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Original Paper

Housing of rabbit does: reproductive performance, health and bone quality

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The objective of the present paper was to evaluate the health status reflected in haematological and biochemical traits of the blood, reproductive performance and bone quality of rabbit does on first parturition housed in several housing systems with or without mirrors. The animals were randomly allocated into 6 experimental groups: pens with soil covered by deep litter with the possibility of digging burrows (A), pens with soil covered by deep litter with the possibility of digging burrows enriched with a mirror (B), pens with plastic slatted floor with elevated resting area (C), pens with plastic slatted floor with elevated resting area enriched with a mirror (D), combi-park system (E) and combi-park system enriched with a mirror (F). Litter size after birth and the number of weaned kits was found to be highest in group C, D, E and F compared to other groups. However, the biochemistry analyses showed every investigated trait as significantly affected by housing conditions except the UREA and CHOL, it has to be stated that values of the blood traits were in accordance with the reference values of rabbits. The effect of housing system on *tibia* and *femur* characteristics was found to be significant in fracture toughness, where the lowest value of this trait was detected in group E and F compared to other groups in both investigated bones. According to these findings, it is possible to recommend every tested housing system from a health point of view for farmers dealing with alternative housing systems.

Keywords: biochemistry, bones, haematology, housing system, reproductive performance

1 Introduction

Breeding rabbit does is now the subject of scientific research (Szendrő et al., 2019; Huang et al., 2021) due to welfare consequences of their housing. In farming systems, individual wire cages are mostly used, where the does are housed with their kits until weaning (Huang et al., 2021). During the last few years, the continuous and part-time collective housing of rabbit does was exposed to research resulting in non-acceptable conditions of rabbit does and their offspring (injuries in does or injured and dead kits) (Szendrő et al., 2019) or the health of the animals in general. For the description of the health status or for the prediction of disease or infection, the serum analysis is standardly used (Rehman et al., 2017). In addition, the decline in reproductive performance in collectively housed females is reflected in the number of pseudo-pregnancies or sharing the same nest box by more animals, which is usually followed by injuries in does and dead kits (Szendrő et al., 2019). To avoid these problems, part-time collective housing seems to be perspective (Zomeño et al., 2018). Several types of housing systems with different enriching elements were investigated ((e.g., hiding places; Rommers et al. (2014) or combi systems with removable walls; Dal Bosco et al. (2019)). Furthermore, the use of mirrors was investigated during the fattening period of growing rabbits resulting in a decreased incidence of stereotypical behaviour (Piller et al., 1999). When rabbits have the opportunity to choose, they always chose the part of the housing system, where the mirror was present (Dalle Zotte et al., 2009a). Another

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benefit of alternative housing usage is that rabbits have more space for movement and thus they have a higher fracture toughness of their bones compared to their counterparts from cages (Krunt et al., 2021).

The objective of the present paper was to evaluate the health status reflected in haematological and biochemical traits of the blood, bone quality and reproductive performance of rabbit does housed in several housing systems with or without mirrors. The overarching hypothesis was that the rabbit does housed in different housing conditions will show different blood profiles and different reproductive performances.

2 Material and methods

The present study was approved by the ethics committee of the Czech University of *Life Sciences* Prague. The whole study was carried out in harmony with the guidelines of Act No. 246/1992, which focuses on the protection of animals against cruelty.

2.1 Animals and husbandry

The study was conducted using 72 20-week-old rabbit does (Hyplus genotype) on first parturition. The animals were randomly allocated into 6 experimental groups according to housing system. The entire observation took place under natural conditions from June until September 2021. The rabbits were fed *ad libitum* by a hopper feeder with a standard fattening pelleted diet (17.80% CP, 9.9 Mj.kg^{-1} of digestible energy). Water was available *ad libitum* from nipple drinkers. The litter was supplemented *ad libitum* (groups A and B). Young rabbit does (11 weeks of age) were from weaning until the star of the experiment housed individually on plastic slatted floor, and at 20 weeks of age were divided into experimental groups (A, B, C, D, E, F) depending on the floor type. Every group consists of 6 animals. After one week, artificial insemination was performed. Each treatment consisted of 2 replications/6 rabbits.

Regarding the housing systems, pens with soil covered by deep litter with the possibility of digging burrows (A), pens with soil covered by deep litter with the possibility of digging burrows enriched with a mirror (B), pens with plastic slatted floor with elevated resting area (C), pens with plastic slatted floor with elevated resting area enriched with a mirror (D), combi-park system (E) and combi-park system enriched with a mirror (F) were used. The mirrors (50 × 120 cm) were installed permanently for the experimental period. Pens (A, B, C, D) had the top open and the average size was 2.5 × 3.0 m with a minimum surface area of 1.4 m^2 per doe after resting platforms and other enriching elements were counted in. Pens C and D were designed to have the same parameters as previously mentioned systems with two floors The floors were connected by an elevated step, which was used by rabbits to jump onto and then jump to the second floor. Under the second floor was a space divided into individual sections, which looked like a corridor. At the end of each corridor was a nest box. Each section was separated from the next one by a wire net wall to keep the rabbits in contact. The E and F systems were classic combi-park systems with removable walls designed for six rabbit does and their kits. All housing systems with rabbits were exposed to natural lighting and temperature, which was 16 : 8 L/D and 16 °C in average. Rabbit does were weighed at the start (21 weeks of age) and at the end of the experiment (31 weeks of age). Rabbit kits were weighed at birth and every week until weaning (35 days of age). At the end of the experiment, rabbit does were slaughtered (mechanically stunned) to obtain *tibia* and *femur* bones for analysing.

2.2 Haematological and biochemical blood sampling

Haematocrit (HCT), haemoglobin (HGB), erythrocytes (ERY), leukocytes (LEU), neutrophiles (NEU), lymphocytes (LYM), neutrophiles (NEU), monocytes (MO), eosinophiles (EOS), basophiles (BAS), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), plasma albumin (ALB), total protein (TP), urea (UREA), glucose (GLU), creatinine (CREA), globulin (GLOB), ALB/GLOB, alanine aminotransferase (ALT), alkaline phosphatase (ALP) and cholesterol (CHOL) contents were measured to indicate health status and body fitness of examined rabbits.

In the end of the experiment, blood samples from 4 animals (randomly selected) from each group and replication (48 samples) were taken from the ear vein. Collected samples were centrifuged at 700 × g for 20 min at 4 °C to obtain blood serum. Then the blood films were made using the slide method, previously described in Schalm et al. (1975). Determination of blood haemoglobin and haematocrit was done according to Dukes and Schwarte (1931). MCV, MCH and MCHC were calculated as described in Al-Daraji et al. (2008). Pappenheim May-Grunwald Giemsa stain was used for staining the blood films. ERY were determined by hemacytometer as well as NEU, MO, EOS and BAS. Three horizontal edge fields followed by two fields towards the centre were done to obtain a differential amount of LEU. Two more fields were lead in a horizontal direction and another two fields in a vertical direction to get the edge and obtain the crisscross shape with right angles. The rest of the blood serum was stored until the biochemical analysis, which was done using commercial kits (Erba Lachema, s. r. o., Czech Republic) on the automatic analyzer XL – 200 (Erba Lachema s. r. o., Czech Republic).

2.3 Bone quality analyses

The *tibia* and *femur* were frozen (-20 °C) after slaughter, for future analysing, in individual plastic bags. A day before the analyses, the bones were taken from the freezer and thawed overnight. Next, they were boiled for 15 min in 95 °C water, further stripped of flesh and dried at 25 °C for 24 h. Subsequently, the maximum shear force until initial structural failure (i.e., the breaking of the bone) was determined by a three-point flexure test using a Instron® Model 3342 (Instron, Norwood, Massachusetts, US) and the load rate was 12 $mm.min^{-1}$. The distance between the two fulcra points maintaining the bones was 45 and 38 mm. The bones were constantly oriented for testing with their natural convex shape downwards.

2.4 Statistical analyses

The statistical analyses were processed using the computer application SAS 9.4 (SAS Institute Inc. Cary. NC. USA). All the data were analysed using the General Linear Model:

$$Y_{ij} = \mu + HS_i + e_{ij}$$

where: Y_{ijk} – value of trait; μ – general mean; HS_i – effect of housing system (i = A–F); e_{ij} – random residual error

Housing system was considered as a fixed effect. Differences between means were determined by the Duncan's test. The value of $P \leq 0.05$ was considered significant for all measurements. All the data are expressed in tables as means.

3 Results and discussion

During the reproductive period no females died. In two females (one from group D and one from group E) were detected footpads. The initial weight of live females was between 4,413 and 4,613 g. The final live weight after weaning was between 4 643 and 4 986 g. The weights did not statistically differ among the groups in these two observation periods. Litter size after birth was found to be the highest in group C, D, E and F. The highest number of weaned kits was observed in the same groups as live born kits. Weaning weight of kits was found to be the highest in A, B, E and F groups. It has to be stated that does from group A and B had the lowest number of kits and their weaning weight was probably influenced by this factor. Typically, scientific literature compares group and individual housing with the conclusion of a decreased productive performance in group housed rabbit does compared to individually housed does (Maertens & Buijs, 2016; Dal Bosco et al., 2019). Maertens & Buijs (2016) reported a decline in the number of weaned kits and in their weaning weight in groups of females. Dal Bosco et al. (2019) found decreased litter sizes after birth and after weaning in group housed does compared to these from individual cages. In addition, the decline in reproductive performance in collectively housed females is reflected in the number of pseudo-pregnancies or sharing the same nest box by more animals, which is usually followed by injuries in does and dead kits (Szendrő et al., 2019). Sharing of the same nest box was observed also in the present study once in group D. We solved that problem by dividing little kits into two nest boxes, where females were separately housed with them for three days. Separated females still had contact with other members of the group through the wire walls. Then they were released and incorporated back into the group.

In general, the haematological and biochemical blood traits are used to indicate disease, infection or health condition (Rehman et al., 2017). The results of the effect of the housing system on haematological parameters of the rabbit does blood are displayed in Table 2. The investigated traits did not significantly differ among the groups. In a study by (Musco et al., 2019) it was found that rabbits, who were exposed to mirrors had higher values of RBC, MCV and MCH compared to their counterparts, who did not have mirrors in their housing system. The authors see a link between feed intake and growth rate,

Table 1 The effect of housing system on reproductive performance of rabbit does on first parturition

Trait	Housing system						*P*-value	SEM
	A	B	C	D	E	F		
LW of does 21 wks (g)	4,445	4,501	4,543	4,613	4,413	4,535	0.2634	45.486
LW of does 31 wks (g)	4,643	4,797	4,812	4,986	4,792	4,825	0.0561	41.553
Live born kits (n)	7.50[b]	8.00[b]	10.00[a]	9.50[a]	9.50[a]	9.00[a]	0.0001	0.1781
Weaned kits (n)	7.00[b]	7.00[b]	9.00[a]	9.00[a]	8.50[a]	9.00[a]	0.0001	0.1639
Weaning weight of kits (g)	972[a]	985[a]	872[b]	866[b]	967[a]	960[a]	0.0001	8.6247

A – pens with soil covered by deep litter with possibility of digging burrows; B – pens with soil covered by deep litter with possibility of digging burrows enriched by a mirror; C – pens with plastic slatted floor with elevated resting area; D – pens with plastic slatted floor with elevated resting area enriched by a mirror; E – combi-park system; F – combi-park system enriched by a mirror; LW – live weight; SEM – standard error of mean; values with significance of $P \leq 0.05$ were considered as significant which is (when the growth rate declines) connected with feed utilization and reduced absorption of iron.

Furthermore, the biochemistry analyses showed (Table 3) every investigated trait as significantly affected by housing conditions except the UREA and CHOL. Rabbits from A and B group had the highest values of GLU, while the lowest values were found in C and D group. GLU is the main energy source (Gallenberger et al., 2012). Musco et al. (2019) observed the lowest values of GLU in rabbits, who were the most active. Unsurprisingly, our data indicates that rabbits from C and D had a high energy output thus they could jump from lower to upper floor, meanwhile the rabbits from digging systems obviously did not spend energy on movement as the previously mentioned rabbits. Additionally, according to measured values, we can state that rabbits were not exposed to stress, because values elevated above the optimum range indicate stress factors in rabbit's environment (Özkan et al., 2012). Moreover, CREA was significantly highest in B, D and F group, which are the groups with mirrors, and the lowest in A group. Musco et al. (2019) also found the highest values of CREA in mirrored groups compared to the rest of the groups. According to Bush (1991), the pre-renal failure is manifested by elevated in CREA Our results do not indicate any problems with rabbit does' kidneys. Also, measured TP was in the optimum range when the highest values were in A and C and the lowest values in B and E groups. It is well known that total protein and albumin are protein utilization indicators (Pavlík et al., 2007) and reflect the health condition of the animal (Marono et al., 2017). ALB values of group C, D and F were a bit above the optimum range, declared by Özkan et al. (2012). Significantly higher levels of GLB were observed in B, C, D and F groups than in other groups. The level of globulin in blood serum serves as an index immune reply. The higher globulin values or the higher ALB/GLB (in optimum range) the higher disease resistance and better immune response occurs in animals (Griminger, 1986). In the present paper, the highest value of ALB/GLB was detected for groups A and F and the lowest was in group B. The difference could be explained by some non-specific immune response initiated by environmental causes (El-Shafaei et al., 2016) thus the animals from group B had contact with the soil. Other studied parameters were ALT and ALP. These indicators are considered to reflect liver function similar to aspartate aminotransferase (Musco et al., 2019). Specifically, ALP serum levels come from the liver and bones and thus differ among the ages of animals and changes could be caused by bone growth (Kaneko et al., 1997). The obtained values in this study were found in the reference range, when ALT values were highest in groups A, B, D and F compared to the rest of the groups and ALP was detected as being higher in groups A and C compared to other groups. In addition, CHOL content did not significantly differ among the groups. However, in our study the presence of mirrors did not affect the CHOL content, Musco et al. (2019) referred to the highest amount of CHOL in mirrored groups of rabbits compared to these without mirrors.

Table 2 The effect of housing system on some haematological blood traits of rabbit does on first parturition

Trait	Housing system						*P*-value	SEM
	A	B	C	D	E	F		
HCT (%)	39.7	38.9	39.3	40.4	40.5	40.9	0.4242	0.580
HGB ($g.l^{-1}$)	126	120	119	122	123	125	0.6041	2.451
ERY ($10^{12}.l^{-1}$)	6.60	6.30	6.40	6.5	6.54	6.42	0.4782	0.064
LEU ($10^{9}.l^{-1}$)	7.30	7.10	7.34	7.73	7.51	7.54	0.6410	0.324
NEU ($10^{9}.l^{-1}$)	3.69	3.23	3.34	3.33	3.41	3.32	0.7641	0.201
LYM ($10^{9}.l^{-1}$)	3.11	2.89	3.13	3.02	2.90	3.14	0.3678	0.086
MO ($10^{9}.l^{-1}$)	0.730	0.840	0.738	0.748	0.761	0.745	0.6458	0.064
EOS ($10^{9}.l^{-1}$)	0.312	0.291	0.300	0.333	0.294	0.289	0.5841	0.005
BAS ($10^{9}.l^{-1}$)	0.239	0.211	0.242	0.220	0.232	0.236	0.8342	0.008
MCV (fl)	63.8	63.2	63.2	63.3	63.1	63.1	0.4132	0.194
MCH (pg)	19.6	19.6	19.3	19.9	19.6	19.4	0.6347	0.052
MCHC ($g.l^{-1}$)	305	311	307	310	311	310	0.4246	1.102

A – pens with soil covered by deep litter with the possibility of digging burrows; B – pens with soil covered by deep litter with the possibility of digging burrows enriched by a mirror; C – pens with plastic slatted floor with elevated resting area; D – pens with plastic slatted floor with elevated resting area enriched by a mirror; E – combi-park system; F – combi-park system enriched by a mirror; HCT – hematocrit; HGB – hemoglobin; ERY – erythrocytes; LEU – leukocytes; NEU – neutrophiles; LYM – lymphocytes; MO – monocytes; EOS – eosinophiles; BAS – basophiles; MCV – mean corpuscular volume; MCHC – mean corpuscular hemoglobin concentration; SEM – standard error of mean; values with significance of $P \leq 0.05$ were considered as significant

Table 3 The effect of housing system on selected biochemical traits of rabbit does on first parturition

Trait	Housing system						*P*-value	SEM
	A	B	C	D	E	F		
GLU (mmol.l^{-1})	7.42^{a}	7.21^{a}	5.79^{c}	5.55^{c}	6.23^{b}	6.28^{b}	0.0135	0.301
CREA (µmol.l^{-1})	73.3^{c}	95.3^{a}	80.0^{bc}	94.5^{a}	86.5^{b}	93.24^{a}	0.0225	2.421
UREA (mmol.l^{-1})	8.38	8.50	8.33	8.43	7.62	7.324	0.2310	0.305
TP (g.l^{-1})	83.0^{a}	67.4^{c}	85.8^{a}	77.3^{b}	64.4^{c}	78.2^{b}	0.0001	1.421
ALB (g.l^{-1})	41.2^{b}	35.0^{c}	49.0^{a}	47.6^{a}	36.1^{bc}	47.1^{a}	0.0001	1.234
GLB (g.l^{-1})	28.3^{bc}	35.3^{a}	37.0^{a}	35.5^{a}	28.4^{c}	32.4^{a}	0.0001	0.749
ALB/GLB	1.45^{a}	0.99^{c}	1.32^{b}	1.34^{ab}	1.27^{b}	1.46^{a}	0.0002	0.062
ALT (UI)	88.0^{a}	91.0^{a}	76.0^{b}	97.5^{a}	69.7^{c}	95.5^{a}	0.0001	2.824
ALP (UI)	46.2^{a}	34.6^{b}	43.8^{a}	22.0^{cd}	33.0^{bc}	31.4^{bcd}	0.0001	2.159
CHOL (mmol.l^{-1})	1.32	1.32	1.25	1.30	1.13	1.29	0.0682	0.002

A – pens with soil covered by deep litter with the possibility of digging burrows; B – pens with soil covered by deep litter with the possibility of digging burrows enriched by a mirror; C – pens with plastic slatted floor with elevated resting area; D – pens with plastic slatted floor with elevated resting area enriched by a mirror; E – combi-park system; F – combi-park system enriched by a mirror; GLU – glucose; CREA – creatinine; TP – total protein; ALB – albumin; GLB – globulin; ALT – alanine transaminase; ALP – alkaline phosphatase; CHOL – cholesterol; SEM – standard error of mean; values with significance of $P \leq 0.05$ were considered as significant

Table 4 The effect of housing system on quality of *tibia* and *femur* bones in does on first parturition

Trait	Housing system						*P*-value	SEM
	A	B	C	D	E	F		
Tibia								
Length (mm)	109	110	108	110	112	110	0.7561	2.124
Width (mm)	8.3	8.42	8.15	8.24	8.16	8.21	0.547	0.214
Fracture toughness (N)	480^{a}	498^{a}	486^{a}	485^{a}	398^{b}	405^{b}	0.036	19.226
Femur								
Length (mm)	103	105	104	106	104	105	0.684	2.025
Width (mm)	8.71	8.68	8.59	8.64	8.62	8.67	0.267	0.242
Fracture toughness (N)	348^{a}	354^{a}	361^{a}	348^{a}	290^{b}	295^{b}	0.042	17.125

A – pens with soil covered by deep litter with the possibility of digging burrows; B – pens with soil covered by deep litter with the possibility of digging burrows enriched by a mirror; C – pens with plastic slatted floor with elevated resting area; D – pens with plastic slatted floor with elevated resting area enriched by a mirror; E – combi-park system; F – combi-park system enriched by a mirror; SEM – standard error of mean; values with significance of $P \leq 0.05$ were considered as significant

The effect of housing system on *tibia* and *femur* characteristics (Table 4) was found to be significant in fracture toughness, where the lowest value of this trait was detected in groups E and F compared to other groups in both investigated bones. The bone quality in rabbit does is marginally studied. Nevertheless, Buijs et al. (2014) published a paper, where they compared semi-group wire pen, semi-group plastic pen and individual cage system with results of significant differences among the groups in cortex thickness of *tibia* and *femur* bones. Rabbit does from individual cages had lower measured values than the other groups. They did not differ in the rest of the parameters (length, width, or breaking strength). The study of Krunt et al. (2021) compared collective pens with cages resulting in thinner bones in growing rabbits from cages compared to their counterparts. Both studies have common intersection-increased movement. The authors from the last cited study proved that bones of rabbits from pens contain more calcium and magnesium (*tibia* bone) or just magnesium (*femur* bone), which are crucial elements for determining bone quality and fracture toughness. Moreover, Dalle Zotte et al. (2009b) found, except the higher fracture toughness in penned rabbits compared to caged rabbits, that pen housed rabbits had heavier bones than those from cages.

4 Conclusions

Housing conditions significantly affected the number of live born and weaned kits and the weight of weaned young rabbits. Based on haematological and biochemical blood results, we can state that rabbit does were, during the experimental period, healthy, and the obtained values were in the optimum range of physiological blood values for rabbits. According to these findings, it is possible to recommend every tested housing system from a health point of view. Moreover, fracture toughness was found to be the lowest in the combi-park system compared to the rest of the investigated systems, which is not the best option for dynamical movement of rabbit does. For future research, we can recommend focussing on stress hormone levels in similar group housing systems to obtain more detailed results and implement them into practise.

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