the prevalence of pododermatitis for does (Buijs et al., 2014). Pododermatitis (sore hocks) reduces animal welfare by causing pain, deep unresolving infection, and reducing movement (Rosell & de la Fuente, 2009). Because flooring type is the most significant risk factor for pododermatitis, many studies have been conducted to examine the impact of the addition of resting mats to wire mesh floors in rabbit cages (for example, see Rosell & de la Fuente, 2009, 2013). When provided with a slatted plastic resting mat, only 15% of does developed pododermatitis by their fifth lactation compared with 71% of does without the resting mat (Rosell & de la Fuente, 2009). Rommers and de Jong (2011) found similar results when does were housed with or without plastic resting mats in wire cages. Without a plastic resting mat only 13% of rabbits had intact footpads, whereas with a resting mat 81% had intact footpads at the fifth parity. Similarly, providing rabbits with early pododermatitis with plastic resting mats may aid their recovery. Rosell and de la Fuente (2009) found that more than 80% of rabbits with early pododermatitis that were given plastic resting mats recovered. Results from Mikó et al. (2014) are also in agreement with the previous studies: with access to a plastic resting mat, 85% of does had no or minimal pododermatitis after five reproductive cycles. Additionally, they determined that does housed in cages with plastic resting mats were heavier than those housed in the same cages without resting mats (Mikó et al., 2014). The researchers hypothesized

that provision of resting mats resulted in increased comfort, leading to increased resting behaviour and feed consumption.

Rosell and de la Fuente (2013) also studied the prevalence of pododermatitis on commercial rabbit farms in Spain and Portugal over a 12-year period. At the start of the period, 28% of farms were using plastic resting mats, increasing to 75% by 2012 (Rosell & de la Fuente, 2013). During that time period, the prevalence of does with pododermatitis decreased from 11.4% in 2001 to 6.3% in 2012. Overall, 13.7% of rabbits housed without resting mats had pododermatitis, compared to only 4.9% of those with plastic resting mats.

While some producers not using plastic resting mats cite hygiene concerns for their reluctance to adopt the inserts (Rosell & de la Fuente, 2013), Rommers and de Jong (2011) found very little evidence of plastic resting mats becoming significantly soiled, even after five reproductive cycles, and only 1% of resting mats had evidence of gnawing.

1.3 Space Allowance

Although commercial stocking densities often approach 20 growing rabbits/m², the European Food Safety Authority (EFSA, 2005) recommends maintaining stocking densities at lower than 16 growing rabbits/m². More recent scientific literature is inconsistent with regard to the effects of density on rabbit welfare, although differences may be partly due to the fact that most studies calculate stocking density as rabbits/m². This may contribute to differences among studies as significant differences in body weights may influence the amount of actual space available for the animals (Aubret & Duperray, 1992). Numerous researchers have examined the effect of stocking density on performance and behaviour, and rabbit preferences (see *Table 2*). For growing rabbits, Buijs and colleagues (2011b, 2012), Trocino and colleagues (2004, 2008, 2015), and Onbaşilar and Onbaşilar (2007) studied the physiologic implications of changing stocking density. In their studies, Buijs et al. (2011b, 2012) incrementally altered the stocking density from 5 to 20 rabbits/m² by housing 8 rabbits in either 40 x 100 cm to 160 x 100 cm cages. There was no effect of increasing animal density on mortality rates, although overall mortality from weaning to slaughter at 68 days was low in all housing densities (1.8%; Buijs et al., 2011b). These researchers also assessed bone strength and bone fluctuating asymmetry, which is a measure of deviation in bilateral symmetry. These variables represent the effects of weight-bearing exercise and adverse stress (created by overcrowding or insufficient space to move or rest naturally, for example) during skeletal growth (Buijs et al., 2012). Rabbits in larger cages housed at lower stocking densities had an increase in tibiofibular diameter, a tendency for increased tibiofibular weight, and decreased fluctuating asymmetry (i.e., more symmetrical leg bones). Rabbits in the largest cage (160 x 100 cm) had a 3.1% increase in bone diameter and 3.6% increase in bone weight compared to rabbits maintained in the smallest cage (40 x 100 cm). This suggests that rabbits housed at lower cage densities have improved bone quality, as measured by several different parameters. No differences were found in rabbit body weight between the different cage sizes that could explain bone quality differences, although rabbits housed in the smallest cages consumed 9 g of feed/day less than those in the largest cage (Buijs et al., 2011b). The authors attributed the improvements in bone quality to the ability of rabbits in the largest cages to perform more load-bearing activities, such as hopping, compared to those housed in smaller cages. However, using the same stocking densities and cage sizes, Buijs et al.

(2011b) found no differences among treatments in fecal glucocorticoid concentrations. They surmised that fluctuating asymmetry and fecal corticosterone are sensitive to different stressors, highlighting the need for measuring multiple indicators of welfare. Onbaşilar and Onbaşilar (2007) housed 1, 3, or 5 rabbits in 70 x 60 cm cages and measured growth and plasma corticosterone and glucose levels. Rabbits housed at the highest density (5 rabbits; 11.9 rabbits/m²) gained the least weight throughout the study and had higher plasma corticosterone and glucose levels at the end of 6 weeks compared to rabbits housed at lower cage densities.

Trocino and colleagues (2004, 2008) housed rabbits in groups of 6 to 8 and altered the cage sizes to result in either 12 or 16 animals/m². They measured performance, health status, bone quality (as determined by bone fracture resistance), and carcass quality. When rabbits were housed in groups of 6, there was no effect of cage density on average daily gain, feed intake, carcass traits, or bone characteristics and strength (Trocino et al., 2008). Using groups of 8 rabbits, they found that rabbits housed at higher density had higher feed efficiency throughout the 71 days of age, and reduced feed intake during the last two weeks (Trocino et al., 2004). They hypothesized that the rabbits housed at 16 rabbits/m² consumed less feed at the end of the growing period due to the reduced space allowance. In a later study, they found that rabbits housed at 16 rabbits/m² had a density of 48 kg/m² by the end of the trial, which is much higher than the 40 kg/m² recommended by EFSA (2005). However, the lower observed feed intake did not translate into differences in final body weight or femur dimensions and strength (Trocino et al., 2004). Using the same stocking densities (12 or 16 rabbits/m²), Trocino et al. (2015) altered the group size from 20 to 27 and assessed rabbit growth rates, skin lesions, and bone strength (as measured by bone weight, length, and resistance to fracture). Unlike the previous two studies, they found that the higher 16 rabbits/m² stocking density resulted in decreased growth from 55 days of age until slaughter, resulting in a lower final live weight in this group. They also found significantly more scratches and other skin lesions due to aggression in rabbits housed at the higher density compared to lower density pens (Trocino et al., 2015).

There are few clear trends concerning beneficial behavioural effects of stocking density variation for growing rabbits housed in cages or pens. This may, in part, be due to the different methodologies used to change stocking density (see *Table 2*). Buijs et al. (2011a) and Trocino et al. (2004, 2008) maintained the number of rabbits per enclosure and altered the floor area to change stocking density while others (Morisse & Maurice, 1997; Onbaşilar & Onbaşilar, 2007; Jekkel & Milisits, 2009) maintained the floor area and altered the number of animals per enclosure. Because of these different approaches, it is difficult to separate stocking density from group size, and the resulting effects on behaviour such as resource use, aggression and maintenance behaviour (Estevez et al., 2007). Buijs et al. (2011a) recorded the behaviours, postures, and space use of rabbits housed at 5, 7.5, 10, 12.5, 15, 17.5, and 20 rabbits/m² at 6 and 9 weeks of age. They found that cage density affected social contact, sternal lying, sitting, standing, and feeding behaviours. As cage density decreased, sternal lying decreased. The authors hypothesized that sternal lying was a “filler” behaviour, performed more often in smaller space envelopes. Sitting behaviour increased with decreasing density, although the change was minimal (Buijs et al., 2011a). Trocino and colleagues (2004) also observed few consistent trends in behaviour between groups of 8 rabbits housed at either 12 or 16 rabbits/m². Rabbits housed at the higher density were minimally more reactive than those housed at the lower density in an open field test. However, it is unclear how open field behaviour relates to animal welfare in the home cage, as there were no differences in feeding, resting, or locomotor behaviours in the home

cage. In contrast, Morisse and Maurice (1997) found that increasing the stocking density from 15.2 rabbits/m² to 23.0 rabbits/m² by changing the group size from 6 to 9 rabbits increased resting behaviour. In addition, rabbits in the lowest density cages displayed more social and investigative behaviours than those housed at higher densities. Interestingly, rabbits housed at the lowest density demonstrated more agonistic behaviour than those housed at higher densities, possibly because the increased space envelope permitted more territoriality to occur. In pens, the relationship between animal welfare and stocking density is equally ambiguous. Jekkel and Milisits (2009) reared rabbits in pens at one of three densities ranging from 8.24 rabbits/m² to 15.29 rabbits/m² from 5 to 11 weeks of age. Rabbits housed at higher density demonstrated decreased feeding and increased comfort and locomotor behaviours.

There are few studies that have examined the effect of space allowance on doe welfare, and none on buck welfare. For laboratory rabbits, the Canadian Council on Animal Care (CCAC; 2003) recommends housing individual rabbits weighing less than 4 kg at 0.37 m², and rabbits weighing greater than 4 kg at 0.46m². They also recommend housing lactating does and their litters at greater than 0.93 m² (CCAC, 2003). EFSA (2005) recommends a minimum floor space of 0.35 m² for individually caged does. In Belgium, breeding does must be provided with a minimum of 0.30 m² (Federal Public Service, 2014). In the Netherlands, breeding bucks must be housed at a minimum of 0.40 m² (Rommers et al., 2014). Prola et al. (2013) provided does with two different space allowances (0.32 m² vs 0.52 m² with a plastic floor mat) and studied fecal corticosterone levels at different phases of the reproductive cycle. Does in larger cages had lower fecal corticosterone levels than those kept in the smaller cages prior to artificial insemination, immediately pre-partum, and the day after weaning (Prola et al., 2013). When given access to both a small (0.22 m²) and a large (0.44 m²) cage between which they could move freely, non-pregnant does spent one third of their time in the small cage and two thirds of their time in the large cage, proportional to the cage areas, although they increased the time spent in the large cage over time (Mikó et al., 2014). Pregnant and lactating does spent more time in the large cage, although location of their parturition influenced their cage preferences. If they kindled in the small cage, they increased their time in the large cage compared to those that kindled in the large cage, presumably to rest away from the litter.

Using 10 week-old nulliparous does, Bignon et al. (2012a) examined whether providing additional cage space on two levels influenced doe behaviour and kit live weight. They housed the does individually in one of three cages: a 0.12 m² cage on a single level, a 0.23 m² two-story cage, or a 0.34 m² cage on a single level with a 0.088 m² platform. When does had access to a platform, they spent 17% of their time on it, and they were more active in the larger pens, demonstrating increased locomotion. Cage size did not influence gnawing or grooming behaviours, but the total litter live weight was 136 g heavier for the largest cages compared to the standard cages (Bignon et al., 2012a).

1.4 Enclosure Height

For laboratory rabbits, CCAC (2003) recommends a minimum enclosure height of 40 cm for rabbits weighing less than 4 kg, and 45 cm for rabbits weighing more than 4 kg. Martrenchar and colleagues (2001) studied the behaviour of fattening rabbits housed in either wire-floor pens or cages at the same stocking density. The housing types differed in group size and height as the

pen did not have a ceiling while the cage was 30 cm high. Within the pens, rabbits were observed to spend more time hopping, and also performed “watching” behaviour, in which they stood in a full upright posture. Rabbits housed in the cages were not observed performing this behaviour, and the authors hypothesized that the 30 cm cage height was restrictive (Martrenchar et al., 2001).

Princz and colleagues (2008c) performed a series of studies to assess growing rabbit preference for different cage heights, as well as the effect of cage height on weight gain, feed intake, mortality, and ear lesions. In their first study, rabbits housed at either 12 or 16 rabbits/m² were given free choice between cage segments with ceilings at 20, 30, or 40 cm, or an open top. Regardless of housing density, the open top cage segment was least preferred. At the higher housing density, there was no difference in time spent in cage segments of different heights, although preference varied by age, with fewer rabbits choosing 20 cm height as they aged (Princz et al., 2008). At the lower housing density, slightly more rabbits chose the 40 cm cage segment than the 20 cm and 30 cm, which were both selected in similar proportions. Regardless of housing density, most rabbits chose to be in the 40 cm segment during their active period and the 20 cm segment during their resting period.

In a subsequent study, Princz and colleagues (2008c) assessed growing rabbit preference between two intermediate cage heights: 30 cm vs 40 cm. While significantly more rabbits chose the 40 cm cage segment, the overall proportion of rabbits in the two segments differed by less than 3%. The preference for the higher cage height was more apparent at a density of 16 rabbits/m² than at 12 rabbits/m². In their third study, they examined rabbit production parameters (as measured by growth rate and feed intake) and health when animals were housed in cages with heights of 20, 30, or 40 cm, or an open top, at a density of 13 rabbits/m² (Princz et al., 2008c). There were no overall effects of cage height on growth rates, feed intake, or mortality. However, the percentage of rabbits in the 20 cm cages that had ear lesions (20%) was significantly increased over that of rabbits housed in 30 cm cages (5%). Rabbits in 40 cm and open top cages had intermediate percentages of ear lesions (10.3% in both types).

For does, there has been limited research into the effect of cage height on welfare. Rommers and Meijerhof (1997) housed nulliparous does in large (0.60 m²) or small (0.30 m²) cages, using plastic or wire flooring, with cage heights of 50 or 30 cm, and studied them through four parities. Increasing cage height had a positive effect on reducing kit mortality rates, and when used in conjunction with the plastic flooring and large cage, body weights of kits were heavier at weaning (Rommers & Meijerhof, 1997). In addition, when given the opportunity to do so, does were observed to stand on their hind legs in 50 cm high cages.

1.5 Platforms

EFSA (2005) recommends that housing conditions should provide enough space for rabbits to retreat from potential threats. A number of studies have been conducted to examine the use of platforms by growing rabbits and breeding does and have evaluated animal production, behaviour, and space use. However, studies comparing housing with and without platforms often confounded platform use with enclosure size (Bignon et al., 2012a; Mikó et al., 2014) or group

size (Postellec et al., 2008), making it difficult to draw conclusions on the impact of platforms on rabbit welfare.

Postellec et al. (2008) housed growing rabbits in conventional cages (0.39 m²; 6 rabbits/cage), in small pens (0.503 m² plus a platform of 0.159 m², 30 cm above the floor; 10 rabbits/pen), or in large pens (3.67 m² plus a platform of 0.39 m², 30 cm above the floor; 60 rabbits/pen). The stocking density was the same in all three housing types (15 rabbits/m²). Rabbits housed in cages had greater average daily gain than those housed in pens, although the differences were attributed to decreased space allowance and activity rather than the presence of a platform (Postellec et al., 2008). In the small pens, the platform was used as an additional surface for resting, while in the large pens, it was used for short bouts of exercise, including jumping and hopping. However, there was no effect of platform on numbers of skin lesions, morbidity, mortality, or overall time spent feeding and drinking, resting, or active.

Bignon et al. (2012a) found that when primiparous does had access to a 35 x 25 cm platform, 30 cm above the cage floor within their individual cages, they sat on it for 17% of the time. Examining platform use of lactating does over five production cycles, Mikó and colleagues (2014) housed does and their kits in pens with either a plastic (41.5 x 52.5 cm, 25 cm above the cage floor) or wire mesh (28.5 x 38 cm, 26.5 cm above the cage floor) platform. Does housed with a plastic platform used it during their active period and spent their resting period beneath it. These does also used the platform 25% more than those with a wire platform, although the plastic platform was more than twice the size of the wire one (Mikó et al., 2014). Provision of a platform, whether wire or plastic, significantly decreased the severity of pododermatitis: without the platform, 48% of does had moderate to severe pododermatitis while 0–5% of does with platforms had moderate to severe pododermatitis (Mikó et al., 2014). As the kits became more active, does increased their use of the platform, regardless of the material. However, when the kits were able to use the platforms (after day 21), doe use of the platforms decreased while kit use increased until weaning (Mikó et al., 2014).

Szendró et al. (2012) examined growing rabbits' preferences between platform floor types. At a stocking density of 11.1 rabbits/m², Pannon White rabbits were housed in groups of 14 in 0.84 m² wire cages, with a 0.42 m² platform placed 30 cm above the floor. Platforms had either a deep-litter floor or an open wire-net floor (Experiment 1), or a deep-litter floor and a wire-net floor with a manure tray underneath (Experiment 2). In both experiments, rabbits with the wire-net platforms spent 12–13% more time on the platform than those with the deep-litter platform (Szendró et al., 2012). In the first experiment, more rabbits were found underneath the platform with the deep-litter compared to the wire-net without a manure tray. When the manure tray was added for the second experiment, rabbits spent more time than expected under the platform, regardless of whether the platform was deep litter or wire-net with a manure tray. The authors concluded that provision of a wire-mesh platform with a manure tray permits rabbits to fully utilize their space (Szendró et al., 2012).

1.6 Group Housing for Does

Many studies have examined the possibility of housing breeding does in groups rather than individually. Buijs and colleagues (2014) housed does for 4 reproductive cycles either in

individual cages or in semi-group housing on plastic slatted or wire flooring. In the semi-group housing, pens were separated into individual housing for short periods around the time of kindling. Compared to does housed in individual cages, group-housed does had improved bone quality, indicative of greater locomotory activity. However, when behaviour was studied in detail (Buijs et al., 2015), levels of locomotion (excluding locomotion linked to agonistic behaviour) and positive social interaction were only mildly increased in group housing systems as compared to the individual housing. The authors suggested that either does are not motivated to perform significant allogrooming (social grooming) and other affective and physical behaviours in the specific gestational stage at which they were group-housed, or that the semi-group system did not elicit such behaviours due to other social or spatial constraints. The semi-group system did not have a positive impact on adverse stress indicators (as measured by paired adrenal gland weights and weight loss during lactation), and 20% of the grouped does received severe wounds (Buijs et al., 2015). Szendrő et al. (2013) studied the welfare of does housed either individually or in groups with four does and one buck. Group housed does had lower kindling rates (45% vs 78–85%), higher suckling kit mortality (38% vs 14–15%), and lower survival rates (50% vs 71–81%) than individually housed does. Group housed does also had significantly increased (three-fold) fecal corticosterone levels compared to individually housed does (Szendrő et al., 2013).

EFSA (2005) concluded that there is not enough evidence as to how to best group- or pair-house does to make this an industry-wide recommendation. However, some researchers have examined interventions to reduce the negative effects of group housing. Mugnai and colleagues (2009) compared housing does in individual cages to housing them in groups of four and trained does to recognize their own nest box or not. Group-housed does performed a wider variety of behaviours and exhibited less stereotypic behaviour than individually housed does (Mugnai et al., 2009). However, does without prior training to recognize their nest box demonstrated higher levels of aggression and dominance, and there were higher numbers of severely injured does in this group compared to trained does. These does also had lower sexual receptivity, fertility, and gave birth to fewer live kits compared to individually housed does, with trained group-housed does being intermediate for these variables. Singly-housed does demonstrated the highest reproduction and fertility parameters.

Rommers et al. (2014) examined possible means to mitigate the adverse effects of group housing. They either provided semi-group housed does with a hiding place, straw as enrichment, familiarity with the cage prior to grouping, or different combinations of these three strategies. While does that were familiar with their cage prior to mixing displayed more comfort behaviours (self grooming, stretching, yawning), neither does defended their territory. More than half of all does sustained skin wounding and there was no effect of treatment on the prevalence of injury. The percentage of does with severe injuries ranged from 13% to 39% for the different treatments, with less severely injured does seen when does were provided access to a hiding place.

When group housing of does is used, enclosure size and familiarity with conspecifics were found to be important considerations for housing success. Valuska and Mench (2013) evaluated pairs of unfamiliar does in small and large enclosures in which barriers were placed to prevent direct aggression. When does were unfamiliar, less aggression was seen if they were first placed into the larger enclosure rather than the smaller enclosure. However, once rabbits were known to each other, those that had first been placed in the smaller enclosure engaged in more aggression when

they were later in the larger enclosure (Valuska & Mench, 2013).

1.7 Environmental Refinement

Most commercial meat rabbits are housed in barren wire cages with limited opportunities to express the full repertoire of species-specific behaviours. A number of studies have been conducted to examine the effect of adding complexity to the environment on rabbit production parameters, health, behaviour, and preferences. The provision of gnawing sticks or blocks has been more extensively studied than any other type of refinement.

Many studies have found few or no adverse effects of provision of a gnawing stick on rabbit production parameters. In one of the few studies that found significant differences in production with or without gnawing sticks, Rizzi et al. (2008) found that individually housed growing rabbits gained 3.5 g/d more and consumed 10 g/d more when provided with wooden gnawing sticks than without. However, Zucca et al. (2012) and Verga et al. (2004) found no effect of inclusion of a gnawing stick on any performance indicator through 79 and 75 days of age, respectively. Princz et al. (2007, 2008a) also found no effect of provision of different types of wooden sticks on any performance indicators. While Princz et al. (2009) found no effect of gnawing stick on feed intake, they did note heavier body weights at 11 weeks in growing rabbits reared with gnawing sticks. Bignon and colleagues (2012b) examined the inclusion of wood fibre blocks in growing rabbit cages and found no effect on growth rates or mortality, but they found better feed efficiency in cages with the blocks. There was also no effect of provision of a wood fibre block to does on maternal performance, nest mortality, milk production, or kit body weight (Bignon et al., 2012b).

Maertens et al. (2013) compared the performance and behaviour of does and their kits when given one of three types of blocks (wood mash, chicory pulp in wood, and inulin in wood). There was no effect of any of the blocks on litter size or weight. Overall mortality for the study was low, although litters of does without gnawing blocks experienced 12.5% mortality (Maertens et al., 2013). At parturition, does without gnawing blocks were heavier than those provided with a wood mash block. Because the does with the wood blocks were consuming significant but highly variable amounts of the block, the authors hypothesized that the lack of nutritional value of the block negatively impacted doe body weight. No difference in doe body weight was found between the chicory pulp or inulin blocks and those without blocks (Maertens et al., 2013).

Growing rabbits were found to have lower tibial calcium levels when they were housed with wooden gnawing sticks, which the authors hypothesized was related to levels of tannin in the wood (Rizzi et al., 2008). Princz and colleagues (2008a) found a significantly lower prevalence of ear lesions in growing rabbits housed with wooden gnawing sticks (1.9% of rabbits with linden leaf; 7.7% with white locust) compared to those housed without a gnawing stick (17.3% of rabbits). In another study, Princz et al. (2009) found that the presence of gnawing sticks in cages or pens significantly reduced the percentage of injured growing rabbits, from 18.5% without sticks to 1.2% with sticks.

In several studies, results have suggested that provision of wooden gnawing sticks may reduce aggression and oral stereotypies, and affect overall behavioural time budgets for growing rabbits

(Verga et al., 2004; Princz et al., 2007, 2008b). This was not found in all studies (Zucca et al., 2012). Zucca and colleagues (2012) saw no differences in general behaviour, temperament, or coping styles (as assessed through Tonic Immobility and Emergence tests) between growing rabbits housed with or without wooden gnawing sticks, although there may have been habituation and loss of interest in the items with time. In other studies, group housed growing rabbits provided with gnawing sticks were more active, performed more grooming, hopping, and allogrooming, and less aggressive behaviours and oral stereotypies compared to those without sticks (Verga et al., 2004; Princz et al., 2007, 2008b). Rabbits also prefer to have wooden gnawing sticks in their environment. When given the choice to move freely among wire and plastic floor cages with or without gnawing sticks, rabbits spent 6% to 8% more time in the cages with gnawing sticks (Princz et al., 2008b). They also spent more of their active time budget in, and consumed more of their feed from, the cages with the gnawing sticks.

Differences in results between these studies may be related, in part, to the type of wood used as a gnawing stick. Lidfors (1997) compared the inclusion of hay, grass cubes, or peeled aspen gnawing sticks on 83-day-old buck behaviour and enrichment use. Bucks in this study consumed an average of 71 g/d of grass cubes and 31 g/d of hay, but virtually ignored the gnawing sticks. The author hypothesized that the type of wood may have influenced the rabbits' behaviour. Rizzi et al. (2008) also suggested that the chemical structure of the wood used for gnawing sticks is important in variables such as calcium levels in the bone. Princz and colleagues (2007) assessed growing rabbit preferences among nine different types of wood. Among the types assessed, rabbits preferred gnawing sticks made of little leaf linden, white willow, and white buckeye to other kinds. Rabbits housed solely with little leaf linden sticks also consumed more of the wood than those housed with either Norway spruce or common oak. However, those provided with the Norway spruce sticks spent the most time actually gnawing the wood. In another study, Princz et al. (2008a) compared the wood consumption and health of growing rabbits penned either with a white locust gnawing stick, a little leaf linden gnawing stick, or no stick. Rabbits provided with the linden gnawing stick consumed more of the stick than those with the white locust, although there were no differences in productivity. However, rabbits with the linden wood gnawing sticks had significantly fewer ear lesions than those with the locust wood gnawing sticks, with both gnawing stick treatments having fewer ear lesions than rabbits penned without any gnawing stick (Princz et al., 2008a).

Other types of environmental refinements, including wooden structures (Buijs et al., 2011a; 2011b), mirrors (DalleZotte et al., 2009; Edgar & Seaman, 2010), roughage (Lidfors, 1997), and other food and non-food items (Harris et al., 2001), have been assessed for their impact on rabbit welfare. Buijs and colleagues (2011a, 2011b) housed rabbits at different stocking densities, with or without a U-shaped wooden structure consisting of two wooden walls connected by a wooden floor (40 x 20 x 20 cm). As the wooden structure could potentially be used for two distinct purposes (as a gnawing structure and a physical structure to divide the cage into separate functional areas), the authors hypothesized that inclusion of the structure would decrease aggression and cage manipulation. Rabbits spent 4% of their time gnawing, licking, or sniffing the structure (Buijs et al., 2011a), and their interest in it did not wane with time. With the structure, rabbits performed less cage manipulation (Buijs et al., 2011a) and had lower levels of fecal glucocorticoids (Buijs et al., 2011b). These rabbits also decreased their contact with conspecifics (Buijs et al., 2011a). Although social contact is generally assumed to be positive for

animal welfare, the authors surmised that the structures permitted the rabbits to avoid unwanted interactions.

In the aforementioned study, Lidfors (1997) found that rabbits interacted with hay more than with other cage provisions (i.e., grass cubes or peeled aspen gnawing sticks). In pens provisioned with hay or grass cubes, rabbits performed less abnormal behaviours compared to those housed in control (barren) pens. There was also a greater weight gain in rabbits housed in pens with grass cubes compared to control pens, due, in part, to the daily consumption of 71 g/d of grass cubes.

Studies have also have examined whether the inclusion of mirrors in individual cages could serve as a substitute for social contact. Dalle Zotte and colleagues (2009) gave individually housed rabbits the choice between a mirrored and non-mirrored cage. Rabbits spent more time and consumed more of their feed in the mirrored cage. Similarly, group housed rabbits preferred the mirrored cage to a non-mirrored cage, although this preference decreased as they aged (and potentially became crowded within the preferred area; Dalle Zotte et al., 2009). Edgar and Seaman (2010) also provided individually housed rabbits with the choice between a mirrored and non-mirrored cage area. There were differences between male and female rabbits in their behavioural response to the mirrors. Females responded to the mirror by decreasing their grooming behaviour and increasing investigatory behaviour, while males exhibited increased overall vigilance behaviour as well as increased stereotypic behaviour in the first two days with the mirror. The authors suggested that males may have perceived competition in their equally-sized mirror reflection.

Finally, Harris and colleagues (2001) investigated the provision of food or non-food items to rabbits for a short period of time (1 hour) each day. Interest in some of the non-food items (i.e., Jingle Ball, Kong toy) was high initially but decreased rapidly. Rabbits' interactions with the food items (i.e., Bunny Blocks, celery) peaked after about 4 to 7 days, although rabbits continued to interact with the Bunny Stix for the duration of the 15-day trial.

1.8 Outstanding Issues Not Addressed by Current Literature

- 1. Management of aggression in group housed does.**
- 2. Shelter use and preference.**
- 3. Refinement of buck housing in terms of space allowance and enrichment.**
- 4. Optimum stocking densities at different stages of production.**

Table 1. Flooring type comparisons from reviewed scientific literature

Reference	Stage of Production	Floor types	Variables measured
Buijs et al. 2014	Does	Wire Plastic slats	Bone quality Spinal deformations Pododermatitis
Princz et al. 2008	Growing rabbits	Plastic net Wire	Behaviour Preference
Dal Bosco et al. 2002	Growing rabbits	Wire net Straw litter	Growth Feed intake Mortality Behaviour Carcass quality
Trocino et al. 2015	Growing rabbits	Wood slats Plastic slats	Growth Feed intake Carcass quality Skin lesions
Morisse et al. 1999	Growing rabbits	Wire net Concrete with litter	Growth Feed intake Mortality Health Behaviour Open field Preference
Gerencsér et al. 2014	Growing rabbits	Wire mesh Plastic mesh Deep litter	Growth Feed intake Mortality Preference
Siloto et al. 2008	Growing rabbits	Wood with straw Wire	Behaviour
Trocino et al. 2004	Growing rabbits	Wire net Steel slats	Growth Feed intake Bone strength Carcass quality Behaviour Open field
Trocino et al. 2008	Growing rabbits	Plastic slats Wire net Wire net with litter Steel slats	Growth, Feed intake Health Tonic immobility Open field Carcass quality

Table 2. Enclosure size, group size, and space allocation comparisons from reviewed scientific literature

Reference	Stage of production	Dimensions (L x W), cm	Floor area, m ²	# rabbits per enclosure	Space allowance per rabbit, m ²	Rabbits/m ²	Variables measured
Bignon et al. 2012a	Does	25 x 46	0.12	1	0.12	0.87	Behaviour
		33 x 68.5 ^a	0.23	1	0.23	0.44	Mortality
		38 x 90 ^b	0.34 ^b	1	0.34 ^b	0.29	Production
Prola et al. 2013	Does	83 x 38	0.32	1	0.32	0.32	Fecal corticosteroid
		113 x 46	0.52	1	0.52	0.19	
Mikó et al. 2014	Does	51.5 x 38	0.22	1	0.22	0.46	Preference
		57.5 x 76	0.44	1	0.44	0.23	
Buijs et al. 2011a	Growing rabbits	40 x 100	0.40	8	0.05	20	Behaviour
		46 x 100	0.46	8	0.058	17.5	Posture
		53 x 100	0.53	8	0.066	15	Space use
Buijs et al. 2011b	Growing rabbits	64 x 100	0.64	8	0.08	12.5	Mortality
		80 x 100	0.80	8	0.10	10	Bone strength
Buijs et al. 2012	Growing rabbits	107 x 100	1.07	8	0.13	7.5	Fluctuating
		160 x 100	1.60	8	0.20	5	asymmetry Fecal glucocorticoid Growth
Villalobos et al. 2010	Growing rabbits	50 x 100	0.50	8	0.063	16	Performance
		50 x 50	0.25	4	0.063	16	
Jekkel and Milisits 2009	Growing rabbits	50 x 170	0.85	7	0.12	8.24	Behaviour
		50 x 170	0.85	10	0.085	11.76	
		50 x 170	0.85	13	0.065	15.29	
Morisse and Maurice 1997	Growing rabbits	77 x 51	0.39	6	0.066	15.3	Behaviour
		77 x 51	0.39	7	0.056	17.8	Growth
		77 x 51	0.39	8	0.049	20.4	
		77 x 51	0.39	9	0.044	23.0	
Onbaşilar and Onbaşilar 2007	Growing rabbits	70 x 60	0.42	1	0.42	2.38	Growth
		70 x 60	0.42	3	0.14	7.14	Plasma corticosterone
		70 x 60	0.42	5	0.084	11.90	Glucose
Postollec et al. 2006	Growing rabbits	77 x 50	0.39	6	0.064	15	Performance
		95 x 70 ^c	0.67	10	0.067	15	Skin lesions
		193 x 190 ^c	3.67	50	0.073	15	Mortality Bone strength Behaviour

Postollec et al. 2008	Growing rabbits	77 x 50	0.39	6	0.064	15	Performance
		53 x 95 ^d	0.66	10	0.066	15	Skin lesions
		193 x 190 ^e	4.05	60	0.068	15	Morbidity Mortality Behaviour
Princz et al. 2008	Growing rabbits		0.12	2	0.061	16	Behaviour
			0.86	13	0.066	16	
Trocino et al. 2004	Growing rabbits	100 x 50	0.50	8	0.063	16	Performance
		110 x 60	0.66	8	0.083	12.1	Behaviour Open field Tonic immobility Bone strength Carcass quality
Trocino et al. 2015	Growing rabbits	120 x 140	1.68	20	0.084	12	Performance
		120 x 140	1.68	27	0.066	16	Bone strength Carcass quality
Trocino et al. 2008	Growing rabbits	78 x 64	0.50	6	0.083	12.1	Performance
		58 x 64	0.37	6	0.062	16.2	Health Tonic immobility Open field Carcass quality

^a Stacked on two levels

^b 0.089 m² platform at 30 cm

^c Pens without ceilings

^d Pen without ceiling. Had additional platform of 0.16 m² at height of 30 cm above floor

^e Pen without ceiling. Had additional platform of 0.39 m² at height of 30 cm above floor

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2. Reproduction

Conclusions

- 1. Does need to be healthy, with a normal body condition score, prior to first breeding to limit body weight loss during gestation and lactation. Otherwise, a longer rebreeding interval may be necessary to permit does to recover.**
- 2. Under current management practices, breeding doe mortality rates remain high because of intercurrent diseases.**

As seasonal breeders with a polygynous mating system (Southern, 1948; SurrIDGE et al., 1999), rabbit does are naturally prolific. Rabbits are induced ovulators, and does are receptive to and will mate with bucks in the postpartum estrous period that lasts for approximately 48 hr after kindling (Bell, 1984; Diaz et al., 1988; Beyer & Rivaud, 1969). Gestation lasts 31 to 33 days and during this time, circulating plasma concentrations of estradiol and progesterone increase (González-Mariscal et al., 1994), inducing maternal behavioural patterns that are necessary for survival of their kits (Zarrow et al., 1961; González-Mariscal et al., 2007). Kits are altricial, meaning they are completely dependent on their dams for nutrition and survival for at least the first 3 weeks of life. They are born hairless, with closed eyes and ears, and have a large surface area to body mass ratio, necessitating a nest and/or the presence of littermates for thermoregulation (Bautista et al., 2008). In natural or semi-natural conditions, does begin building a nest one week prior to parturition (González-Mariscal, 2007) from plant fibres (when available) and hair that is plucked from the doe's body (Zarrow et al., 1963; Canali et al., 1991). Parturition is a relatively rapid process in rabbits (Hudson & Distel, 1982) and does only return to the nest for approximately 3–5 minutes once (or, less frequently, two or three times) a day to nurse the kits, usually at dawn or dusk (Selzer et al., 2004; Hoy & Selzer, 2010). This behaviour is thought to minimize detection of the nest by predators.

Does differ in their nest building behaviour depending on age and experience: primiparous does are more likely not to build a nest (González-Redondo, 2010) or to build an inadequate nest (Ross et al., 1956; Canali et al., 1991) than multiparous does. Genetics also influences nest building behaviour. Hamilton et al. (1997) found that New Zealand (NZW) and California (CAL) does differed in their nest building abilities. NZW does used more fur in their nests (18 g) than CAL does (11.8 g) and had greater nest structure and better fur placement within the nest (Hamilton et al., 1997). Breed differences may be, in part, due to differences in fur coverage, as Szendrő et al. (1988) found that NZW does have more fur than CAL does. The lack of a suitable nest increases mortality rates in both wild and domestic rabbits (Canali et al., 1991; González-Redondo, 2010). However, while Hamilton et al. (1997) found that nest traits accounted for 21 to 35% of the variation in neonatal mortality, only 5% of the pre-weaning mortality could be explained by nest traits. The presence of littermates is also important for kit survival: litters with single kits are at a significant survival disadvantage compared to those with 2, 4, or 6 kits (Bautista et al., 2003), which may imply that cross-fostering can play a role in reducing mortality. Bautista et al. (2008) reported that kits that died within the first 5 days of life had spent less time huddling with littermates, had lower mean body temperatures (33.8° vs 36.1°C), and obtained less milk (1.9 g vs 9.5 g) than their littermates. Cross-fostering can also be used to reduce the variation in body weights among kits, which can have lasting positive effects on

growth rates. Bautista et al. (2015) reported that kits that have significantly lower body weights compared to their littermates have lower body temperatures, milk intake, and growth rates.

2.1 Breeding Methods

In commercial meat rabbit production in Canada, natural or hand mating is used, with artificial insemination (AI) used by a minority of producers. Few studies have compared the welfare implications of natural mating to AI on either doe or buck welfare. Gil and colleagues (2004) compared the prevalence of abdominal pregnancies in does from a farm that used AI exclusively to a farm that predominantly employed natural mating. Although abdominal pregnancies in does can remain undetected for long periods and can occur concurrently with viable pregnancies, they can lead to uterine rupture, hemorrhages and infections. The authors found an abdominal pregnancy prevalence of 7.8% on the farm using AI exclusively, compared to 1.7% prevalence on the farm that used AI sparingly (Gil et al., 2004). While numerous other management practices may have caused the difference in prevalence, the authors suggested that improper technique during insemination could cause perforation of the vaginal wall, resulting in abdominal pregnancies (Gil et al., 2004).

Bucks begin performing sexual behaviour around 60–70 days of age, although they do not reach sexual maturity for another 60–70 days (Alvariño, 2000). When offered *ad libitum* opportunities to mate, mature bucks remained highly motivated to engage in sexual activity even after multiple mounts including ejaculations (Jiménez et al., 2012), which suggests that the act of natural mating, in and of itself, does not have negative welfare implications on bucks. Perhaps because of bucks' high motivation to mate, Gacek and colleagues (2012) failed to find significant differences in time to mating and efficiency of mating among bucks characterized as timid, tame, or aggressive through two behavioural tests. Further research is necessary to compare the welfare implications of natural mating to AI on both doe and buck welfare.

2.2 Age at First Breeding

Young female rabbits are still developing at the time at which they reach puberty, and the first four breeding cycles represent a critical period for the development of energy and protein reserves (Rommers, 2004). Multiple variables affect rabbit development during rearing, including their birth weight and the feeding strategy used (Szendró et al., 2006). Particularly for primiparous does, there is a significant disparity between the dietary energy intake and energy output in the form of milk production (Xiccato et al., 2004). This discrepancy leads to larger losses in body condition during lactation for younger does than for older does (Xiccato et al., 2004). In their survey of 130 Spanish rabbitries, Rosell and de la Fuente (2009) found the average age at first breeding was 147 d (21 weeks), with no one breeding does younger than 114 d (16.3 weeks). While they found no relationship between age at first insemination and doe mortality rate (Rosell & de la Fuente, 2009), the mortality rate after first kindling was 8.7%. Different management practices for females prior to first mating can help maximize their feed intake and body reserves (Rommers et al., 2006). Rommers (2004) hypothesized that does that are “well developed” in terms of skeletal growth and body fat reserves have improved reproductive performance and experience reduced culling rates. In 2004, Rommers summarized

her previously published and unpublished reports on age at first insemination (14.5 or 17.5 weeks of age) and feeding method (*ad libitum* or restricted). Age at first insemination influenced productivity, particularly when does weighed significantly less than their adult weight (4 kg for New Zealand white does) at the time of insemination (Rommers, 2004). When inseminated at 14.5 weeks of age, does weighing less than 4 kg had smaller litter sizes than heavier does, and there were more stillborn kits from does inseminated at 14.5 vs 17.5 weeks of age. Does inseminated at 17.5 weeks of age and limit fed after 10 weeks of age had heavier kits at 16 days postpartum compared to does inseminated at 14.5 weeks or at 17.5 weeks and fed *ad libitum*. Rommers and colleagues (2006) suggested that limiting fat deposition while encouraging body weight growth during sexual development is integral in primiparous doe fitness. In their experiments, culling rates of does did not differ in the first three parities, although limited numbers of animals were used (Rommers et al., 2006).

Using two different breeds, Matics and colleagues (2008) examined the age at first insemination in conjunction with limit feeding. They found that does that were inseminated at 15 weeks of age, after *ad libitum* feeding during rearing, had litters with higher pre-weaning mortality, compared to does inseminated at 19 weeks of age after a 7 week feed restriction period. This was most apparent for the heavier genotype studied (Matics et al., 2008).

It can be concluded that young does are particularly vulnerable to diseases, and age at first breeding should be such to limit body weight losses during lactation.

2.3 Synchronization of Estrus

Since rabbit does are induced ovulators and behave similarly whether they are sexually receptive or not (Stoufflet & Caillol, 1988), producers often synchronize the estrus cycles of their does to facilitate insemination practices and improve fertility rates (Boiti et al., 2006), and optimize biosecurity practices with all-in/all-out management. First used some 50 years ago to induce superovulation in rabbits (Kennelly & Foote, 1965), pregnant mare serum gonadotrophin (PMSG) has been regularly used for the last 15 years to both induce and synchronize estrus in commercial rabbits (Theau-Clément, 2007).

Compared to does that did not receive PMSG treatment, multiparous does given a 25 IU injection of PMSG two days prior to AI were 26% more receptive and had a higher kindling rate (72% with PMSG, 62% without; Theau-Clément & Lebas, 1996). Fertility was higher with PMSG, but only through the first four cycles of use. Theau-Clément and Lebas (1996) also found no effect of treatment on the mortality rate of does or kits. However, Maertens and Luzi (1995) found a significantly higher kit mortality rate when does were given 30 UI PMSG (14%) compared to control does that did not receive hormone injections (6%), which could, in part, be attributed to differences in litter size. Does treated with PMSG had more small (<5 kits) and more large (>12 kits) litter sizes compared to untreated does (Maertens & Luzi, 1995); however, differences in mortality rates were observed in “normal” sized litters as well. In addition to the large variation in litter size with PMSG treatment, repeated use of PMSG (at 25 IU) resulted in the development of anti-PMSG antibodies (Lebas et al., 2010). After seven parities induced with PMSG injections, more than 30% of does developed significant immunity to PMSG, lasting for

up to 18 weeks after treatment.

2.4 Bio-Stimulation

Because of the concern surrounding the use of hormones, steroids, and other xenobiotics in the food chain (EFSA, 2005), there has been interest in finding alternatives to PMSG for synchronizing estrus in does. There have been multiple scientific investigations into approaches to maintain reproductive performance without the use of PMSG. Most commonly, different periods of dam-litter separation have been studied (see *Table 3*). Researchers have examined different durations of separation, from 24 to 48 h, prior to artificial insemination, typically at 11 d postpartum. When does and litters were separated for a 24 h period, there was no difference in the reproductive performance or kit growth rates for multiparous does compared to does and litters with free nest box access (Castellini et al., 2010). However, when does were removed from the home cage for 24 h periods during each of their lactations for their entire reproductive life, there was significantly higher pre-weaning mortality (22.6%) in the separated litters compared to does with free nest box access (14.7%; Castellini et al., 2010). There was no difference in the number of kits born alive (8.8 vs 8.3 kits), number of weaned rabbits (6.8 vs 7.1 rabbits), or the individual kit weaning weight (658 vs 670 g) between these two groups (Castellini et al., 2010). Rebollar et al. (2006) also studied the effect of 24 h dam-litter separation. They compared the effects of dam-litter separation to hormonal stimulation of doe fertility with equine chorionic gonadotropin (eCG) injections on kit mortality. Using second parity does on a short (35 day) reproductive cycle, Rebollar et al. (2006) compared injecting does with 25 IU eCG to separating the doe and litter for either 24 or 48 h before AI on day 4 postpartum. Dam-litter separation was as effective as eCG in increasing fertility rates compared to non-treated does. However, by 4 days of age, kits in the separated litters had 30% lower body weights than control litters, and those separated for 48 h had the highest mortality rate, reaching 12% by 4 days postpartum (Rebollar et al., 2006).

Other researchers have assessed longer separation periods, often in comparison to free access nursing or other bio-stimulation methods (see *Table 3*). Maertens (1998) compared the effects of a 40 h dam-litter separation to PMSG or nutritional flushing. Does in the nutritional flushing treatment received a concentrated diet for 4 days prior to insemination. PMSG was injected at 20 IU at 9 days postpartum, and AI was performed at 11 d. Maertens (1998) found similar high fertility rates for PMSG and dam-litter separation (77–78%), compared to control (67%) or flushing (55%). However, the effectiveness of PMSG or separation disappeared by the fourth parity, and kits from the separated litters had 7–8% lower body weights than any other treatments (Maertens, 1998).

Multiple studies compared a longer separation period to free access nursing (Bonanno et al., 2004, 2010; Rebollar et al., 2004; Ilés et al., 2013; Ubilla et al., 2000a, 2000b). Bonanno and colleagues (2004, 2010) compared kit mortality and growth rates when does were given free access to nurse, separated from their litters for 44–48 hr with the separation split into one or two periods, or moved to another cage with their litter and then separated for 44 h. When litters were subjected to a 44 h separation, kit mortality and body weights were comparable to litters that had only a change of cage (Bonanno et al., 2010). When a 48 h separation period was split into two 24 h periods, kits grew comparably to control (free access nursing) litters, and better than litters

separated for 48 h continuously (Bonanno et al., 2004). The control litters experienced a higher pre-weaning mortality from d 14 to 35 (9.6%) than the separated litters (4.3–5%). Unlike litters from young does, litters from multiparous does separated for 48 h continuously had compensatory weight gains after the separation (Bonanno et al., 2004).

Using multiparous does, Ubilla et al. (2000a, b) evaluated separating does from their litters with the use of a metal screen for a 48 h period prior to insemination. Compared to litters permitted to nurse freely, litters that were separated had lower kit body weights from d 9–11, although the differences disappeared by d 21. Rebollar and colleagues (2004) also studied multiparous does, but compared controlled nursing (once daily) to controlled nursing with a 48 h dam-litter separation on kit body weight and mortality and dam feed intake. Does from the separated treatment produced 11% less milk than the controlled does through 21 d. Kits from the separated treatment had lower body weights than control kits after the separation, but differences disappeared by d 16 (Rebollar et al., 2004). Does from the separated treatment also consumed 40% less feed on d 10–11 than the control does. The authors hypothesized that the short term change in feed intake could be related to pain or discomfort from intramammary pressure. However, this has not yet been explored.

Ilés et al. (2013) studied the effect of a 48 h dam-litter separation on the adrenal activity and behaviour of primiparous does. Does had free access to the nest box (control) or had free access to the nest box but were separated from the litter completely from 9–11 d postpartum (DLS). There was no difference in plasma cortisol levels between the control and DLS treatments before or after the separation days. Control does had low mating acceptance (54.2%) compared to DLS does (88.5%) when presented with a buck on d 11. However, 60% of the DLS does refused mating prior to nursing. Similar to Rebollar et al. (2004), Ilés et al. (2013) suggested that the 48 h separation period led to some degree of discomfort, as the does had a full mammary gland. However, the stress associated with the separation was not enough to modify cortisol secretion (Ilés et al., 2013). Dam-litter separation, whether performed for 24 or 48 h, reduced kit growth rates compared to non-separated litters (Rebollar et al., 2004, 2006; Maertens, 1998; Ubilla et al., 2000a), which influenced their mortality rates in some (Castellini et al., 2010; Rebollar et al., 2006 with 48 h separation) but not all (Bonanno et al., 2004) studies. More research including other behavioural and physiological indicators of welfare is necessary to conclude whether dam-litter separation practices are acceptable for doe and kit welfare.

2.5 Ovulation Induction

With the use of artificial insemination (AI), ovulation needs to be induced artificially. Induction of ovulation in does is commonly done through an intramuscular injection of gonadotropin-releasing hormone (GnRH) immediately before or after the AI procedure. Because injecting the does prior to or immediately after insemination adds extra handling and potentially increases stress, Quintela et al. (2004) studied whether a GnRH agonist (buserelin) could be added directly to the seminal dose to decrease handling but maintain fertility. While fifteen times the dosage of buserelin was needed in semen to maintain high productivity, there was no negative effect of this dose on doe mortality rate (Quintela et al., 2004).

2.6 Restricted Access to Kits

Domestic rabbits differ from their wild counterparts in that they are typically housed in cages, with an open nest box attached to or inside the doe's cage. During the first 7–10 days, if provided the opportunity, does will leave the nest and their kits when not nursing, although they may maintain visual, auditory, and olfactory contact with the kits during this time, which may influence both kit and doe welfare. After the kits' eyes open, around 10–12 days of age, they begin exploring their environment and they will leave the nest box, gaining access to the doe and their feed.

In some commercial units, does are given access to the nest box and litter for a short period of time each day to nurse, mimicking natural conditions (Selzer et al., 2004; Hoy & Selzer, 2010). Otherwise, access to the nest box and litter is restricted. Different methods to separate litters have been studied (see *Table 4*), with varying effects on welfare. Baumann and colleagues (2005a, 2005b, 2005c) performed a series of experiments to compare different methods of controlled nursing. In their first experiment, they compared the behaviour of does given free access to the nest box to those given controlled access (Baumann et al., 2005a). Does with restricted access either had the closed nest box placed adjacent to the home pen, or removed completely except for once daily intervals of 15 min access for nursing. When does had free access, they performed significantly more nest approaches and checked the litter in 40% of those approaches. Does with restricted access to a closed nest box performed nest activities (nest opening and closing behaviour) throughout the entire 24 h period, whereas does with restricted access and a removed nest box restricted their nest activity to the hour prior to nursing. There was no significant effect of nest box access variations on kit weight or mortality, although litters with restricted access to a closed nest box grew more slowly than kits from litters in which does had free access. The authors suggested that the presence of an inaccessible nest box resulted in greater disturbances to the dam and litter than the nest box removal (Baumann et al., 2005a).

In a second experiment, Baumann et al. (2005c) studied whether the use of a cat flap which would permit free, but visually obstructed, access would improve doe and kit welfare. Does with free access (i.e., no cat flap) had higher serum corticosterone levels and had a higher frequency of putting their head fully in the nest outside of nursing times compared to does with the cat flap. The litters from does with free access had higher pre-weaning mortality rates (11.8%) compared to those with the cat flap (6.3%), which may have been due to the kits' ability to leave the nest. At 8 d of age, 39% more kits from the free access litters were observed outside the nest compared to the litters with the cat flap. While the cat flap had a positive effect on both doe and kit welfare, does in the cat flap treatment performed repeated nest closing behaviour, characterized by scratching on the floor and tapping at the nest entrance, after nursing bouts (Baumann et al., 2005c), which may imply that the does find these nests inadequate. The authors suggested that the presence of olfactory cues from the kits in both experiments may have had a negative effect on doe welfare by causing unsettled behaviour (Baumann et al., 2005a, c). In their follow-up experiment, Baumann et al. (2005b) tested does with restricted nest access with either an empty, clean nest or an empty nest with kit odours. The empty nest box with kit odours elicited more nest-related behaviour, such as repetitive digging movements at the entrance to the nest, scratching on the floor, and blocking the entrance to the nest, than the empty, clean nest. As wild rabbits typically close their burrows after nursing bouts to mask odours as anti-predator behaviour, the authors suggested that the inability of does in laboratory conditions to escape the

presence of kit odours in standard breeding cages may reduce their welfare (Baumann et al., 2005b).

Eiben et al. (2007) compared free access to nursing to four variations of restricted access to nursing on productivity and kit mortality. Kits were able to either nurse freely for the entire 35 d (control), were limited to nursing once a day for the first 14 d of lactation (farm practice) with free access thereafter, or were limited to nursing once a day from 8–10 days postpartum with visual, olfactory and acoustic contact otherwise (B2), olfactory and acoustic contact otherwise (B1), or no other contact otherwise (B0). Prior to 8 days and after 10 days postpartum, B1, B2, and B0 treatments were able to nurse freely. Does were inseminated at 11 d postpartum. Does with no contact with their kits outside the restricted nursing (B0) had the highest fertility (89.5%) and kindling rates (88.2%), but kits from these litters had lower body weights at weaning than B2 and B1 litters. Kits from the farm practice litters had the highest mortality during the first 8 days postpartum and weighed 4–7% less than kits from other litters weaned at 35 d. Growing rabbits from the farm practice (13.5%) and B0 (11.9%) treatments also experienced higher mortality rates from weaning to processing compared to the offspring of control (7.9%) and B2 (7.9%) treatments, although the reason is unclear. The authors concluded that it was not necessary to restrict access to nursing for the first 14 d as the kits grew slower and there was minimal added benefit to fertility (Eiben et al., 2007). They recommended permitting some olfactory and acoustic contact outside of the nursing period (B1 treatment). In a second experiment, Eiben et al. (2008) compared free nursing (control) to restricted access to nursing (once a day for the first 14 d of lactation with free access thereafter; farm practice) to restricted access to nursing with a 24 h fasting period for the does between d 8–9 (B). All does were inseminated on d 11. During lactation, kits from the B litters had slower growth rates (4.98 g/d) compared with 8.25 g/d for the farm practice litters. This difference continued through the fattening period, with the kits from B does growing more slowly until d 70 (36.1 g/d compared to 40.5–40.8 g/d for the farm practice and control rabbits; Eiben et al., 2008). There was no effect of treatment on pre-weaning mortality, although mortality rates from d 35–70 were higher for kits from does in the farm practice (13.5%) and B (12.3%) treatments compared to control kits (7.9%). Restricted access to kits postpartum may reduce kit mortality, particularly in litters from primiparous does. However, this restriction may reduce kit body weights at weaning. More research is needed to understand the long term implications of this practice on rabbit health and welfare.

2.7 Re-breeding Interval

In the wild, the weaning process begins with emergence behaviour at approximately 21 days (Richardson & Wood, 1982). If pregnant and lactating concurrently, does become aggressive towards their older kits in the pre-partum period (Mykytowycz & Rowley, 1958). While some suckling may continue through 6 weeks (Lloyd & McCowan, 1968) and wild rabbits may attempt suckling through 7 weeks (Lehmann, 1991), most domestic rabbits are weaned around 4 weeks of age as milk yield decreases after 21–25 days (Scapinello et al., 1999; Schlolaut et al., 2013). In Spanish and Portuguese commercial rabbitries, most producers inseminate does on day 11 postpartum and wean on day 31, using a 42 day reproductive cycle (Rosell & de la Fuente, 2009; de la Fuente & Rosell, 2012; Sánchez et al., 2012). However, some producers use shorter breeding intervals or earlier weaning ages to maximize the number of kits produced per doe per year.

Xiccato et al. (2006) compared the body compositions of multiparous does that had been re-bred at 2, 11, or 26 days postpartum. Those that were re-bred at 2 days postpartum exhibited significant body weight loss and energy deficit from first to last kindling (Xiccato et al., 2006). Those re-bred at 11 or 26 days maintained their energy balance, while either sustaining their body weight (11 day re-breeding) or gaining weight (26 day re-breeding). In a similar study, Gerencsér et al. (2011) compared the welfare of does and kits on a standard 11 d rebreeding interval (with weaning at 35 d) to a longer rebreeding interval (25 d postpartum, with weaning at 23 d). Does were studied through six to eight parturitions. With the longer reproductive cycle, there was a higher kindling rate (89.3 vs 82.0%) and heavier doe body weight at kindling (4474 vs 4188 g). Does also had a higher survival rate at 336 d with the longer rhythm (26 vs 13%). With the shorter rebreeding interval (and later weaning age), litters were heavier at birth (564 vs 528 g). However, early weaned kits experienced compensatory gains, and weighed more than those with the later weaning age at 11 weeks (2694 vs 2632 g; Gerencsér et al., 2011).

In their survey of Spanish farms, de la Fuente and Rosell (2012) found that 75% of breeding does were considered healthy, with an average body weight of 4.81 kg and a body condition score (BCS) of 4.5 (on a scale from 1 [emaciated] to 9 [obese]). BCS also varied with lactation status, genetics, and farm (de la Fuente & Rosell, 2012; Sánchez et al., 2012). After the first 5 lactations, BCS remained steady until the 20th lactation when it began to decline (Sánchez et al., 2012). Further research into rebreeding intervals and culling rates is needed, particularly during the first few parities when the mortality risk is the highest.

Lactating does differ in their sexual receptivity and fertility rates, depending on the day they are rebred postpartum (Stoufflet & Caillol, 1988; Ubilla & Rebollar, 1995). This, in turn, can affect kit welfare. Castellini et al. (2003) compared different re-breeding intervals on doe and kit survival. Half the does were bred on a fixed cycle every 42 d (insemination at 11 d postpartum). The other half were rebred on an alternating cycle: for one cycle, they were rebred at 1 day postpartum and for next cycle, on day 27 postpartum. Litter sizes were standardized to either 6 or 8 kits, and all kits were weaned at 26 days (Castellini et al., 2003). Pre-weaning and post-weaning kit mortality was highest when litters had 8 kits, particularly for litters from does on a fixed rebreeding cycle of 11 days postpartum. The same kindling interval was found for both rebreeding cycles even though the rebreeding interval of the alternating cycle was 3 days longer, on average. The authors concluded that alternating rebreeding cycles was better for rabbit welfare when kits are weaned at less than 35 d of age, particularly when in combination with larger litter sizes (Castellini et al., 2003).

2.8 Outstanding Issues Not Addressed by Current Literature

- 1. Association between AI techniques and doe welfare, including morbidity and mortality.**
- 2. Effect of bio-stimulation methods on the welfare of does and kits.**
- 3. Effect of early and late weaning ages on doe and kit welfare.**
- 4. Welfare implications of free access to kits in the early postpartum period.**
- 5. Effect of extended rebreeding interval on doe body condition and health.**

6. Litter size and doe and kit welfare (i.e., effect of balancing litters).

Table 3. Comparisons of dam-litter separation studies on kit welfare from reviewed scientific literature. $a \neq b$, $x \neq y$ denote statistical differences ($P < 0.05$) within studies within columns

Reference	Treatments compared	Weaning age	Kit mortality, %	Kit weaning weight, g
Bonanno et al. 2010	1. Control	31 d	2.8 ¹	664 ^b
	2. Changed cage for mating (free access nursing)		7.9	651 ^{ab}
	3. 44 h separation		3.9	631 ^{ab}
	4. 44 h separation and changed cage for mating		4.5	613 ^a
Bonanno et al. 2004 ²	1. Control (free access nursing)	35 d	2.6, 5.0 ^a	780 ^a
	2. 48 h separation		3.4, 4.3 ^a	732 ^b
	3. Two 24 h separation periods		3.7, 9.6 ^b	777 ^a
Castellini et al. 2010	1. Control (free access nursing; Exp. 1)	30 d	9.5	615
	2. 24 h separation with nest box closed (Exp. 1)		12.7	640
	1. Control (free access nursing; Exp. 2)	30 d	14.7 ^a	670
	2. 24 h separation with doe moved to new cage (Exp. 2)		22.6 ^b	658
Maertens 1998	1. Control (free access nursing)	29 d	5.9 ¹	670 ^a
	2. PMSG administration		6.4	668 ^a
	3. 40 h separation		5.8	623 ^b
	4. Flushing (does received concentrated diet from d 8–11)		3.9	663 ^a
Rebollar et al. 2004	1. Control (controlled daily nursing)	21 d ³	--	375
	2. 48 h separation		--	315
Rebollar et al. 2006 ⁴	1. Control (free access nursing)	25 d	4.0 ^b , 11.5 ^x , 1.2	428 ^a
	2. eCG injection on d 9		6.6 ^b , 5.5 ^y , 0.9	439 ^a
	3. 24 h separation		2.4 ^b , 10.1 ^x , 0.3	406 ^b
	4. 48 h separation		12.1 ^a , 15.4 ^x , 1.1	441 ^a
Ubilla et al. 2000b	1. Control (free access nursing)	30 d	19.0	--
	2. 48 h separation (with metal screen)		13.7	--

¹ Represents a value converted from original paper

² Mortality rate split into time periods: 9–16 days and 16–35 days

³ Measurements ended at 21 days

⁴ Mortality broken down into periods: 2–4 days, 5–16 days and 17–25 days

Table 4. Summary of restricted nursing studies on kit welfare from reviewed scientific literature. $a \neq b \neq c$ denote statistical differences ($P < 0.05$) within studies within columns

Reference	Treatments compared	Weaning age	Kit mortality, %	Kit weaning weight, g
Baumann et al. 2005a ¹	1. Control (free access nursing)	35 d	0.0, 0.0	874
	2. Restricted nursing (once daily; else, door closed to nest box)		0.0, 5.5	792
	3. Restricted nursing (once daily; else, nest box removed from pen)		12.7, 0.0	853
Baumann et al. 2005c ²	1. Control (free access nursing)	35 d	11.8 ^a	933
	2. Visual restriction (free access nursing but door at entrance to nest to restrict visual access)		6.3 ^b	958
Coureaud et al. 2000 ³	1. Control (free access nursing; primiparous does)	28 d	18.0 ^a	289 ^a
	2. Restricted nursing (once daily) from 0–3 d (primiparous does)		7.3 ^b	267 ^b
	3. Restricted nursing (once daily) from 0–5 d (primiparous does)		8.9 ^b	261 ^b
	1. Control (free access nursing; secondiparous does)	28 d	2.1	299
	2. Restricted nursing (once daily) from 0–3 d (secondiparous does)		3.6	289
	3. Restricted nursing (once daily) from 0–5 d (secondiparous does)		3.6	289
Eiben et al. 2007 ⁴	1. Control (free access nursing)	35 d	1.6 ^{ab} , 4.3	1033 ^c
	2. Restricted nursing (once daily) to d 14		2.4 ^a , 3.7	966 ^a
	3. Restricted nursing from d 8–10 with contact otherwise through wire mesh separator		0.5 ^b , 2.7	1017 ^{bc}
	4. Restricted nursing from d 8–10 with contact otherwise through metal separator		1.4 ^{ab} , 3.0	1024 ^{bc}
	5. Restricted nursing from d 8–10 with no contact otherwise		1.4 ^{ab} , 4.8	1004 ^b
Eiben et al. 2008	1. Control (free access nursing)	35 d	4.3	1033 ^b
	2. Restricted nursing (once daily) to d 14		3.7	966 ^a
	3. Restricted nursing to d 14 with doe fasted for 24 h from d 8–9		5.0	944 ^a

¹ Mortality rate split into time periods: 1–15 days and 16–35 days

² Mortality rates only different from 16–35 days of age

³ Weight measured at 21 d

⁴ Mortality rate split into time periods: 1–8 days and 1–35 days

2.9 References

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3. Health Management

Conclusions

- 1. Infectious enteric and respiratory diseases are significant causes of morbidity and mortality for all age groups of commercial rabbits.**
- 2. Intensive management practices and lack of implementation of common on-farm biosecurity practices used for other food animal sectors contribute to ongoing disease.**
- 3. Emerging techniques, such as Body Condition Scoring and a novel Rabbit Grimace Scale may be implemented with minimal cost as routine assessment tools for monitoring rabbit health and welfare.**
- 4. Rabbits are susceptible to heat stress, which may be partially offset by provision of space that allows postural adjustments. Regardless of space availability, extreme high temperatures may result in increased mortality depending on the stage of production.**

Compared with other food animal species, domestic rabbits have high mortality rates from birth until the end of production, averaging around 25% in Canadian herds (Kylie et al., 2016a), and mortality rates have changed little over the past few decades (EFSA, 2005). Both infectious and non-infectious causes contribute to morbidity and mortality of pre-weaned kits, growing rabbits, and breeding rabbits resulting in reduced welfare. Mortality rate is commonly used as an index of welfare in commercial rabbitries (Hoy & Verga, 2006). This section will focus on factors influencing the health of rabbits, and thus their welfare, in different production stages.

3.1 Tools to Assess Health and Well-Being

Rabbits are notoriously difficult to assess for health and welfare because they are prey species and are programmed to mask signs of sickness, weakness, and pain until disease is in its final stages. Changes in the behavioural time budget, feed and water intake, or body condition of a rabbit can indicate that there are threats to their well-being; however, this can be difficult to assess on an individual basis in intensive animal rearing conditions. While few validated assessment tools exist for commercial rabbits, researchers have validated the use of changes in behaviour, facial expressions, and body temperature for detecting pain in rabbits in experimental settings (Leach et al., 2009; Farnworth et al., 2011; Keating et al., 2012). Male rabbits that underwent abdominal surgery displayed reduced feeding and drinking, general grooming, and lying, but spent considerable time grooming the abdominal incision site (Farnworth et al., 2011). Rabbits also performed behaviours that were interpreted to be pain-specific, such as sitting with their back arched and fore and hind limbs drawn in tightly (referred to as “tight huddle”), walking at a very slow pace (“shuffle”), and partial hop movements (forward extension of forelimbs as if to hop, without movement of hind limbs; Farnworth et al., 2011). Following ovariohysterectomy, female rabbits also performed slow postural adjustments and shuffling, staggering, and twitching movements that were not observed prior to surgery (Leach et al., 2009). Weaver et al. (2010) studied rabbits after ovariohysterectomy and found a significant decrease in feed and water intake and fecal output after surgery regardless of analgesia used, a

75% reduction in distance travelled in the home pen, and a 98% reduction in rearing activity. However, Leach et al. (2009) cautioned against using high levels of inactivity as an indicator of pain following surgery because of the potential sedative impact of anesthesia or analgesia on locomotion and the “freezing effect” that direct observation causes in rabbits. Following ear tattooing, Keating and colleagues (2012) found no effect of the procedure performed with or without a topical anesthetic cream (EMLA, a topical eutectic mixture of 2.5% prilocaine and 2.5% lidocaine) on home pen behaviour, and an increase in ear grooming behaviour that occurred as a result of the EMLA application with or without tattooing. Changes in other pain-specific indicators were noted in this study (see below, Rabbit Grimace Scale) and could be used to differentiate between rabbits that had received a topical anesthetic prior to tattooing and those that had not.

Other behavioural and physiologic indicators of welfare have been studied in rabbits. Body weight changes and body condition scores (BCS) are useful indicators of health, pain, and disease in many other species (Bonde et al., 2004; Pritchard et al., 2005; Roche et al., 2009) and have been used in rabbits. de la Fuente and Rosell (2012) assessed the body condition scores of 2,775 breeding rabbits and found that sick rabbits had marginally but significantly reduced BCS compared to healthy rabbits. The problem with using body weight or body condition scoring for assessing animal health and welfare is that the method is always retrospective and can only be detected when significant pain or disease have been present over a period of time.

Keating et al. (2012) validated the use of a novel Rabbit Grimace Scale in a study on ear tattooing in rabbits. The Rabbit Grimace Scale assesses five different facial action units (orbital tightening, cheek flattening, nose shape, whisker position, and ear position) to create an overall score that increases when rabbits experience pain, which occurred in this study when rabbits were tattooed without topical anesthetic (Keating et al., 2012). Certain procedures (such as ear tattooing) may invalidate one or more of the facial actions (i.e., ear position) based on the procedure itself (Keating et al., 2012); however, the scale is a useful cage-side tool that may be used for a rapid but nonspecific assessment of animal wellness. Minimal training and no equipment is required for this scale to be implemented and it is a promising tool for assessing rabbit well-being on-farm.

Infrared thermography has been validated for use in assessing adverse stress (Ludwig et al., 2010) and heat stress (de Lima et al., 2013) in rabbits. Ludwig et al. (2010) subjected adult mixed breed rabbits to a social stressor (being placed in a cage with unfamiliar rabbits), a sudden noise, and tonic immobility. They recorded external eye and ear temperatures with an infrared camera before and after the stressors and collected blood samples before and after tonic immobility to assess serum corticosterone levels. Eye temperatures did not change between basal and stress conditions whereas ear temperatures decreased following each of the stressors. Corticosterone levels were higher after tonic immobility than before (Ludwig et al., 2010). The authors suggested that ear vasoconstriction following stressful conditions could serve as an adaptive coping strategy. In other species, physiologic responses to different stressors are dependent on whether or not the individual has the opportunity to influence the outcome of the stressor (Sherwood et al., 1990). Under social or auditory stress, ear vasoconstriction may serve to protect the ears from threat. de Lima et al. (2013) also used infrared thermography to detect differences in surface body temperature due to heat stress. They exposed rabbits to control environmental conditions (20–30°C) or extreme heat stress conditions (32°C) and photographed

their eyes, internal and external ears, and noses. They also took fecal samples to assess fecal corticosterone levels. Under heat stress, temperatures in the eyes increased most (up to 3.36°C above basal levels), followed by temperatures in the internal ear, external ear, and nose. As ear temperatures in this study contradicted the findings by Ludwig et al. (2010), more research is necessary to understand the effect of different types of stressors on rabbit physiologic response. While IR thermography may be correlated with physiologic stress during heat stress, the equipment is specialized and expensive and is unlikely to be implemented in farm settings.

3.2 Health Considerations at Different Production Stages

3.2.1 Pre-Weaned Kits

The behaviour of farmed neonatal kits is similar to that of their wild relatives. During the first 5 days of life, rabbit kits spent more than 90% of their time immobile in the nest, with lighter kits being more active than heavier kits (Bautista et al., 2008). However, they perform dynamic huddling behaviour, with a near constant circulation of kits to the centre of the huddle where temperatures are the highest (Bautista et al., 2003).

Pre-weaning mortality in farmed rabbits is highly variable, with reports ranging from 5–46% (Coureaud et al., 2000a; Drummond et al., 2000; Bautista et al., 2008; Rödel et al., 2009; Garrido et al., 2010; González-Redondo, 2010; Huneau-Salaün et al., 2015; Kylie et al., 2016a). Unlike mortality in growing and breeding rabbits, which is often due to infectious causes, much of the mortality that occurs prior to weaning can be attributed to litter size and weight (Bautista et al., 2003), doe behaviour (González-Redondo, 2010), genetics (Whitney et al., 1976; Lukefahr et al., 1983), management factors (Garrido et al., 2010), and doe health. Common causes of death include chilling, inadequate nest, poor maternal care, insufficient milk, and cannibalism. The first day of life is particularly important: Coureaud et al. (2000b) found that kits born from primiparous does that did not consume colostrum within the first 12 hours after birth had a 16% mortality rate through 10 days of age compared to 6.8% mortality rate for those that did consume colostrum. However, the difference disappeared when kits were born from multiparous does, and mortality rate overall was lower (2.2–2.5%; Coureaud et al., 2000b). Thus, while early colostrum consumption is important for kit health, it is not the sole determinant of survival, since kits receive partial transfer of maternal antibodies from their dams in utero (Peri & Rothberg, 1986).

Although kits are covered by maternal antibodies through to the time of weaning (Yoshiyama & Brown, 1987; Milon & Camguilhem, 1989), they are very susceptible to developing subclinical infections with various infectious agents, such as *Pasteurella multocida* and *Staphylococcus aureus*, particularly when there is a high environmental burden, when conditions are unhygienic, and when kits are exposed to contaminated secretions from the doe during routine activities, such as nursing and grooming. This early exposure to infectious agents, while not immediately detrimental to kit health, sets them up for more significant disease later in life, during periods of growth and reproductive stress. On-farm biosecurity practices remain at a low level overall in the Canadian meat rabbit industry, contributing to this ongoing problem (Kylie et al., 2016a).

Young kits are at risk for developing splay leg, and the condition was observed on the majority of commercial farms visited in one study (Rosell et al., 2009). Splay leg is associated with hip dysplasia and can range from mild, where the kit displays some gait abnormalities but is

otherwise unaffected, to severe, where the kit cannot ambulate. In general, rabbits with splay leg are otherwise clinically normal, with no other physical or neurological disorders (Joosten et al., 1981; Owiny et al., 2001; Fallahi, 2014). While the exact pathogenesis of splay leg is unknown, genetics (Joosten et al., 1981) and housing (Owiny et al., 2001) have been implicated. Joosten et al. (1981) studied the prevalence of splay leg in a closed breeding colony of Dutch rabbits. Kits with splay leg did not display any other clinical signs of disease, but developed splay leg by 3–4 weeks of age. They found that 4% of litters had one or more kits with splay leg and the condition was inherited through the male line (Joosten et al., 1981). However, the inheritance pattern was not straightforward: while selective breeding for the condition increased the prevalence to 10% of affected litters, the observed number of affected kits was lower than expected, leading some to believe that splay leg is multifactorial. Owiny et al. (2001) studied the effect of nest box flooring and dichlorodiphenyltrichloroethane (DDT) exposure on the prevalence of splay leg. Dutch-belted does were exposed to 0, 25, or 250 $\mu\text{mol/kg}$ of DDT during gestation and through 4 weeks of lactation. The does and their kits were housed in cages with three types of flooring in the nest boxes: waxed cardboard, smooth plexiglas, or plexiglas with textured non-slip plastic strips. There was a significantly higher prevalence of splay leg in kits housed with smooth plexiglas (21.8% prevalence) compared to those with cardboard (6.7%) or plexiglas with textured strips (0%). Doe exposure to DDT increased the prevalence of splay leg, but only in the plexiglas treatment. Owiny et al. (2001) suggested that exposure to the neuromuscular toxin may have weakened the kits and increased the prevalence of splay leg when they were housed on suboptimal flooring. The prevalence of splay leg can be reduced through genetic selection (Fallahi, 2014) and providing kits with appropriate nest box flooring material.

3.2.2 *Post-Weaned, Growing Rabbits*

Enteritis is common in young post-weaned growing rabbits. Rabbits in this age group are highly susceptible to infections as they change from a primarily milk-based diet to a cellulose-based diet and their gut acquires new flora. Reported mortality rates for growing rabbits are quite variable. In their survey of 95 French farms, Huneau-Salaün et al. (2015) found an average annual mortality rate of 7.1%, ranging from 1.9 to 11.3% on different farms, and Whitney et al. (1976) reported an average post-weaning mortality rate of 5.5%. However, in their study on weaning ages, Chen et al. (1978) observed post-weaning mortality rates ranging from 17.8 to 23.8%. Nonetheless, most studies agree that primary cause of mortality for growing rabbits in Canada and elsewhere is enteric disease (Chen et al., 1978; Percy et al., 1993; Badagliacca et al., 2010; Garrido et al., 2010; Kylie et al., 2016a).

3.2.3 *Breeding Does*

Young breeding does are at the highest risk for mortality and early culling in the first three parturitions (Rosell & de la Fuente, 2009a), in part due to high prevalence of respiratory disease, specifically, pasteurellosis (Sánchez et al., 2012; Rosell & de la Fuente, 2016). Because mortality rates in young does are high, average doe longevity is less than 8 months (Lukefahr & Hamilton, 2000), and the annual replacement rate for does is very high, compared to other food animal species (120–140%; Sánchez et al., 2006; Gil et al., 2007). Rosell and de la Fuente (2009a, 2016) surveyed Spanish farms and found the monthly spontaneous mortality risk for does was 2.8 to 3.4%. Gestational mortality rate was 4.5% and the highest risk was between days 10–15 and 25–

33 of gestation (Rosell & de la Fuente, 2009a), due primarily to respiratory and digestive diseases.

3.2.4 Bucks

In their survey of breeding rabbits, de la Fuente and Rosell (2012) found bucks generally to be less healthy than does (25.6% vs 18.5% with rhinitis; 4.4% vs 3.9% with sore hocks) and to have lower body condition scores than does (4.1 vs 4.69; 5 being the ideal in a 9-point scale). In two other studies, Rosell and de la Fuente (2009, 2016) found the mean monthly mortality risk for bucks to be 1.2% and 1.87%, respectively, with a monthly culling risk of 4.2%. Culling was due primarily to low productivity (Rosell & de la Fuente, 2009). More than 25% of bucks had rhinitis (de la Fuente & Rosell, 2012) and respiratory infections were the main cause of mortality (0.88% overall; 65% of mortalities due to pneumonia; Rosell & de la Fuente, 2009, 2016). Bucks were also susceptible to gastrointestinal diseases: 5.9% of deaths reported in Rosell and de la Fuente (2009) were due to enteritis and the mean monthly mortality risk due to enteric disease was 0.35% (Rosell & de la Fuente, 2016).

3.3 Health Conditions Affecting Rabbits at All Stages of Production

3.3.1 Rabbit Enteritis Complex

Whitney (1970) first described “enteritis complex” as nonspecific intestinal infections in growing rabbits that often results in death without any prior clinical signs of disease. Enteropathitis peaks at 10–14 days after weaning (Badagliacca et al., 2010; Garrido et al., 2010), and rabbits typically die within 24 to 72 hours after onset of anorexia or diarrhea (Whitney, 1970; Peeters et al., 1984). Multiple bacterial, viral, and parasitic agents have been reported to contribute to rabbit enteritis complex (REC), including *Escherichia coli*, *Clostridium spiriforme*, *Lawsonia intracellularis*, rotavirus, coronavirus, astrovirus, and coccidiosis, with more than two agents typically present at one time (Whitney, 1976; Peeters et al., 1984; Percy et al., 1983; Badagliacca et al., 2010; Dewrée et al., 2010). While some agents, such as rotavirus, are thought to be mildly pathogenic (Peeters et al., 1984), other agents, such as *E. coli*, *C. spiroforme*, and various coccidial species are associated with more severe enteritis, and moderate to high mortality rates (Peeters et al., 1984; Badagliacca et al., 2010). Clinical signs of REC include mild to severe watery diarrhea with or without blood, abdominal distention, and borborygmus (bowel rumbling noises; Whitney, 1976; Coudert & Licois, 2005; Badagliacca et al., 2010).

As REC is multifactorial, there is no single treatment for it. However, some management practices can reduce the risk of enteritis. Older weaning ages (≥ 35 days) are associated with increased risk for enteritis (Le Bouquin et al., 2009; Huneau-Salaün et al., 2015), possibly because of increased animal stress. Dirty, contaminated cages (Garrido et al., 2010) or deep litter in animals housed on the ground (Schlolaut et al., 2013) can increase coccidial transmission from older rabbits to young growing animals (Le Bouquin et al., 2009). Producers may introduce antimicrobial agents into feed in an attempt to manage REC, but this can further upset the delicate balance of the gastrointestinal flora of rabbits, resulting in fatal dysbiosis and routine use of antimicrobials can contribute to antimicrobial resistance in animals and humans (Kylie et al., 2016b). Thus, there is a need for evaluation of non-pharmacologic methods and practices to reduce the prevalence of REC in growing rabbits.

3.3.2 *Pasteurellosis*

Pasteurella multocida (“snuffles”) is the most common respiratory pathogen of rabbits, and it is associated with rhinitis and sneezing, otitis media/interna, conjunctivitis, systemic disease, pneumonia, abscesses, metritis, and genital infections (Percy et al., 1984; Deeb et al., 1990; Rougier et al., 2006). Clinical signs of the disease include mucopurulent oculonasal discharge, matted forepaws, sneezing, and head tilt (Kunstýř & Naumann, 1985), although infections may also be subclinical (Deeb et al., 1990). In one study, 29% of breeding rabbits were carriers for *P. multocida* (DiGiacomo et al., 1991). However, there are anecdotal reports that this rate is much higher on commercial farms since the probability of infection with *P. multocida* increases with age (Deeb et al., 1990), and Rosell and de la Fuente (2009a) found that clinical respiratory disease accounted for 30% of mortalities on breeding farms. Similarly, Sánchez and colleagues (2012) surveyed 103 breeding farms and reported 32.1% of the breeding doe population was sick, with a 22.7% prevalence of overt respiratory disease and 3.3% prevalence of co-morbidities. In another study, Rosell et al. (2009) reported that respiratory diseases contributed to 7.2% of urgent veterinary visits to Spanish and Portuguese rabbitries over a 10 year period, with increased visits during summer months. Virag et al. (2004) suggested that stressors, including poor ventilation, high ambient temperatures, transportation, and relocation can activate a latent infection. Although no research has examined the impact of *P. multocida* on behaviour and welfare specifically, or the impact of various stressors on disease rate, the high mortality rates associated with the infection signify reduced welfare.

3.3.3 *Heat Stress*

Rabbits have a normal core body temperature between 38.1–40.8°C (Chen & White, 2006) and cannot regulate their body temperature at ambient temperatures above this range (Nicol & Maskrey, 1997). As a result, they are particularly susceptible to heat stress. Amici et al. (2000) found that acute exposure to high heat stress (42°C for one hour) increased body temperature for the next 2 hours, and reduced feed intake for up to 5 days, and rabbits exposed to 43.3°C reached a body temperature of 41.7°C and respiration rate of 812 breaths/minute after 40 minutes of exposure. Although temperatures in Canada’s temperate climate rarely reach 40°C, rabbits begin to show signs of moderate heat stress at 30°C. Gonzalez et al. (1971) observed a significant increase in respiration rate in rabbits at 30°C compared to 25°C (172 breaths/minute vs 84 breaths/minute). Similarly, Kasa and Thwaites (1990) found that rabbits housed at 32.2°C increased their respiration rate to 204 breaths/minute within 20 minutes of heat exposure, and increased to 610 breaths/minute after 4 hours. A 24 day exposure to 33.5°C resulted in lower feed intake and body weight, higher rectal temperature and reduced immunoglobulin concentrations compared to housing rabbits at 18°C (Franci et al., 1996). However, rabbits may be able to acclimate to moderate heat stress. Dalmau et al. (2015) housed rabbits either at 18.4°C or at 20.1°C from 16:00 to 9:00, and 27.9°C from 9:00 to 16:00. During the hotter daytime, heat stressed rabbits spent more time lying prostrate (45–54% more time than control animals). However, their overall behavioural time budget differed little from the control animals and the authors concluded that the predictable rhythm of the heat stress allowed the rabbits to adapt their behaviour to the conditions. If housed at temperatures above their thermoneutral zone of 15 to 25°C, rabbits require adequate space within which to perform heat reducing behaviour. Regardless of space availability, extreme high temperatures may result in increased mortality depending on the stage of production.

3.3.4 *Ulcerative Pododermatitis*

Ulcerative pododermatitis also represents a significant threat to breeding doe welfare. The disease begins with hair loss and callus formation (also referred to as plantar hyperkeratosis), progresses to cracked and open calluses, and becomes most severe when open wounds or ulcers have formed on the hocks. While there is debate as to whether plantar hyperkeratosis is painful for rabbits (Rommers & de Jong, 2011), pododermatitis is generally accepted as a painful condition (Rosell & de la Fuente, 2009b; Rommers & de Jong, 2011; Buijs et al., 2014; Mancinelli et al., 2014). Although not identified as a leading cause of mortality (Rosell & de la Fuente, 2009a), pododermatitis is a major risk factor for culling (Cervioli et al., 2008; Huneau-Salaün et al., 2015), particularly as rabbits age, and the condition is significantly affected by housing. Further details on pododermatitis can be found in *Section 1: Housing*.

3.3.5 *Staphylococcosis*

As rabbit production becomes more intensive, the risk for pathogen spread increases. *Staphylococcus aureus* is one of the most common opportunistic pathogens present on commercial rabbitries, with one study finding *S. aureus* on 95% of farms tested (Hermans et al., 1999). Most rabbits are carriers of low virulence strains and bacteria are commonly isolated from the nose, skin, perineum, preputium, and vagina of rabbits (Hermans et al., 1999, 2003). However, *S. aureus* infections can also result in high mortality rates and the bacteria is virtually impossible to eliminate without complete depopulation (Hermans et al., 2003). *S. aureus* can cause exudative dermatitis, subcutaneous and internal abscesses, conjunctivitis, and purulent rhinitis in suckling rabbits, subcutaneous and internal abscesses in growing and breeding rabbits, and mastitis in breeding does (Hermans et al., 2003). Gil et al. (2007) found that *S. aureus* was responsible for 79% of the mastitis cases they examined, 79% of abscesses cultured, and 95% of pododermatitis cases. Since mastitis is one of the most common causes of culling for breeding does (Gil et al., 2007; Rosell & de la Fuente, 2009, 2016; Sanchez et al., 2012), the presence of *S. aureus* on farm significantly reduces rabbit welfare. Mastitis can occur at any time during lactation, resulting in infected does becoming lethargic and agalactic (Adlam et al., 1976). In its chronic form, mastitis causes abscess formation and discharge of pus. In its acute form, mastitis causes gangrenous lesions (also referred to as “blue breast”) and the condition can be rapidly fatal (Adlam et al., 1976; Gil et al., 2007). *S. aureus* can be transmitted from a doe with mastitis to a suckling kit, and then passed to an uninfected doe during cross-fostering (Adlam et al., 1976); therefore, care needs to be taken when there is an active outbreak of mastitis to limit the practice of cross-fostering and the introduction of new animals (Hermans et al., 2003).

3.3.6 *Encephalitozoonosis*

Encephalitozoonosis is caused by an intracellular parasite, *Encephalitozoon cuniculi* (Valencakova et al., 2008). The disorder is associated with neurological problems, most notably a head tilt, ocular disease, and kidney disease. Significant kidney disease and ocular lesions are less common in large breed rabbits than dwarf rabbits. Researchers have examined the disorder in pet rabbits and found that 78–100% of pet rabbits with neurological signs, and 35–71% of asymptomatic rabbits, were seropositive for *E. cuniculi* (Künzel et al., 2008; Valencakova et al., 2008). Correlation does not imply causation, however, and there are many potential causes underlying neurologic conditions in rabbits. Santaniello et al. (2009) surveyed rabbits on 40

commercial farms in Italy, and found the parasite present on all farms. Seroprevalence was higher in older (>4 months of age; 48% prevalence) rabbits compared to younger (<4 months of age; 15% prevalence) rabbits, perhaps due to temporary protection of younger rabbits by maternal antibodies transferred in the does' milk (Santaniello et al., 2009). Overall, 31% of the rabbits in their study were seropositive for *E. cuniculi*, despite having healthy body condition (Santaniello et al., 2009). In general, the disease progresses rapidly and up to 10% of rabbits have concurrent kidney failure (Kunzel et al., 2009).

3.4 On-Farm Biosecurity

With all infectious diseases, early recognition is necessary to minimize animal suffering and to reduce environmental burden of the parasite, thus improving animal welfare. Equally as important as early recognition is maintaining biosecurity on farm. Increased biosecurity measures significantly reduced the prevalence of *Campylobacter* spp. in broiler chickens (Gibbens et al., 2001) and porcine reproductive and respiratory syndrome (PRRS) virus in pigs (Dee et al., 2004). However, in a recent survey of Ontario rabbit producers, Kylie et al. (2016) found that cage cleaning practices were irregular and only 55% of farms quarantined new animals upon their arrival at the farm. In addition, 63% of producers kept other livestock species, most often within the same barn as rabbits. As sanitary status on farm is related to rabbit body weight and body condition (de la Fuente & Rosell, 2012) and can alter the prevalence of infectious diseases (Bennegadi et al., 2001), it is imperative for producers to increase the sanitation status of their farms.

3.5 Outstanding Issues Not Addressed by Current Literature

- 1. Non pharmacologic methods of reducing or minimizing REC in growing meat rabbits.**
- 2. Extent of encephalitozoonosis in commercial meat rabbits and contributions to animal distress.**
- 3. Impact of biosecurity practices on infectious diseases.**
- 4. Buck health and disease prevalence.**

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4. Feed and Water Management

Conclusions

- 1. Fibre, in particular acid-detergent fibre (ADF), is a critical nutrient for gut health and can reduce the incidence of enteric disease when provided in sufficient quantity.**
- 2. Qualitative or quantitative feed restriction practices, within limits, can improve welfare by reducing digestive disorders and obesity.**
- 3. Unrestricted access to clean drinking water is imperative for rabbit well-being.**
- 4. The presence of mycotoxins in feed can have negative effects on rabbit welfare.**

Rabbits are strict herbivores that, when given the opportunity, are highly selective in their diet choice, maintaining adequate fibre and protein levels based on feed quantity and quality (Cooke, 2014). Rabbits also produce and consume cecotrophs (a soft type of feces), which represent approximately 15% of their total feed intake, and are thought to be an important source of protein, micronutrients, and B vitamins (Gidenne & Lebas, 1987). When fed *ad libitum*, commercial rabbits consume their feed in 30 to 40 small meals per day, usually concentrated in the late afternoon and at night (Prud'hon et al., 1975). Sixty percent of the dry matter intake is consumed during the dark period, with a peak in intake at the start of the dark period (Gidenne et al., 2010).

Rabbit kits are entirely dependent on the doe's milk for nutrition for the first 16–18 days of life. The doe suckles the kits at least once per day, for about 3 to 5 min (Zarrow et al., 1965; Hoy & Seltzer, 2002). During their first week of life, kits consume approximately 15–25% of their live body weight in milk, which is high in lipids and proteins (Gidenne et al., 2010). Kits consume little solid feed prior to about 21 days of age (Gidenne et al., 2002). They begin investigating solid feed around 16–18 days of age and producing small quantities of cecotrophs (Gidenne & Lebas, 1987), but the majority of their feed intake continues to be milk. After weaning at approximately 4–5 weeks of age, rabbits consume about 84 g/d of solid feed, and begin producing larger quantities of cecotrophs (Maertens, 2010). Intake of cecotrophs increases to about 55 g/d until the rabbits reach 2 months of age, when intake becomes stable (Gidenne & Lebas, 1987).

The nutrition requirements of rabbits have been previously described in detail (NRC, 1977). This section will focus on the welfare implications of feed and water management practices.

4.1 Feed Quality

When given the opportunity, rabbits are selective in their diet and regulate their feed intake to optimize protein and digestibility (Somers et al., 2008; Gidenne et al., 2010; Cooke, 2014). de Blas and Mateos (2010) outlined the energy and nutrient requirements of growing and breeding rabbits, and defined the metabolizable energy requirement to be approximately 10 MJ/kg, on an as fed basis, for intensively reared rabbits. Krohn et al. (1999) compared the behaviour of female growing rabbits fed two diets differing in energy density, but provided in different quantities to maintain the same overall energy content, that is, 10.1 MJ/kg vs 7 MJ/kg, on an as fed basis of

metabolizable energy. They found no effect of diet on resting behaviour, but higher levels of exploratory and abnormal behaviour (bar biting and scratching in the corner of the cage) in animals fed the lower volume but higher energy density diet. They surmised that the larger volume of feed needed for the lower energy density diet led to longer feeding times, which left less time for performing exploratory and abnormal behaviours. When fed grasses cut to different lengths, domestic rabbits preferred forages with the highest crude protein content, regardless of the length (Somers et al., 2008). However, there is no data on whether these preferred forages increase other measures of animal welfare.

There has been little other research looking at the effect of feed additives on rabbit welfare. While there is some debate as to whether tannin extracts are non-nutritive and toxic or beneficial (Mueller-Harvey, 2006), two studies have examined the welfare impact of adding hydrolysable tannin extracts to the diets of growing rabbits (Maertens & Štruklec, 2006; Liu et al., 2012). Maertens and Štruklec (2006) examined whether hydrolysable tannin extracts, through their potential role in reducing protein digestibility, could reduce mortality rates related to digestive disorders. In 2 of 3 trials, they found reductions in mortality rates of growing rabbits through 57 d of age with the addition of 5 g/kg chestnut tannin extract. Liu and colleagues (2012) compared the serum cortisol levels of rabbits fed diets with or without the addition of hydrolysable chestnut tannins, natural antioxidants thought to reduce the negative effects of high environmental temperatures. They housed male growing rabbits (45 days of age) at 20°C without tannins or 33°C with 0, 5, or 10 g/kg chestnut tannins. Compared to growing rabbits housed at 20°C, those housed at 33°C without chestnut tannins had lower average daily gains (22.8 vs 26.6 g/d) and higher cortisol levels (2078 pg/mL vs 1067 pg/mL) after 3 weeks. However, the effect of heat stress on weight gain and cortisol levels disappeared with the inclusion of chestnut tannins in the diet (at 5 g/kg: average daily gain = 25.9 g/d, cortisol = 1332 pg/mL; at 10 g/kg: average daily gain = 24.7 g/d, cortisol = 1236 pg/mL). These results suggest that inclusion of chestnut tannins at 5 or 10 g/kg reduces some of the adverse impact of heat stress on growing rabbits. There is increasing research into the use of other additives such as probiotics, prebiotics, enzymes, and amino acids as alternatives to antimicrobials (Dalle Zotte et al., 2016), but there are no reports yet on their effects on rabbit welfare.

Commercially raised rabbits are typically fed pelleted concentrates. Rabbits prefer consuming pelleted rather than meal diets (Harris et al., 1983) and alternative feed formulations such as meal, mash, and roughage decreased average daily feed intake (Maertens, 2010). They also have a preference for pellet size, with pellets of at least 0.8–1.0 cm in length preferred (Gidenne et al., 2010). However, little else is known about the welfare implications of different feed forms.

4.2 Fibre

Using newly weaned rabbits, Gidenne et al. (2000) studied the effect of changing the diet composition from 12 to 20% acid-detergent fibre (ADF), through the inclusion of wheat bran, dehydrated lucerne meal, and dehydrated beet pulp. ADF is the least digestible portion of the forage, and as dietary ADF increases, digestible energy decreases. Gidenne et al. (2000) found that there was a significant effect of fibre inclusion on morbidity from 42–70 days of age, in that growing rabbits fed a lower fibre diet experienced higher morbidity than those fed the higher fibre diets. Similarly, Bennegadi et al. (2001) fed newly weaned rabbits a pelleted diet with

either 19% or 9% ADF and assessed the morbidity and mortality through 70 days. Rabbits fed the low fibre diet had twice as many days with diarrhea and 2.7 times the mortality rate (25% vs 9.4%), as those fed the higher fibre diet.

However, not all fibre affects morbidity and mortality similarly, and it may be that dietary fibre source, fibre content, and the ratios of high to low digestible fibre (Gidenne, 2003) and energy to protein are also important (de Blas et al., 1981). Champe and Maurice (1983) studied the source and fibre content of different dietary fibres for newly weaned rabbits. Alfalfa and coastal Bermuda grass meal were included in the diets at 3, 6, 9, or 12%. The authors saw a dose dependent decrease in mortality with increased feeding of alfalfa. However, mortality rates of rabbits fed the Bermuda grass ranged from 50–70%, mostly due to nonspecific enteritis, regardless of the inclusion rate (Champe & Maurice, 1983). de Blas and colleagues (1981) assessed the effects of different crude fibre levels in combination with different crude protein levels on mortality rates for growing rabbits. In general, they found that diets with low crude fibre content were associated with high mortality, but this was also dependent on the protein levels as the lowest mortality rate was found when the energy to protein level was 24.35 kcal digestible energy/g digestible crude protein.

Feugier and colleagues (2006) performed a series of experiments to assess the effects of different fibre and protein levels on growing rabbit health from 23 to 50 days of age. In their first experiment, they altered the dietary fibre level from 160 to 220 g/kg ADF by changing the starch and fat content in the diets. Increasing the fibre tended to negatively affect growth rate in the first 2 weeks after weaning, but there was no effect in the later growing period. There was less than 4% mortality overall, with a tendency for an inexplicable higher mortality rate when growing rabbits received 190 g/kg ADF. In the second experiment, Feugier et al. (2006) altered the protein (crude protein; CP) content from 150 to 210 g/kg CP by changing the starch and fat contents. Overall, there was high morbidity (32%) due to an outbreak of enteritis, but there was no effect of protein level on mortality rate. In their third experiment, Feugier and colleagues (2006) formulated a diet to match the best performing diets from the first two experiments (160 g/kg ADF and 210 g/kg CP, with 110 g starch) and compared this to a control diet (160 g/kg ADF and 180 g/kg CP, with 170 g starch). Growing rabbits on the experimental diet had a higher mortality (20.4%) than those on the control diet (6.8%), and grew 1.7 g/d slower. The authors suggested that, while fibre and protein levels within the ranges used did not affect mortality, the protein to starch ratio played an important role in determining mortality rates.

Pascual and colleagues (2013) reviewed the findings of multiple studies on increasing fibre for young does; they reported that high fibre diets (neutral detergent fibre greater than 40% dry matter), when fed to does prior to 60–70 days, had a positive effect on reproductive performance. However, data is lacking on the effect of qualitative feed restriction on the welfare of does.

Prebble and colleagues (2015) studied the effect of supplemental fibre in the form of intact hay on the behaviour of Dutch pet rabbits observed over a period of 17 months. The rabbits were fed an extruded diet with hay, muesli with or without hay, or hay only. Rabbits fed only hay spent more time feeding, and less time inactive, than those fed muesli without hay. They also spent less time in contact with other rabbits, perhaps because they spent 50% of their time engaged in feeding behaviour. Rabbits fed muesli without hay spent significant time chewing their rubber mat, while those fed the extruded diet with *ad libitum* access to hay did not differ in their

behaviour from those fed only hay (Prebble et al., 2015). Similarly, Berthelsen and Hansen (1999) investigated the behaviour of mature rabbits (16–31 months old) housed individually in either standard wire cages (46 x 77 x 40 cm) or enriched cages (standard cage with raised height in half the cage to 80 cm, and addition of a wooden box, 44 x 25 x 19 cm with a perforated plastic roof) when provided with *ad libitum* access to hay. Without hay, rabbits in both cage types performed grooming up to 16% of their time. When they had hay, the rabbits spent more time sniffing at their surroundings while keeping their hind limbs in place, and spent less time grooming and gnawing than when they did not have hay. Rabbits in the standard cages also interacted more with the hay than those in the enriched cages (Berthelsen & Hansen, 1999). It is unclear if differences in behaviour when rabbits are fed hay are due to satiety from increased fibre content or because a higher proportion of the time budget is utilized through interactions with the hay. No differences were observed in mortality rates.

Leslie et al. (2004) assessed the preference of rabbits for grass or coarse mix feeds. When they had no previous experience with grasses, rabbits chose the grass more than the coarse mix. In a motivation test requiring the rabbits to circumnavigate a maze, all rabbits were willing to work more to gain access to grass than the coarse mix, but not by much (11.9 trips around the maze for grass; 10.3 trips around the maze for coarse mix). This finding led the authors to hypothesize that the novelty of the grass may have played a significant role in rabbit preference (Leslie et al., 2004).

Overall, feeding diets with sufficient levels of the correct types of fibre can improve gastrointestinal health, protecting rabbits from enteritis complex. There may be additional positive effects of dietary fibre on rabbit behaviour.

4.3 Feed Restriction Practices

Production and feed management systems vary significantly between European countries and Canada, due in large part to significantly higher per capita consumption of rabbit in Europe. As a result, commercial rabbit production in Canada is on a smaller and less intensive scale. Unlike the situation in many European countries (Maertens, 2010), most commercial rabbits are fed *ad libitum* in Canada. Conditions that may warrant energy restriction include outbreaks of digestive disorders (e.g., rabbit enteritis complex [REC]; Gidenne et al., 2012) or excessive weight gain in reproductive does (Manal et al., 2010). Energy can be restricted either by increasing the digestible fibre in the diet as discussed in the previous section or restricting feed quantity (Fernández-Carmona et al., 1996) and/or controlling access to feed at specific times of the day.

In meat rabbits, restriction of feed quantity for a period after weaning has been utilized in countries where REC is prevalent, and this practice reduced morbidity and mortality rates (Foubert et al., 2008; Martignon et al., 2010; Romero et al., 2010). Foubert and colleagues (2008) weaned rabbits at 32 days of age and induced REC in half the cages. When reared under good sanitary conditions, the rabbits had a 3.5% mortality rate that was not influenced by feeding level (Foubert et al., 2008). However, when REC was induced, the mortality rate spiked to 29%, with the feed-restricted treatment experiencing lower mortality rates in the first 3 weeks after disease induction (Foubert et al., 2008). Martignon et al. (2010) restricted rabbits weaned at 28 days to 72% of *ad libitum* feed access for the first 25 days after weaning. They observed a

tendency for lower mortality rates in restricted rabbits compared to *ad libitum* fed rabbits. Similarly, Romero and colleagues (2010) studied newly weaned rabbits that had *ad libitum* access to feed or those that had access to feed for 8 h/day for the first two weeks following weaning. When there was a *Clostridium* spp. outbreak during the experiment, feed restricted rabbits experienced lower mortality rates than those fed *ad libitum* (Romero et al., 2010).

Quantitative feed restriction practices have an effect on rabbit behaviour. Using rabbits weaned at 34 days of age, Gidenne and Feugier (2009) incrementally restricted feed to 60, 70, 80, and 100% of *ad libitum* access. They observed immediate changes in feeding patterns. Full-fed rabbits consumed the majority of their feed during the dark period, and never consumed more than 10% of their ration within any hour (Gidenne et al., 2012). Restricted rabbits, fed at 8:00 AM, had finished their daily ration completely within the light period (Gidenne & Feugier, 2009). This, in turn, changed the circadian rhythm of cecotrophy. While full-fed rabbits eliminated the majority of cecotrophs during the dark period, rabbits fed at 60% *ad libitum* eliminated the majority of their cecotrophs during the morning and early afternoon, with no cecotrophs produced overnight. The effect of changing feeding patterns on rabbit welfare is unknown.

Martínez-Paredes et al. (2012) examined the effects of either qualitatively or quantitatively restricting feed intake for young does during rearing on their performance and mortality. Using 190 young does beginning at 9 weeks of age, they compared *ad libitum* access to a control diet (11.03 MJ/kg dry matter [DM] of digestible energy [DE]; 114 g/kg DM of digestible protein [DP]), restricted access to the control diet (140 g/d), *ad libitum* access to an alternative diet with lower energy and higher fibre (8.72 MJ kg⁻¹ DM DE; 88 g kg⁻¹ DM DP), and two combinations of *ad libitum* access to the control and alternative diets. The control and alternative diets had similar ratios of DP:DE (10.3 and 10.1 g/MJ, respectively). At the time of insemination, the rabbits fed the control diet *ad libitum* had significantly higher estimated body energy (MJ/kg) than those fed restricted amounts of the control diet or those on the alternative diet, with rabbits fed the combination of the two diets being intermediate. However, at the time of parturition, there was no effect of treatment on body mass. Rabbits fed the control diet *ad libitum* had significantly higher mortality (34%) from 9–12 weeks of age compared to those on the alternative diet (3%) due to REC and higher mortality (14%) than all other treatments (3%) during the last 3 weeks of pregnancy.

Menchetti and colleagues (2015) studied the timing and degree of restriction on primiparous doe body condition. They fed primiparous does either a controlled amount throughout pregnancy (130 g/d; equivalent to 1.2 times the maintenance requirements), or restricted the does to 90 g/d (0.8 times the maintenance requirements) during the first 9 days (R1), the second 9 days (R2), or the final 9 days of pregnancy (R3). Does in the R1 group lost weight during feed restriction but had compensatory gains upon refeeding; however, they were unable to match the final body weights of the control does (Menchetti et al., 2015). Does in the R2 group had the lowest perinatal mortality rates, but pre-weaning mortality was higher for those restricted in mid- (27%) or late (37%) pregnancy compared to control (10.6%) or early pregnancy (9.6%). In late gestation, does in the R1 and R2 groups had lower body condition scores compared to control does, and control does produced 23–32 g/d more milk than any of the restricted does.

Voluntary feed intake for reproductively active does varies widely, from 150 to 450 g/d depending on where they are in the reproduction cycle (Maertens, 2010). Aside from their maintenance requirements, the energy requirements are dictated by growth, pregnancy, and lactation. During early gestation, does can easily fulfill these requirements (Maertens, 2010) and if they are not restricted there is a risk of excess fat stores, which decreases embryonic survival (Manal et al., 2010). Yet, lactating does often have difficulty meeting their energy requirements in their first few lactations (Xiccato et al., 1996, 1999). Although there has been little welfare research into the area, young primiparous does are typically feed restricted to 40 g/kg live weight from 11 weeks until first kindling (Rommers et al., 2001; Pascual et al., 2013). If not feed restricted during parturition, young does may become obese, have reduced fertility (Rommers, 2004), and become at increased risk for developing pregnancy toxemia (Flatt et al., 1974). Manal and colleagues (2010) restricted pregnant primiparous does to 1.32 times their maintenance requirements during the first 10, 15, or 20 days of gestation and compared them to does fed *ad libitum*. While they found no effect of restriction on fertility or kindling rates, total litter size, or the number of kits born live, there were fewer stillbirths in the restricted group. Restriction also had positive effects on pre-parturient maternal behaviour; restricted does began nest building and nest lining earlier than those fed *ad libitum*. The kits from does restricted for 15 and 20 days were also heavier at birth and weaning (Manal et al., 2010). They concluded that the short-term feed restriction led to compensatory feed intake in late gestation, which is generally a time of weight loss for does as it parallels the period of greatest fetal growth.

Data on young bucks and feeding regimens is lacking. While Maertens (2010) suggested that adult bucks (>18 weeks) are typically feed restricted to 40 g/kg, data on the effectiveness of feed restriction on reproductive traits are equivocal (Alvariño, 2000), and there has been no research on the welfare implications of restricting bucks.

While feed restriction and controlled feeding practices have a positive impact on rabbit health with the reduction of enteric diseases, the effect of these practices on hunger and aspects of behaviour that may impact welfare are unknown.

4.4 Mycotoxins

Mycotoxins are secondary metabolites produced by fungi, and they are often found in animal feeds. There are many different types of mycotoxins and their presence is determined by field and storage moisture and temperature conditions. There has been significant research into understanding the toxicologic effects of mycotoxins on rabbits (e.g., Mézes & Balogh, 2009), resulting in recommendations on the maximum levels of mycotoxins that can be present in rabbit feed (EFSA, 2014). Deoxynivalenol (DON; vomitoxin) is the most prevalent mycotoxin in North American forage. Hewitt and colleagues (2012) compared growing rabbits fed a diet with corn naturally contaminated with *Fusarium* mycotoxins with growing rabbits fed a control diet. DON was the major contaminant in the feed, present at 4.2 µg/g. There was no effect of feed contamination on feed refusal, but growing rabbits fed the contaminated feed had increased water intake and lower serum alkaline phosphatase activity than control rabbits (Hewitt et al., 2012). In the absence of other differences, the authors concluded that mycotoxin-contaminated feed negatively affected rabbits, but that rabbits appear less sensitive to feed-borne mycotoxins than other livestock species (Hewitt et al., 2012).

Aflatoxins A and B and ochratoxin A (OTA) are highly teratogenic, resulting in physical, skeletal, and organ abnormalities in kits born to does fed the toxins (Wangikar et al., 2005, 2010). Rabbit kits can be exposed to OTA through the does' milk, and Ferrufino-Guardia and colleagues (2000) found significant correlations between the amount of toxin the does consumed and the concentrations of toxins found in their milk. This resulted in twice the levels of OTA in the kidneys of kits as in does (Ferrufino-Guardia et al., 2000).

The effects of T-2 toxin, a metabolite of *Fusarium*, which is often found in cereals and plant products, were determined in a number of studies (Gentry & Cooper, 1981; Glávits et al., 1989; Kovács et al., 2013). Gentry and Cooper (1981) gave a single intravenous injection of 0.05 mg/kg BW of T-2 toxin to adult male and female rabbits. The injection caused a rapid decrease in hematocrit, white blood cell count, and serum alkaline phosphatase levels. They then studied the effects of orally administered toxin on the rabbits (Gentry & Cooper, 1981). When dosed orally with 2.0 mg/kg BW, rabbits developed oral lesions, diarrhea, anorexia, hair loss, and nasal discharge. Glávits et al. (1989) assessed a wider range of toxin concentrations and administered a single oral dose between 1 and 15 mg/kg BW to adult rabbits. Doses higher than 4 mg/kg BW led to mortality within 24–48 h. Lower doses of the toxin resulted in liver problems and damaged cells involved in both humoral and cell-mediated immunity (Glávits et al., 1989). In their follow-up experiment, they administered an oral dose of 2 mg/kg BW and observed gastritis, emaciation, and adrenocortical hypertrophy (a nonspecific sign of severe systemic stress) in the rabbits.

To determine the No Observed Adverse Effect Level (NOAEL), Kovács and colleagues (2013) studied the effects of chronic exposure to T-2 toxin in rabbits. They gave 9-month-old bucks varying doses of T-2 toxin by gavage over a 65 d trial. They found a dose dependent decrease in feed intake through the first 4 wk of the experiment, but no effect of the toxin on morbidity or mortality. They determined that the NOAEL was between 0.05 and 0.1 mg/rabbit/day (approximately 0.01–0.02 mg/kg/d). Higher concentrations caused temporary hepatic dysfunction and parenchymal damage to the liver (Kovács et al., 2013).

Rabbits are moderately sensitive to the effects of different mycotoxins present in their feed. Excessive contamination should be avoided.

4.5 Water Intake

In the wild, it is rare for rabbits to drink water from open sources, possibly because they are prey species and rarely gather at the edge of large bodies of water in the open (Cooke, 2014). When given the opportunity, they selectively choose food with moisture contents above 55%, which allows their water needs to be met in the food (Cooke, 2014). However, standard pelleted diets have a high dry matter content, requiring growing rabbits to consume about 1.6–1.8 times water as feed, and adults and breeding does to consume twice the amount of water as feed (Gidenne et al., 2010). Cizek (1961) studied the water intake of 146 rabbits, with data on Dutch, English Angora, Rex, Chinchilla, giant Flemish, Himalayan, New Zealand white, and Polish breeds. In general, rabbits consumed less feed and less water with age, but the relationship between feed intake and water intake changed with age, and was dependent on breed (Cizek, 1961). Young male New Zealand white rabbits (BW = 2.3 kg) ingested 104 ml/kg water per day, while females

(BW = 2.5 kg) consumed 99.5 ml/kg water per day. Rabbits of both sexes ate approximately 37 g/kg of pelleted feed per day (Cizek, 1961).

Without *ad libitum* access to either drinking water or high moisture-content feed, rabbits restrict their feed intake (Boisot et al., 2004; Tschudin et al., 2011b; Bovera et al., 2013) and have decreased urine output and higher dry matter content of urine and feces (Tschudin et al., 2011a). Bovera et al. (2013) also found higher serum cholesterol levels in water-restricted rabbits. These findings underscore the welfare concerns of water restriction.

Research on water intake of commercial meat rabbits is limited, and there is no data on preferences for drinker types. However, insight may be gained through studies with pet rabbits. Tschudin et al. (2011a) studied the preference of pet rabbits to drink from either a nipple drinker or an open water dish mounted at different heights. All of the rabbits preferred to drink from the open water dish, and drank more rapidly from the open water dish than the nipple drinkers (Tschudin et al., 2011a). When they had *ad libitum* access to water and were fed a diet with fresh parsley, adult Dwarf rabbits consumed 106 ml/day of water from the drinkers in 30 drinking bouts per day. Rabbits did not display a preference for nipple drinker height, although there were significant individual differences (Tschudin et al., 2011a). While rabbits preferred open dishes on the floor (Tschudin et al., 2011a), they became contaminated quickly (Tschudin et al., 2011b), which could increase the spread of pathogens.

Water is necessary for survival. Rabbits must be given unrestricted access to clean drinking water for their well-being.

4.6 Outstanding Issues Not Addressed by Current Literature

- 1. Potential adverse effects of feed restriction and feed control practices on hunger and behaviour.**
- 2. Effects of feeding practices on buck welfare.**
- 3. Effect of fibre length on gut health.**

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5. Pre-Transport

Conclusions

- 1. Wire flooring may help maintain cleanliness on upper levels of trucks, but may increase soiling in lower levels, which may decrease welfare.**

Most rabbits are transported at least once in their life, yet transportation between farms and to slaughter is a major risk factor for reduced rabbit welfare in Canada. However, rabbits are not referenced in the current Code of Practice for Transportation (Canadian Agri-Food Research Council, 2001), and the rabbit sector lacks a national board from which guidance can be provided. Although the transportation period, including lairage, will not be covered in this review, the period prior to transportation, which may include feed and water withdrawal, handling, and placement in crates or containers, includes many critical points that may lead to behavioural and physiological signs of poor welfare, and negative affective states.

5.1 Feed and Water Withdrawal

In Canada, on-farm lairage periods occasionally last longer than 24 hours, with abattoir lairage durations sometimes longer than 48 hours. During this time, feed and water are not typically provided. According to Canadian legislation (Health of Animals Regulation, 2015), rabbits, as a monogastric species, cannot be contained for longer than 36 hours without access to feed and water. It has been suggested that rabbits may be more resistant to thirst and hunger than other non-ruminating monogastric species due to their ability to produce and consume cecal feces (cecotrophy; Jolley, 1990); however, the production of cecotrophs follows a circadian rhythm (Jilge, 1982) that is easily disrupted under stressful conditions (Mugnai et al., 2009). Therefore, feed and water withdrawal prior to transport is considered among the most important risk factors impacting welfare (Cavani et al., 2006; EFSA, 2011).

In one of the only studies on feed and water withdrawal independent of transport, Purdue (1984) examined the effects of feed, water, or feed and water withdrawal for up to 36 hours on the weight loss of 2.2 kg rabbits. After a 6 hour feed and water fast, rabbits had lost 2.7% of their body weight (BW). After 12 hours, rabbits lost 4.9% BW with no access to feed, and 7.1% BW with no access to feed or water. Weight loss reached its maximum after 24 hours without feed or water access (13.9% BW lost). Purdue also observed the presence of hair in the stomach contents of transported and fasted animals. Although fur licking behaviour was not observed, the accumulation of hair could have been the result of drinking water droplets off their or conspecific's fur, normal grooming behaviour, or a behavioural response to stress.

Transport and lairage durations can be used as indirect measures of feed and water withdrawal, as feed and water are not typically supplied either on truck or in lairage. Although not solely a predictor of feed and water withdrawal time as there may be a fasting period at the farm prior to transport, lairage durations can provide insight into rabbit responses to extended feed and water restriction. In a study assessing lairage durations, María et al. (2005) transported rabbits for 3 hours, lairaged them for either 2 or 6 hours at the slaughter plant, and measured their physiological responses to the treatments. They found higher corticosterone and lactate levels in

the rabbits that were lairaged for 2 hours. Similarly, Liste and colleagues (2009) transported rabbits for 3 hours, and lairaged them for either 2 or 8 hours prior to slaughter. They assessed physiological indicators of stress, and found that rabbits in the longer lairage treatment (associated with longer fasting times) had significantly lower corticosterone, glucose, and lactate concentrations, and fewer hematomas than those in the shorter lairage treatment. While it is impossible to tease out the effect of transportation and handling on these findings and the authors did not report the duration of fasting prior to transport, the longer period of lairage (and, presumably, fasting) did not negatively impact the physiological indicators of stress in this study.

In contrast, Petracci et al. (2008) found negative effects of longer transport and lairage durations. They studied 831 herds of growing rabbits transported to slaughter. Rabbits were fasted for approximately 5 hours before being loaded. As both trip and lairage durations increased, mortality rates significantly increased. Trips longer than 320 minutes had more than twice the mortality rate of those shorter than 220 minutes, and had higher live weight loss and more bruising than medium and short trips. Mortality rates increased incrementally as lairage durations increased, with the highest mortality rates associated with lairage durations greater than 4 hours.

5.2 Containers

Size and height of the container (hereto referred to as crate) are considered among the most important animal welfare risk factors during transport (EFSA, 2011). Transportation crate size can be quite variable although the standard (European) crate size is 100–110 x 50–60 x 22–30 cm (L×W×H; area = 5000–6600 cm²; Verga et al., 2009; Petracci et al., 2010), with most rabbits transported in plastic crates (Cavani et al., 2006). In Canada and the United States, producers often use standard plastic chicken crates that measure approximately 87.6 x 58.4 x 27.9 cm (area = 5116 cm²). Buil and colleagues (2004) surveyed more than 100 farms, abattoirs, and trucking companies in Spain on their transport practices. All farms used a crate system for containment during transport, with a mean crate area of 4300 cm² (range from 1430 to 10000 cm² per crate). They found that the mean crate density was 353.7 cm²/rabbit, with a range of 179–667 cm²/rabbit, and a mean crate height of 30.7 cm. Most trucks in their survey did not have controlled environmental conditions, and litter was rarely used.

There is no scientific data on the welfare implications of different rabbit transport crate designs. However, the European Union has made recommendations with regard to crate design and placement (EFSA, 2011) and German law legislates crate height (Luzi et al., 2006). According to EFSA, crates should permit “adequate ventilation” during transport, to ensure a temperature within 5–20°C and, when placed on top of other crates during transport, crates should limit urine and feces falling onto crates below. German law requires a minimum crate height of 15 cm for rabbits up to 1 kg, 20 cm for those up to 3 kg, and 25 cm for those >3 kg (Luzi et al., 2006). However, transport conditions in Germany vary considerably from those in Canada as their growing rabbits are transported for less than 4 hours, and crated for less than 8 hours (Luzi et al., 2006).

5.3 Loading Density

Using both males and females at the end of their production cycle, Lambertini et al. (2006) transported rabbits at either high (75.5 kg/m²; 15 rabbits/cage; 340 cm²/rabbit) or low (49.0 kg/m²; 10 rabbits/cage; 510 cm²/rabbit) stocking densities for 1, 2, or 4 hours. While they predominantly studied meat quality parameters, they found no effect of stocking density on weight loss during transport and no interactions between trip duration and stocking density. However, their “high” density treatment is close to what is considered standard within the European industry, and their 3.3% live weight losses, even with the “long transport and high density” were within previously published reports (de la Fuente et al., 2007; Mazzone et al., 2010).

de la Fuente and colleagues (2004) assessed the physiological responses of rabbits transported at two different stocking densities (53.6 and 37.0 kg/m²) and in two seasons (winter and summer). While they found significantly higher concentrations of cortisol, lactate, glucose, and lactate dehydrogenase activity in summer rather than winter, there was no effect of stocking density on the measured parameters. This may have been because the differences in stocking densities were not large enough, because the seasonal effects overrode any differences between densities, or because stocking density may not significantly impact welfare in terms of biological functioning.

5.4 Outstanding Issues Not Addressed by Current Literature

1. Rabbits’ behavioural responses to extended periods of feed and water withdrawal.
2. Welfare response to transportation in different containers.
3. Welfare response to long periods of lairage and transportation (>8 h), as is common in Canada.

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6. On-Farm Euthanasia

Conclusions

1. **When using captive bolt devices, correct placement on the head is critical.**
2. **Blunt force trauma is associated with high rates of failure to cause immediate insensibility.**

On an annual basis, approximately 600,000 rabbits are processed for meat at Canadian slaughter plants. With estimated pre-market losses of 20% to 30% (Kylie et al., 2016), there are roughly 200,000 rabbits that die or are culled yearly on commercial rabbit farms. In Ontario, most producers use blunt force trauma as their method of euthanasia (Walsh et al., 2016b); however, approximately 50% of Canadian rabbit producers do not have a current on-farm euthanasia plan (Walsh et al., 2016b). As such, there is a pressing need for research into the most humane methods for on-farm euthanasia.

Euthanasia is defined as the “ending of the life of an individual animal in a way that minimizes or eliminates pain and distress” (AVMA, 2013). CVMA (2014) stipulates that animals “must be rendered irreversibly unconscious as rapidly as possible with the least possible pain, fear, and anxiety.” Animals can be killed by one of three means: direct or indirect hypoxia to the brain and related tissues, depression of the nerve signalling that is necessary for life, or physical damage to brain that interferes with its activity or that of vital neuronal pathways (AVMA, 2013). Hypoxia occurs when oxygen (O₂) levels are lower than normal. This can occur via exposure to gases such as carbon dioxide (CO₂) or nitrogen (N₂). However, hypoxia can also occur indirectly through asphyxiation and any method that directly interferes with respiration. Depression of the cortical neurons necessary for life can occur through chemical, gas, or physical means, whereas physical damage to brain activity occurs solely through direct trauma to the head.

In rabbits, loss of consciousness can be evaluated through assessment of brainstem and spinal reflexes, including pupillary light and corneal reflexes, righting reflex, tonic/clonic convulsions, response to painful stimuli, rhythmic breathing, and EEG patterns (Szczodry, 2013; Walsh et al., 2016a). Death should be confirmed by cessation of heart beat as well as respiration.

6.1 Chemical Methods of Euthanasia

Chemical methods of euthanasia involve intravenous injection of specific drugs. The Canadian Council on Animal Care (CCAC; 2010), American Veterinary Medical Association (AVMA; 2013), and Canadian Veterinary Medical Association (CVMA; 2014) include intravenous injection of barbiturates as the only unconditionally acceptable method for euthanasia of rabbits. While barbiturates, including sodium pentobarbital, are often considered the most humane method of killing animals, they are controlled substances and currently can only be administered by a licensed veterinarian, limiting their routine use on-farm for euthanasia.

6.2 Gaseous Methods of Euthanasia

Inhalant anesthetics have been assessed for their efficacy and quality of euthanasia (Hayward & Lisson, 1978; Flecknell et al., 1999; Llonch et al., 2012; Dalmau et al., 2016). Flecknell and colleagues assessed the induction of anesthesia via sevoflurane and isoflurane. Delivery of both of these gases at a rate of 4 L/min (up to 5% isoflurane in 100% O₂ or 8% sevoflurane in 100% O₂) induced immediate tachypnea (rapid breathing), followed by apnea (suspension of breathing) for up to 180 seconds, coupled with escape attempts from the chamber (Flecknell et al., 1999).

Hayward and Lisson (1978) assessed two concentrations of CO₂ for causing loss of consciousness. At 30% CO₂, few animals lost consciousness whereas at 40% CO₂, rabbits lost consciousness within 4–10 min. While they did not assess whether rabbits found CO₂ to be aversive, Hayward and Lisson (1978) found differences between wild and domestic rabbits in their response to the gas in that wild rabbits had longer survival times than domestic rabbits (Hayward & Lisson, 1978). Dalmau et al. (2016) studied rabbit aversion to different, high concentrations (70%, 80%, 90%, or 98%, in air) of CO₂. Regardless of the concentration, rabbits displayed nasal discomfort and vocalizations when exposed to CO₂. More animals displayed nasal discomfort and muscular jerks with 90% vs 70% CO₂, but the time to loss of posture was faster with 90% and 98% vs 70% and 80% CO₂. Behaviour indicative of aversion lasted for approximately 15 s before loss of posture and consciousness (Dalmau et al., 2016). In a similar study, Llonch et al. (2012) studied rabbit aversion to 90% CO₂ and 80% N₂ with 20% CO₂. They found a higher percentage of animals in respiratory distress with the 90% CO₂ than with the N₂/CO₂ mixture (97% vs 42%), and a longer latency to loss of posture with 90% CO₂ (28.2 seconds) vs the N₂/CO₂ mixture (24.2 seconds). Although CO₂ exposure is listed as a conditionally acceptable method of euthanasia within the most recent CCAC guidelines (2010) for all species, both the CCAC and the AVMA (2013) stipulate that gradual fill of chambers must be used.

6.3 Physical Methods of Euthanasia

Physical methods of euthanasia, including penetrating and non-penetrating captive bolt devices, cervical dislocation with or without assistance, blunt force trauma, and decapitation induce significant and direct damage to the brain resulting in depression of the central nervous system. Captive bolt devices are included as acceptable methods of euthanasia for all species within the CCAC (2010) and AVMA (2013) guidelines, with the condition that the devices be properly placed, cleaned, and maintained. Although few studies have been performed on the various penetrating and non-penetrating captive bolt devices available, Schütt-Abraham et al. (1992) found that placement of a spring loaded captive bolt device is critical; 95% of 3 kg rabbits were immediately killed or stunned, although misplacement of the device reduced its efficacy to 63%.

More recently, Walsh et al. (2016a) evaluated the brain damage and failure rates of blunt force trauma (BFT), assisted manual cervical dislocation (AMCD) using a wall mounted device, and non-penetrating captive bolt (NPCB) for euthanizing pre-weaned kits, growing rabbits, and adult rabbits. The NPCB caused significantly greater brain damage compared to the other two methods and resulted in immediate insensibility in 100% of the rabbits euthanized (Walsh et al., 2016a). BFT failed in 22% of all rabbits and was ineffective for 43% of adult rabbits (BW = 4.0 kg).

AMCD resulted in an intermediate failure rate of 6%, with most failures on pre-weaned kits (BW = 0.4 kg). The authors concluded that NPCB and AMCD were effective single step methods for on-farm euthanasia, but that blunt force trauma was not humane or effective for all age groups (Walsh et al., 2016a). Regardless of method, training is essential to improve accuracy, efficacy, and operator confidence.

6.4 Outstanding Issues Not Addressed by Current Literature

1. Aversiveness of mixed gas combinations.
2. Effect of low levels of CO₂.
3. Effect of different flow rates of CO₂.
4. Effect of physical methods.

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