Animal (2020), **14:6,** pp 1270–1277 © The Animal Consortium 2020
doi:10.1017/S1751731119003379

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Effect of different housing systems (single and group penning) on the health and welfare of commercial female rabbits

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(Received 12 September 2019; Accepted 9 December 2019; First published online 13 January 2020)

In recent decades, concern about rabbit welfare and sustainability has increased. The housing system is a very important factor for animal welfare. However, information about how different available housing types for female rabbits affect their health status is scarce, but this is an important factor for their welfare. Hence, the objective of this study was to evaluate the health status of female rabbits in five common housing systems: three different single-housing systems with distinct available surfaces and heights; a single-housing system with a platform; a collective system. Female rabbits in the collective and platform cages had greater cortisol concentrations in hair than those in the single-housing system with no platform. Haptoglobin concentrations and kit mortality rates during lactation were greater for the collective-cage female rabbits. The collective group had more culled females and more lesions than in the other groups. The main reasons for culling in all the groups were reproduction problems and presence of abscesses, and the collective group of females was the most affected. In conclusion, it appears that keeping females together in collective systems negatively affects their health status and welfare, while single-housing systems imply lower kit mortality rates during lactation and cortisol concentrations, and fewer lesions in female rabbits.

Keywords: cages, rabbit does, stress, health status, haematological parameters

Implications

There is much controversy about which housing type (individual or collective) is more appropriate for rabbits and how it affects their welfare. The positive effect of collective systems has been highlighted because rabbits can better mimic their natural behaviour. However, health status and stress must also be considered because they strongly affect animal welfare. In this study, the rabbits allocated to higher and deeper cages had the best welfare indicators, while those in collective ones displayed greater levels of stress, morbidity and kit mortality.

Introduction

Animal welfare is currently one of the main challenges facing livestock production as social and commercial demands are increasing. Rabbit farming is also affected by these demands, and there is growing social concern about welfare and sustainability in rabbit production. Therefore, it is necessary to improve current production systems based on scientific knowledge.

The implementation of housing measures to improve animal welfare has a direct effect on the economic benefits of farms. The incorporation of larger cages with fewer animals increases production costs and lowers profits. However, these measures also positively impact animal welfare and health by improving farms' productive results. In fact, stressed or sick rabbits show greater infertility rates, more abortions and greater weight loss (Marai *et al.*, 2002; Rosell and De La Fuente, 2008).

Concern about housing conditions is due to the fact that keeping animals in cages may favour the appearance of different injuries, which may be related to cage characteristics. For example, pododermatitis is associated with the absence of footrests (Rosell and de la Fuente, 2013). It is assumed that the elements present in cages (e.g. straw in the nest) or the cage material itself can increase the risk of injuries, such as purulent dermatitis, subcutaneous abscesses or mastitis (Marcato and Rosmini, 1986). Nonetheless, information about the relationship between other common pathologies

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and cage type is scarce. In general, the most frequent causes of culling in female rabbits on Iberian farms are diseases of the respiratory system (particularly coryza) and the reproductive tract, as well as mastitis, subcutaneous abscesses and pyometra (Segura *et al.*, 2007; Sánchez *et al.*, 2012).

Several authors also recommend placing items for environmental enrichment purposes, such as mirrors (Dalle Zotte *et al.*, 2009), shelters (Hawkins *et al.*, 2008) and platforms (Hansen and Berthelsen, 2000) in cages to reduce stress. The use of platforms has been studied in depth and, although diseases or injuries were not detected, the welfare of female rabbits did not improve (Szendrő and McNitt, 2012; Alfonso-Carrillo *et al.*, 2014). Nevertheless, the same authors later reported the usefulness of platforms for breeding does (Szendrő *et al.*, 2019). Thus, results regarding the use of platforms must be accurately studied with further research by considering the specific conditions of each platform and cage.

In recent years, collective systems have been proposed to improve the welfare of female rabbits as they allow them more mobility, and social contacts between animals increase. However, continuous group housing produces more aggression and injuries among does and kits than group housing systems with temporary separations (Dal Bosco *et al.*, 2019; Szendrő *et al.*, 2019).

This study aimed to evaluate five types of common housing systems, and a new housing system is proposed to improve breeding does' welfare from health and immunological points of view by studying the haematological values, cortisol concentrations, causes of death and culling of does and kit mortality rates at weaning.

Material and methods

Animals and housing

One hundred and fifty 19-week-old breeding female rabbits were used. They were placed in five different cage types 1 week before the first artificial insemination (**AI**; Figure 1). These cages were located in the facilities of the Universitat Politècnica de València, and the study was carried out in 2016 and 2017. All the cages had wire floors, and footrests were placed inside all the cage types. Detailed characteristics of the five cage types are shown in Table 1. The five housing systems were three single-housing systems with different total floor surface areas and cage heights (24 polyvalent (**PV**; 3 920 cm^2 and 38 cm high), 24 higher and deeper (**HD**; 5 250 cm^2 and 50 cm high), and 24 traditional (**TR**; 4 270 cm^2 and 38 cm high)); 24 single housing with a wire platform (**PF**; 4 960 cm^2 and 57 cm high); four collective systems for six females (**COL**; 4 980 cm^2 and 65 cm high). In the collective system, female rabbits were placed in groups of six which, between 28 gestation days and 18 days postpartum (**dpp**), were separated into individual compartments (41 cm wide; Figure 1, COL-2). Female rabbits and kits were monitored throughout five reproductive cycles or until they died or were culled. When a female rabbit died or was culled, another 19-week-old animal was placed in its cage to achieve 30 animals per group. A reproductive management rhythm (parturition interval) of 42 days was used, with insemination at 11 dpp and weaning at 28 dpp. The average temperatures were between 16ºC and 21ºC. The lighting programme was 16 h of light and 8 h of darkness, and the building was artificially ventilated. Animals had free access to fresh water through automatic drinkers and were fed with a commercial pelleted diet *ad libitum* (chemical composition: 91% DM, 8% ash, 17% CP, 34% neutral detergent fibre, 17% acid detergent fibre and 3.2% lignin). Experimental proceedings were approved by the competent authority (Generalitat Valenciana, Spain) as set out in Spanish Royal Decree 53/2013 on protection and use of animals for experiments.

Blood and immune system assessments

Between 19 and 22 female rabbits were sampled per group at first insemination (**1AI**) and between 16 and 17 females at fifth parturition (**5P**) to analyse the haematological parameters. Nine millilitres of blood was drawn from the median artery of each animal's ear using tubes with ethylenediaminetetraacetic acid (**EDTA**) to carry out different analyses. Blood cell counts (erythrocytes, platelets and white blood cells), haematocrit and haemoglobin were analysed using an haematology analyser (MEK-6410; Nihon Kohden, Tokyo, Japan). Intra-assay CV of haematology analyser was 5.3%. Differential leukocyte counts were obtained from Giemsa-stained blood extensions. Haptoglobin concentration was determined from blood plasma, which was extracted from whole blood by centrifugation at 2500×**g**. Subsequently, a colorimetric assay (Phase Range; Tridelta Developments Ltd, Maynooth, Ireland) was used to analyse the haptoglobin concentration according to manufacturer's protocol. Intra- and inter-assay CVs were 1.0% and 2.1%, respectively. In the detection limit tests, the samples containing 0.10 mg/ml were significantly different from the blank controls ($n = 12$; $P < 0.001$).

The phagocytic capacity of heterophils, as a representative cellular type of innate immunity, was evaluated by an *in vitro* cell function test. Heterophil purification was carried out according to the protocol described by Siemsen *et al.* (2014) with some modifications. Briefly, 5 ml of whole blood was lysed with 45 ml of ammonium chloride lysing solution for 6 min at room temperature in the dark. The tubes were centrifuged at 400×**g** for 5 min at room temperature without brake. The supernatant was eliminated, and the pellet was carefully resuspended in 5 ml of rabbit neutrophil buffer. The suspension was transferred carefully with a Pasteur pipette over 7 ml of Histopaque® 1077 (Sigma) in a 50-ml tube, and different washes were performed as described in this protocol. Once heterophils were purified, the number of cells was calculated by counting with a Neubauer chamber. Then they were faced with fluorescein isothiocyanate (**FITC**)-stained *Staphylococcus aureus* for 30 min. The final bacteria : heterophils ratio was 20 : 1. After 30 min, phagocytosis was stopped by adding 1 ml of 1× cold

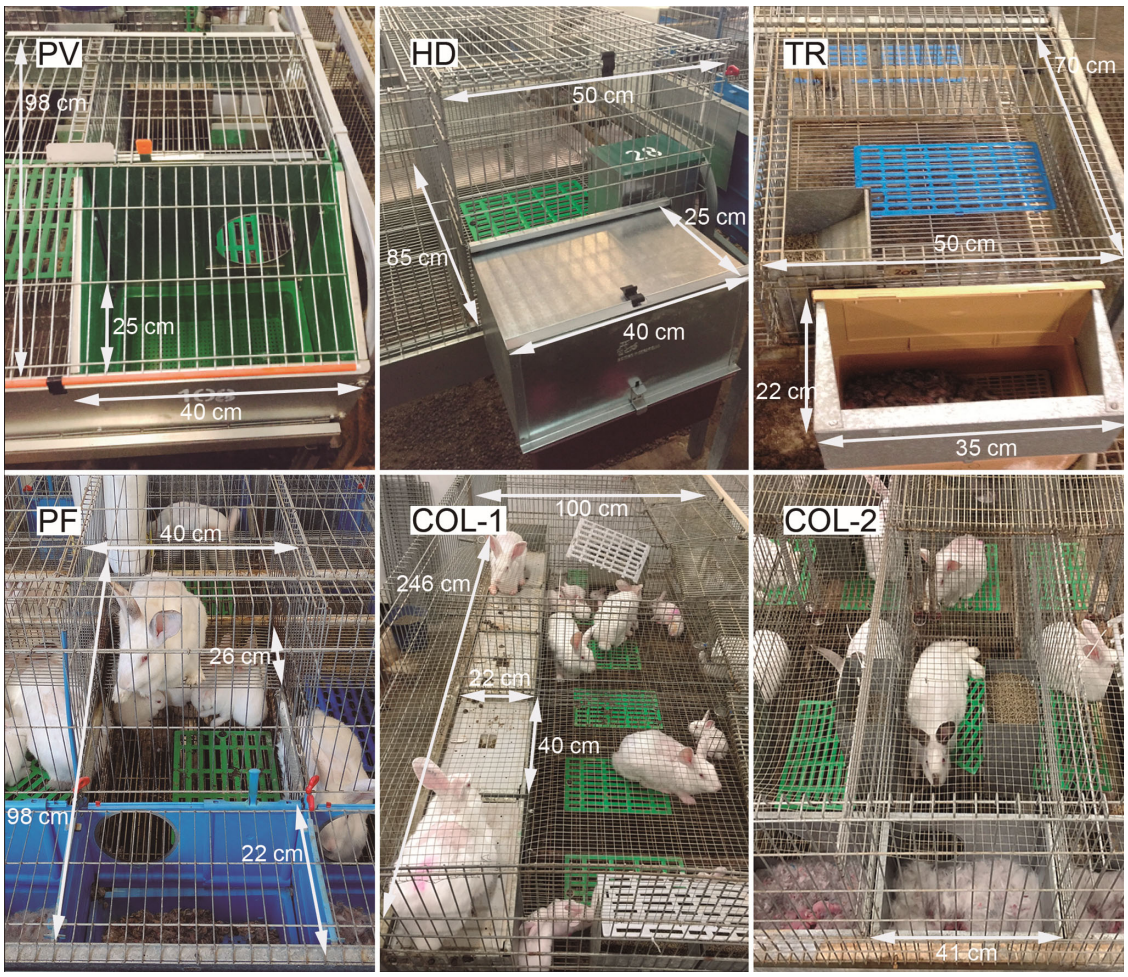

Figure 1 (colour online) Different cage types for female rabbits used in this study: polyvalent (PV), higher and deeper (HD), traditional (TR), with platform (PF), collective (COL-1, all together; and COL-2, individually separated between 28 gestation days and 18 days postpartum).

Table 1 *Characteristics of cages for female rabbits*

	Polyvalent (PV)	Higher and deeper (HD)	Traditional (TR)	With platform (PF)	Collective (COL)
Width (cm)	40	50	50	40	41
Depth (cm)	98	85	70	98	100
Height (cm)	38	50	38	57	65
Nest (cm)	40 × 25 × 47	40 × 25 × 37	35 × 22 × 37	40 × 22 × 39	40 × 22 × 37
Type of nest	Internal	External	External	Internal	Internal
Platform (cm)	No	No	No	40 × 26 × 35	40 × 22 × 30
Floor surface area (cm^2)	3920	5250	4270	4960	4980

phosphate-buffered saline (**PBS**). Then the tubes were centrifuged at 420×**g** for 10 min at 4ºC. The supernatant was eliminated, and the pellet was resuspended in 200 µl of 1× PBS. Ten microlitres of this suspension was placed over a slide and fixed with 4% paraformaldehyde. The cell cytoplasm was stained with Rhodamine Phalloidin® (Thermo Fisher, Waltham, MA, USA) and the nucleus with 4′,6-diamidino-2-phenylindole. The phagocytosis ratio was calculated by fluorescence microscopy (Leica, Solms, Germany) by counting 100 cells.

Cortisol concentrations

Samples of hair from the front of the head were taken at 1AI and the fifth weaning of all the does for chronic stress evaluation. Analyses were performed according to Tallo-Parra *et al.* (2015).

Pathological studies

All the culled and dead female rabbits were evaluated. Females were culled for either evident lesions that seriously affected animal health and welfare (e.g. severe pododermatitis,

mastitis, abscesses and bleeding injuries), or clinical reproductive problems such as three negative AIs, dystocia or abortions. Animals were euthanised by an intravenous injection of T-61 (MSD Animal Health), and a complete necropsy was carried out. Histopathologic samples were taken from those organs showing lesions and were fixed in 10% buffered formalin. Then they were processed routinely and stained with haematoxylin–eosin. Gram and red Congo stains were performed whenever necessary. Samples were also taken from the observed lesions for microbiological studies. The percentage of kit mortality during lactation was recorded in each group.

All the sections were evaluated under a light microscope (Nikon Eclipse E600), and photographs were taken with a digital camera (Nikon DXM 1200).

Microbiological studies

Samples from the observed lesions were taken using swabs. Swabs were sown in Columbia blood agar plates and incubated at 37ºC for 24 h. The presence of *S. aureus* and *Pasteurella multocida* was studied based on the morphological appearance of colonies. Those compatible with *S. aureus* were sown in tryptone and soya broth and were incubated 24 h at 37ºC with shaking. Then DNA was extracted using the Genelute Bacterial Genomic DNA Kit (Sigma, St. Louis, MO, USA) according to the manufacturer's protocol, except that bacteria were lysed by lysostaphin (12.5 mg/ml; Sigma) at 37ºC for 1 h before DNA purification. Molecular typing was performed as previously described by Viana *et al.* (2007). Multi-Locus Sequence Typing (**MLST**) was also performed. The MLST allelic profiles and the corresponding sequence types (**ST**) were determined from the MLST database. To confirm any other colonies that differed from *S. aureus*, such as *P. multocida* and other frequently isolated ones, they were sent to a microbiological laboratory. Isolates were analysed with Gram staining and biochemical tests: catalase, oxidase, indole, urea, citrate, triple sugar iron agar, Slanetz Bartley, esculin and the CAMP test.

Statistical analysis

Leukocyte and haptoglobin values were normalised by applying logarithms due to the asymmetrical distribution of the original data, except for the heterophils : lymphocytes (**H** : **L**) ratio, which was obtained directly from the counts. Data were analysed using the MIXED procedure and the SAS® programme, with the model including cage type and sampling time as fixed effects, as well as the permanent effect of the animal as the random effect (more information about the statistical models is found in Supplementary Material S1). Binomial data such as causes of culling and death, kit mortality, lesions and microbiology were analysed using a generalised linear model (GENMOD procedure) following a binomial distribution with a logistic regression. A least square means comparison test was carried out to determine differences between treatments.

Table 2 *Blood parameters and cortisol concentrations in hair of female rabbits at first insemination (1AI) and fifth parturition (5P)*

	1AI	5P		
No. of animals	104	81	SEM	*P*-values
Erythrocytes ($\times 10^{12}$ cells/l)	6.19	5.43	0.046	<0.001
Haemoglobin (g/l)	133	111	1.179	<0.001
Haematocrit (%)	40.5	34.0	0.342	<0.001
Platelets ($\times 10^9$ cells/l)	267	320	9.171	<0.001
Leukocytes ($\log_{10}$ 10^9 cells/l)	0.88	1.00	0.017	<0.001
Heterophils ($\log_{10}$ 10^6 cells/l)	3.51	3.62	0.024	0.004
Lymphocytes ($\log_{10}$ 10^6 cells/l)	3.56	3.61	0.026	0.199
Monocytes ($\log_{10}$ 10^6 cells/l)	2.70	2.60	0.056	0.266
Eosinophils ($\log_{10}$ 10^6 cells/l)	1.25	1.62	0.121	0.054
H : L ratio[1]	0.95	1.66	0.241	0.057
Haptoglobin ($\log_{10}$ mg/l)	2.54	2.63	0.024	0.018
Cortisol in hair (pg/mg)	0.48	0.93	0.043	0.001
Phagocytosis (%)	24.4	16.8	1.378	0.001

[1]Heterophils : lymphocytes (H : L) ratio directly obtained from counts (no logarithmic transformation).

Results

Haematological parameters and cortisol in hair

Table 2 shows the haematological parameters of all the rabbit does at two different sampling times (1AI and 5P). The values at 1AI were taken as baseline values for the rabbit does in this study as they were not under any housing group effect until 1AI. Blood counts indicated that the total number of erythrocytes, haemoglobin and haematocrit decreased from 1AI to 5P (−12%, −17% and −16%, respectively; $P < 0.001$), while the number of platelets increased (+20%; $P < 0.001$). The number of leukocytes also increased from 1AI to 5P (+14%; $P < 0.001$), mainly due to an increase in the number of total heterophils (+3%; $P < 0.01$). Haptoglobin and cortisol concentrations also increased from 1AI to 5P (+4% and +94%; $P < 0.05$), whereas phagocytic capacity decreased from 1AI to 5P (−31%; $P < 0.001$).

Blood values at 5P were compared to assess the effect of different housing systems on the haematological parameters of rabbit does at an advanced time of their productive lives (Table 3). Female rabbits placed inside the PF cages had greater haemoglobin values than those in HD and TR cages (on average +7%; $P < 0.05$). Female rabbits housed in the COL cages had more platelets than those in HD and PF cages (on average +35%; $P < 0.05$). Females rabbits in the COL cages had greater haptoglobin concentrations than those in HD, PF and TR cages (on average +8%; $P < 0.05$). The PF and COL groups had greater cortisol concentrations than the PV and TR groups (on average +136%; $P < 0.05$), while the HD group had the lowest cortisol concentrations (on average −578% *v.* COL and PF; $P < 0.05$).

Pathological studies

Thirty-five rabbit does were culled for various reasons and 27 died during the study (Table 4). Necropsy was performed on 55 animals and 52 had at least one evident lesion.

Table 3 *Blood parameters and cortisol concentrations in hair of female rabbits located in each housing type at fifth parturition (5P)*

	Types of cages						
	PV	HD	TR	PF	COL		
No. of animals	16	16	17	16	16	SEM	*P*-values
Erythrocytes ($\times 10^{12}$ cells/l)	5.55	5.35	5.42	5.52	5.31	0.12	0.216
Haemoglobin (g/l)	116^{ab}	108^{a}	108^{a}	115^{b}	107^{ab}	3	0.045
Haematocrit (%)	35.3	33.1	33.7	34.9	32.8	0.8	0.094
Platelets ($\times 10^{9}$ cells/l)	312^{ab}	281^{a}	335^{ab}	272^{a}	369^{b}	23	0.014
Leukocytes ($\log_{10}$ 10^{9} cells/l)	1.04	0.96	0.99	0.97	1.04	0.05	0.196
Heterophils ($\log_{10}$ 10^{6} cells/l)	3.65	3.59	3.66	3.60	3.63	0.06	0.329
Lymphocytes ($\log_{10}$ 10^{6} cells/l)	3.63	3.63	3.53	3.62	3.65	0.07	0.181
Monocytes ($\log_{10}$ 10^{6} cells/l)	2.62	2.51	2.67	2.64	2.57	0.14	0.375
Eosinophils ($\log_{10}$ 10^{6} cells/l)	1.68^{ab}	1.12^{a}	2.00^{b}	1.57^{ab}	1.72^{ab}	0.30	0.029
H : L ratio[1]	1.38	1.13	2.68	1.25	1.87	0.52	0.051
Haptoglobin ($\log_{10}$ mg/l)	2.62^{ab}	2.57^{a}	2.57^{a}	2.59^{a}	2.78^{b}	0.06	0.018
Cortisol in hair (pg/mg)	0.61^{b}	0.23^{a}	0.71^{b}	1.67^{c}	1.45^{c}	0.09	0.001
Phagocytosis (%)	13.4	16.2	18.7	16.5	19.0	3.0	0.221

PV = polyvalent; HD = higher and deeper; TR = traditional; PF = with platform; COL = collective.
[1]Heterophils : lymphocytes (H : L) ratio directly obtained from counts (no logarithmic transformation).
[a,b,c]Values in a row with different superscripts differ significantly at $P < 0.05$.

Table 4 *Causes of death and culling of female rabbits in each housing system (number and frequencies) and kit mortality percentage*

	Types of cages											
	PV		HD		TR		PF		COL		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	*P*-values
Total removed	12^{ab}	40.0	10^{a}	33.3	13^{ab}	43.3	9^{a}	30.0	18^{b}	60.0	62	0.041
Dead	5	16.7	7	23.3	7	23.3	3	10.0	5	16.7	27	0.177
Culled	7^{ab}	23.3	3^{a}	10.0	6^{ab}	20.0	6^{ab}	20.0	13^{b}	43.3	35	0.007
Reproductive problems	5^{ab}	16.7	1^{a}	3.3	3^{ab}	10.0	5^{ab}	17.7	7^{b}	23.3	21	0.049
Abscesses	0	0.0	2	6.7	3	10.0	1	3.3	4	13.3	10	0.139
Others	2	6.7	0	0	0	0	0	0	2	6.7	4	0.112
Kit mortality	–	9.3^{ab}	–	6.4^{a}	–	8.2^{ab}	–	7.8^{a}	–	13.1^{b}	–	0.038

PV = polyvalent; HD = higher and deeper; TR = traditional; PF = with platform; COL = collective.
Values in percentages over the total of animals in each group.
[a,b]Values in a row with different superscripts differ significantly at $P < 0.05$.

More animals were removed from the COL group ($n = 18$, 60% of animals; $P < 0.05$) because there were more culled does ($n = 13$, 43.3%; $P < 0.05$) than in individual cages.

When examining all the groups together, the most frequent causes of culling were reproductive problems ($n = 21$) and presence of abscesses ($n = 10$), with greater values in the COL group, although there was only a significant difference in reproductive problems between COL and HD groups ($P < 0.05$). The main causes of death were infectious processes ($n = 19$), especially metritis ($n = 7$), pneumonias ($n = 6$) and peritonitis (n = 4), but no significant differences were found between groups.

Of all the lesions observed during necropsies, reproductive problems (mainly associated with metritis, foetus retentions and presence of corpora lutea), pneumonia and abscesses (Figure 2) were the most frequent. The PV group had the fewest number of reproductive lesions ($n = 2$) compared to the PF, TR and COL groups ($n = 10$, 10 and 9, respectively; $P < 0.05$). More rabbits with abscesses were observed in the COL group ($n = 7$) than in PV and HD groups ($n = 0$ and 1, respectively; $P < 0.05$).

Kit mortality rates during lactation were significantly greater in the COL group than in HD and PF groups (on average +6.0%; $P < 0.05$).

Microbiological analysis

The most frequently isolated bacteria from lesions were *P. multocida* (55% of necropsied animals), mainly from metritis, pneumonia and abscesses, followed by *S. aureus* (18% of necropsied animals), mainly from pododermatitis, and a wide range of different lesions. *Proteus* spp. was isolated from abdominal abscesses (10% of necropsied animals). No intergroup differences were found. One female carried two different genotypes of *S. aureus*: B1/II1/α and A1/II1/δ.

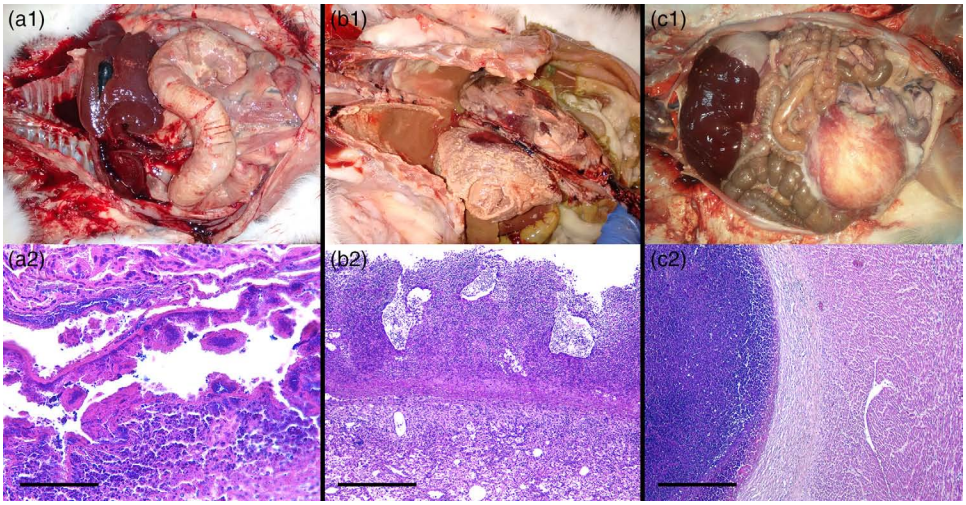

Figure 2 (colour online) Main lesions observed after performing necropsies on female rabbits: (a1) Suppurative metritis (uterine dilation due to the presence of pus) with (a2) numerous bacteria microscopically. Haematoxylin–eosin. Bar, 100 μm. (b1) Fibrinous pneumonia (abundant fibrinous exudate in the thoracic cavity and lung surface) and (b2) severe inflammatory infiltrate affecting the pleura and alveoli. Haematoxylin–eosin. Bar, 500 μm. (c1) Abscesses in different locations (abdominal cavity) and (c2) heart. Haematoxylin–eosin. Bar, 500 μm (c2).

Two other females carried only genotype B1/I1/α, while four carried B1/I1/λ. Finally, two rabbit does carried genotypes B1/I1/α and B1/I1/λ at the same time. Genotypes B1/I1/α and B1/I1/λ belonged to ST96, while A1/II1/δ belonged to ST121.

Discussion

In this study, several parameters associated with the health status affecting rabbits housed in different cages were evaluated as an important factor of animal welfare. First of all, a reduction in erythrocytes, haemoglobin, haematocrit and phagocytosis, and an increase in heterophils between 1AI and 5P suggest possible productive senescence, as previously shown by other studies (Archetti *et al.*, 2008). Haptoglobin is an acute phase protein that markedly increases when animals are subjected to significant immunological challenges (Dishlyanova *et al.*, 2011), and it is used as a biomarker of infection and inflammation in rabbits (Petersen *et al.*, 2004). It can also increase throughout the productive lives of rabbit does (Argente *et al.*, 2014), as indicated by a 4% increase observed herein.

After comparing the five groups at 5P, significant differences were observed in haptoglobin, with the highest values for the COL group. Increased haptoglobin in the COL group could be related to more animals showing with lesions in this group because haptoglobin concentrations rise when a wound or inflammation occurs (Dishlyanova *et al.*, 2011). Nevertheless, a high stress level can also be assumed in this collective system as a result of aggressive interactions between the animals housed together (Dal Bosco *et al.*, 2019; Szendrő *et al.*, 2019).

Despite the significant differences in haemoglobin and platelets, the levels of these parameters remained within the normal range for these animals according to Kriesten *et al.* (1987). Other immunological indicators such as the H : L ratio and phagocytic capacity were not significantly affected.

Cortisol concentration in hair was greater in PF (1.67 ± 0.09) and COL (1.45 ± 0.10) groups, whereas the HD group had the lowest values (0.23 ± 0.09). These results are similar to those obtained by other studies in which faecal corticosterone concentrations were threefold greater in group-housed does than in single-caged ones (Szendrő *et al.*, 2013). Similarly, Rommers *et al.* (2006) and Mugnai *et al.* (2009) reported high corticosterone concentrations for group-housed rabbits. Cortisol is a hormone that indicates a physiological response to stress. Thus, female rabbits placed in both housing types, PF and COL, could have been subjected to greater chronic stress conditions, which may suggest that they were housed under worse welfare conditions. The finding regarding the PF group is surprising because platforms in cages are incorporated as a form of environmental enrichment to reduce animal stress (Hansen and Berthelsen, 2000). However, it should be taken into account that platform cages herein used were narrower than the other cages, and these platforms were made of wire. Therefore, these two characteristics of PF cages could explain greater hair cortisol concentrations in PF does, but not the presence of the platform itself (Masthoff and Hoy, 2019). In fact, this platform cage could be improved using a plastic platform and increasing cage width.

More animals were removed from the COL group ($n = 18$) because more rabbit does were culled ($n = 13$). Previous studies have shown that the survival rates of group-housed does are lower compared to single-caged does (Szendrő *et al.*, 2013). Reproductive problems, which were herein found mainly in the COL group, have also been reported by Dal Bosco *et al.* (2019), who observed poorer reproductive performance in rabbit does housed with temporary separations compared to individual housed ones. The high percentage

of reproduction problems found in the present study does not match what Sánchez *et al.* (2012) observed, who described diseases of the respiratory system, mastitis and ulcerative pododermatitis as the commonest disorders in rabbit does on Iberian farms. However, it should be noted that Sánchez *et al.* (2012) studied diseases on several commercial rabbit farms, while this study was conducted on an experimental farm. Necropsied rabbit does in our study had very few cases of pododermatitis ($n = 8$; 14.5%) and mastitis ($n = 1$; 1.8%). The few pododermatitis cases could be due to the use of footrests in all the cages and the farm's high standard of cleanliness.

In microbiological analyses, *P. multocida* was the bacterium most frequently isolated from pneumonic lungs, metritis and abscesses. This result partially agrees with former studies (Segura *et al.*, 2007) in which the most commonly isolated bacterium from pneumonia and metritis was also *P. Multocida*, while *S. aureus* was the most isolated bacterium from abscesses (Segura *et al.*, 2007). As explained above, this difference could be due to the distinct nature of the analysed farms.

The *S. aureus* genotypes found in this study (mainly B1/I1/α and B1/I1/λ) differed from previous data because Viana *et al.* (2007) described A1/II1/δ as being the most frequent genotype in commercial rabbitries, with 70% of the isolates. B1/I1/α and B1/I1/λ, both isolated herein, belonged to the ST96 clone, which is considered a low-virulence *S. aureus* clone that does not usually cause serious problems on farms, which could explain the few staphylococcal lesions that we detected.

In recent decades, group-housing designs for rabbit does have sought to allow natural behaviour to develop. However, a large number of problems still need to be solved in group housing, such as aggression between females even with escape routes (Rommers and de Greef, 2018), management problems (Machado *et al.*, 2016) or reduced productivity (Cervera *et al.*, 2017). Furthermore, no reduction in stress indicators has been observed, and more rabbit does had skin lesions (Maertens and Buijs, 2016; Zomeño *et al.*, 2018). Nonetheless, making comparisons among several studies is not easy because they involve different cage characteristics.

In wild rabbits, Rödel *et al.* (2008) observed more aggressive interactions per hour between females in close proximity to their breeding burrows on the first 20 days of lactation. This could explain the high percentage of lesions observed in the COL group, mostly injuries and abscesses, which agrees with Mugnai *et al.* (2009).

A greater kit mortality rate during lactation was observed in the present study for the COL group (13.1%) *v.* HD (6.4%) and PF (7.8%) groups. This could be due to the stress and aggressive behaviour of female rabbits housed in this group, as observed by Szendrő *et al.* (2013), who reported 15.2% suckling mortality in single-caged does and 38.5% in group-caged does. However, kit mortality obtained in the present study was lower than that reported by Szendrő *et al.* (2013) and Rödel *et al.* (2009) in wild rabbits at 32.4%.

In conclusion, this is one of the most extensive studies on the effects of different housings on the health of rabbit does. According to the parameters studied, higher and deeper single housing (HD) had better welfare indicators, such as lower haptoglobin and cortisol concentrations, fewer culled rabbit does and lower kit mortality than other housing types studied. Conversely, the COL group had greater haptoglobin and cortisol concentrations and kit mortality, and more culled and injured rabbit does. These results suggest that female rabbits allocated in COL cages suffered more stress under worse health conditions compared to those in individual housing systems herein studied. Thus, neither this collective housing system nor cage with a wire platform is recommended.

Although the high kit mortality and large number of lesions observed in group-housed rabbits resemble those observed under natural conditions, the expression of typical aggressive behaviour should not prevail over animals' health and physical integrity when evaluating the best housing system. Finally, the choice of housing system should also consider each farm's specific characteristics.

Acknowledgements

This study was supported by the INIA-INTERCUN Project (CUN2014-00001-00-00) and by Universidad CEU-Cardenal Herrera (INDI16/07, INDI17/07, INDI18/08). Grants for Sara Pérez Fuentes, Asunción Muñoz Silvestre and Elena Moreno Grua from Universidad CEU-Cardenal Herrera, and also for Sara Pérez Fuentes from Generalitat Valenciana and FSE (ACIF/2016/085), and for Elena Moreno Grua from Ministerio de Educación, Cultura y Deporte (FPU17/02708) are also gratefully acknowledged.

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Declaration of interest

The authors state that there are no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Ethics statement

Experimental proceedings were approved by the competent authority (Generalitat Valenciana, Spain) as set out in Spanish Royal Decree 53/2013 on the protection and use of animals for experiments.

Software and data repository resources

None of the data were deposited in an official repository.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731119003379

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