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DEVELOPMENT OF SIMPLIFIED SAMPLING METHODS FOR BEHAVIOURAL DATA IN RABBIT DOES

ALFONSO-CARRILLO C.*, MARTÍN E.*, DE BLAS C.†, IBÁÑEZ M.A.‡, GARCÍA-RUIZ A.I.*, GARCÍA-REBOLLAR P.†

*Nutreco PRC, 45950, Casarrubios del Monte, Toledo, Spain.
†Departamento de Producción Animal, E.T.S.I. Agrónomos, UPM, 28040, Madrid, Spain.
‡Departamento de Estadística, E.T.S.I. Agrónomos, UPM, 28040, Madrid, Spain.

Abstract: The aim of this study was to compare the results of different simplified sampling methods for behavioural data compared to reference records of 24-h in order to assess rabbit doe behaviours at different physiological stages (gestation and lactation) in animals housed in 2 types of cages (conventional and alternative). In total, we analysed 576 h of continuous video of 12 rabbit does at the end of lactation and the same females after weaning. The behavioural observations were studied using 3 independent categories of classification (location in the cage, posture and functional behaviours). Continuous behavioural recordings of 24 h were considered as the reference method to validate another 4 data collection sampling methods by aggregated video recordings of different frequency and duration [regular short and long methods with 2.4 and 8 h of observation respectively, and irregular (more frequent during the active period) short and long methods with 6 and 8 h of observation, respectively]. The current results showed that, independently of the housing system, the best method to reduce the total observation time required to assess rabbit does' behaviour depends on the trait studied and physiological stage of the does. In gestating does, irregular methods were not suitable to estimate behaviours of long duration such as lying, sitting, resting and grooming. However, in both physiological stages, regular methods were accurate for location behaviours, postures and functional behaviours of long duration. Instead, for the study of infrequent behaviours performed mainly during dark period, where coefficients of variation were high, the irregular long method led to the lowest mean estimation errors.

Key Words: rabbit does, behaviour, type of cage, physiological stage, sampling techniques.

INTRODUCTION

In rabbits, as in other species, welfare conditions may be measured using behavioural, physiological, pathological and productive indicators. Behavioural observation techniques are appropriate to determine space allowances and to identify and evaluate abnormal conducts that could be associated with an impairment of welfare in farmed animals (stereotypies).

Continuous recording is the most accurate method for behavioural measurements, but it is difficult to conduct due to the time needed if a large number of animals and a wide range of behaviours are studied. Thus, in many cases it is necessary to design simplified observation methods. Sampling techniques to reduce the time required to study animal behaviour were described by Altmann (1974). However, these techniques should be validated to ensure proper collection and interpretation of the data. A good estimation of duration of an activity is achieved if the observation period lasts long enough and if the interval between the samples is not too long (Broom and Fraser, 2007). Depending on frequency and length, the limitations and advantages of different types of simplified observation methods of animal behaviour have been examined by Arnold-Meeks and McGlone (1986), Martin and Bateson (1993), Mann (1999) and

Correspondence: C. Alfonso-Carrillo, c.alfonso@nutreco.com. Received *March 2016* - Accepted *November 2016.*
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Mitlöhner *et al.* (2001). These techniques have been studied in animal species such as mice, swine, primates, cattle or cetaceans. Rabbits show an important nocturnal activity and the use of visible light to control rabbits' behaviour can lead to alterations in circadian periodicity that give rise to conduct disturbances. In addition, the presence of an observer can influence behavioural patterns of rabbits. Nowadays, infrared observation techniques allow a continuous view of the animals for 24 h, without disturbing them overnight and not affecting their behaviour. Different recording frequencies and lengths have been used in behavioural rabbit research, such as one every 15 min throughout 24 h (Morisse and Maurice, 1997), one min every hour throughout 24 h (Morisse *et al.,* 1999), 15 min at the end of the light period and at the beginning and in the middle of the dark period (Chu *et al.,* 2004), instantaneous observations (scan sampling) at a 5 min frequency during 6 h for the light period and 6 h for the dark period (Princz *et al.,* 2008) or 5 min in the morning and 5 min in the afternoon (Mugnai *et al.,* 2009). However, these techniques have not been validated in farmed domestic rabbits yet.

The aim of this study was to compare the results of different simplified sampling techniques for behavioural data with respect to reference records of 24-h on the behavioural assessment of rabbit does at different physiological stages (gestation and lactation) housed in 2 type of cages (conventional and alternative).

MATERIAL AND METHODS

Animals and housing

All experimental procedures were approved by the Ethics Committee of the Polytechnic University of Madrid and complied with the Spanish guidelines on care and use of animals in research (Boletín Oficial del Estado, 2013).

The study was carried out at the Poultry and Rabbit Research Centre of Nutreco, in Toledo, Spain. A total of 12 multiparous rabbit does (*Oryctolagus cuniculus*) in their fourth reproductive cycle from a hybrid maternal line (Hy-Plus) were used. Animals weighed on average 4.5 kg live weight, and were inseminated 25 d after kindling, kits being weaned 32 d after kindling. All animals were housed in the same artificially lit room. The light:dark cycle was 15:9 h (light interval from 06:00 to 21:00 h and dark interval from 21:00 to 06:00 h). From the first artificial insemination, half of the rabbit does were individually housed in alternative polyvalent cages (385×995×600 mm) with a wire platform (381×310 mm) raised 400 mm from the floor. The other half of the animals were individually housed in conventional polyvalent cages (385×995×300 mm). All cages were equipped with a feeder and a nipple drinker placed in the lower level and a foot mat (perforated plastic plate) in the middle of the floor. Heating, cooling and forced ventilation systems allowed the building temperature to be maintained between 20 and 23°C throughout the experiment.

Feed

Rabbits were fed *ad libitum* with a commercial pelleted diet (Cunilactal, NANTA, S.A., Spain). Triplicate chemical analysis of feed was performed according to AOAC International (2000) procedures, and the average composition on as-fed basis was: crude protein 18.6%, ether extract 3.8%, starch 22.0%, crude fibre 14.4% and ash 8.2%.

Behavioural measurements

The observations were performed on the same does at the end of the lactation period (24 d after parturition) with 8 kits per litter, and 1 wk before next parturition (3 d after weaning in pregnant not lactating does). All females' records were captured simultaneously for 24 h per day. To avoid disturbances to the rabbit does' behaviour, nobody entered the room while behaviour was being recorded. Behaviours were recorded by infrared video cameras (VCB-3380/Sanyo) and a LED infrared reflector (IR-880/12D) placed on bars 2 m above the cages. Video recordings were analysed in their entirety by one trained person viewing at double speed, whereupon the data was fed into the computer using "The Observer XT 8.0" software (Noldus Information Technology, Wageningen). Observations were classified into 3 exclusive categories (location, posture and functional behaviours) and behaviours were assigned to each category according to the ethogram described in Table 1. Grooming and caecotrophy behaviours were considered as one due to difficulty in distinguishing them, and were simply referred to as grooming.

Table 1: Ethogram of behaviours used per category (location, posture and functional behaviours).

Category	Behaviour	Description
Location		On platform (only in alternative cages)
		On foot mats
		On wire-net
Posture	Lying	Trunk on ground, forelimbs and hindlimbs tucked under the body or outstretched
	Sitting	Forepaws on ground with the forelimbs straight, the thorax and abdomen visible
	Standing	Sitting on hindlimbs with both forepaws off the ground
	Hyperactivity	Hopping in circles around itself or quickly running around in the cage
Functional behaviours	Resting	Sitting or lying without carrying out any activity
	Eating	Consumption of feed from the feeder, gnawing the pellet
	Drinking	Drinking water from nipple drinker
	Caecotrophy	Rabbit doe bowed down, pushed the head between hind legs and ingested caecotrophs. Afterwards they rose and chewed intensively for a few moments
	Grooming	Licking, scratching or nibbling of the body
	Interacting with Neighbours	Physical contact with animals from the adjacent cage by biting, sniffing, licking and removing hair
	Interacting with Kits	Physical contact of the rabbit does with the kits by licking or pushing them with the head
	Nursing	Rabbit doe lying with belly exposed and kits suckling
	Sniffing	Smelling surroundings, with movement of head
	Paw scraping	Rapid scratching with the forepaws on the floor or feeder
	Gnawing	Biting cage and platform wire bars and feeder

Experimental design

Continuous video-records obtained over 24 h (reference method) were cutting out in sequences of different frequency and length, in order to decrease total observation time. The choice of the length and frequency of the recordings depended upon several considerations such as the duration, frequency and distribution throughout the day of the broad range of rabbit behaviours. Thus, a total of four new videos (simplified sampling methods) were designed considering records of different length (2 min or 2 h) in regular frequencies throughout the day (every 20 min or 6 h) and records of 1 h with a higher frequency during the activity period of rabbits. Specifications of these methods were as follows (Figure 1):

Regular-short method (R2.4): records for the first 2 min out of every 20 min. Total recorded time: 2.4 h/d.

Regular-long method (R8): records for the first 2 h out of every 6 h. Total recorded time: 8 h/d.

Irregular short 6h method (I6): records for 1 h, twice during the rest period (at 09:00 and 15:00 h), and 4 times during the active period (at 21:00, 00:00, 03:00 and 06:00). Total recorded time: 6 h/d.

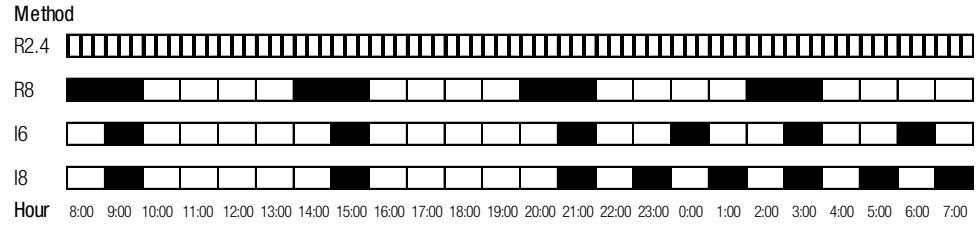


Figure 1: Frequency and duration of video recordings of different sampling methods. Black sections show recording periods.

Irregular long 8h method (I8): records for 1h, twice during the rest period (at 09:00 and 15:00 h), and 6 times during the active period (at 21:00, 23:00, 01:00, 03:00, 05:00 and 07:00). Total recorded time: 8 h/d.

To represent behaviour over the whole day, the time recorded for each behavioural category during the different recording sequences was multiplied by the appropriate factor depending on their frequency and duration in each selected method. In regular methods (R2.4 and R8), times of each behaviour recorded during each sequence (2 min and 2 h) were multiplied by 10 and 3 respectively, and in irregular methods (I6 and I8) by 6 in both cases during rest period sequences and 3 and 2 during the active period sequences, respectively.

Statistical methods

Behavioural measurements effects were analysed in a completely randomised design, using a mixed model with repeated measures, with type of cage, physiological stage, observation method and their interactions as fixed effects and physiological stage within type of cage as the repeated term. Rabbit does nested to type of cage was included in the model as a random effect. Only the effect of methods and their interactions with type of cage and physiological stage are shown, as type of cage and physiological stage effects were discussed in a previous article (Alfonso-Carrillo *et al.,* 2014). When method effect and their interactions were significant, a Dunnet test was used to make pairwise comparisons using the reference method as control treatment. Trait values are reported as average duration (min per day and doe)±standard error. All analyses were performed using SAS (2008). Estimation errors of different simplified sampling methods are represented as:

$$\frac{\textit{Reference method - Simplified method}}{\textit{Reference method}} \times 100$$

RESULTS

Location

No significant effect of observation methods and their interactions with physiological stage and type of cage was detected on the time spent by rabbit does at different locations (Table 2). According to values obtained with the reference method, females spent on average 57.6, 30.9 and 23.0% of the day on foot mats, wire bars and platform, respectively, and the estimation errors observed using simplified methods were low (on av. 1.06, 1.52 and 1.66%, respectively; Figure 2).

Posture

The effect of observation methods and the comparison of the simplified sampling methods with the reference one on the time spent by does performing different postures are shown in Table 3. The observation method affected the estimation of the time spent in lying and sitting postures ($P<0.05$); the differences among the simplified sampling methods with the control were independent of type of cage, but were affected by physiological stage ($P<0.001$). According to the reference method, gestating and lactating does spent on average 77.3 and 79.5% of the day

Table 2: Comparison of the simplified methods with the reference method on the time (min/d±standard error) spent by does on different locations.

Methods[1,2]	Reference	R-2.4	R-8	I-6	I-8	*P*-value[4]
Foot mats	829±63	841±68	831±68	824±62	845±62	0.952
Wire bars	445±49	433±52	449±49	444±47	455±46	0.962
Platform[3]	331±81	331±82	321±83	343±83	331±76	0.827

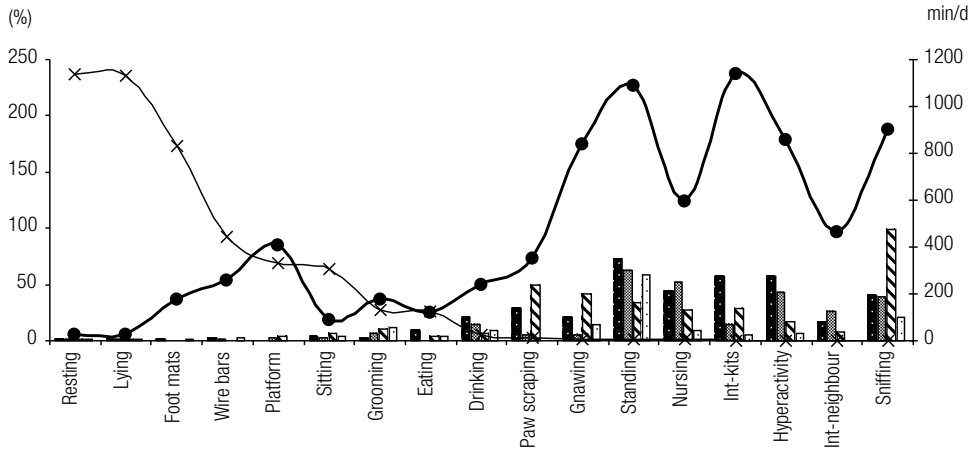
[1]Methods: R-2.4: regular short; R-8: regular long; I6: irregular short; I8: irregular long.

[2]Interactions method x type of cage and method x physiological stage were not significant.

[3]Only does housed in enriched cages were considered.

[4]Effect of observation method.

Figure 2: Estimation error (%) of different simplified behavioural methods: R -2.4; R-8; I-6; I-8. Coefficient of variation (%) and duration (min/d) of behaviours observed throughout 24h: , , respectively.



lying and 22.4 and 20.3% sitting, respectively. In lactating does, the estimates reached by simplified methods for both postures did not differ from the reference method; however, in gestating does, the estimates of the irregular methods were significantly different from the reference method in both postures ($P<0.001$ and $P<0.01$ for I6 and I8, respectively).

The standing posture was only observed in gestating does housed in alternative cages with an average value of 0.49% of the day. Hyperactivity was only observed when rabbit does shared the cage with kits, to flee from kits, with an average value of 0.21% of the day. Both postures were not affected by the observation method, but simplified methods reached high estimation errors (on av. 57.1 and 30.8% for standing and hyperactivity postures, respectively; Figure 2).

Table 3: Comparison of the simplified method with the reference method on the time (min/d±standard error) spent by does in different postures in 2 physiological stages (gestating [G] and lactating [L]).

Methods[1,2]	Reference	R-2.4	R-8	I-6	I-8	*P*-value[6]
Lying	1129±11	1142±12	1116±14	1107±19^{+}	1116±19	0.057
G[3]	1113±18	1136±19	1097±22	1057±29***	1073±27**	<0.001
L[4]	1145±9	1149±16	1135±16	1156±15	1161±17	0.305
Sitting	308±11	294±13	318±15	328±19^{+}	320±19	0.074
G[3]	323±20	298±21	337±23	379±30***	363±27**	<0.001
L[4]	292±9	289±16	301±17	279±15	275±17	0.327
Standing[3,5]	7.0±6.8	12.1±9.7	11.4±11.1	9.4±8.9	11.1±3.5	0.337
Hyperactivity[4]	3.0±0.6	1.3±0.8	4.3±2.1	3.5±1.1	3.2±0.8	0.291

$^{+}$,*,**,*** means differ significantly compared to reference method, $P<0.10$; $P<0.05$; $P<0.01$; $P<0.001$, respectively.

[1]Methods: R-2.4: regular short; R-8: regular long; I6: irregular short; I8: irregular long.

[2]Interaction method×type of cage was not significant.

[3]Only gestating does were considered.

[4]Only lactating does were considered.

[5]Only does housing in alternative cages were considered.

[6]Effect of observation method. When the interaction of method with physiological state was significant also the effect of the method is shown considering gestating or lactating does independently.

Table 4: Comparison of the simplified methods with the reference method on the time (min/d) spent by does performing different functional behaviours in two physiological stages (gestating [G] and lactating [L]).

Methods[1,2]	Reference	R-2.4	R-8	I-6	I-8	*P*-value[6]
Resting	1136±11	1146±20	1119±18	1119±13	1122±14	0.013
G[3]	1117±19	1138±30	1097±26	1064±20***	1073±23**	<0.001
L[4]	1155±10	1153±14	1142±15	1174±17	1171±15	0.128
Grooming	131±10	135±15	140±14	144±12	147±13	0.231
G[3]	160±15	157±22	173±21	192±19**	196±19**	0.001
L[4]	102±5	113±10	107±8	97±11	99±10	0.619
Drinking[5]	23.8±2.4	19.0±3.1	27.1±2.5	25.2±4	26.1±3.5	0.022
Eating	125±6	114±7	125±7	120±8	120±7	0.206
G[3]	115±9	96±11**	115±12	119±9	116±9	0.013
L[4]	136±8	133±9	135±8	121±11	125±10	0.167
Sniffing[5]	0.72±0.27	0.43±0.79	1±0.51	1.43±0.34	0.87±0.35	0.248
Nursing[4]	6.92±2.47	3.88±3.55	10.52±1.43	8.78±3.27	6.33±4.79	0.133
Interacting-neighbour[5]	1.73±0.64	2.02±0.83	2.17±0.66	1.87±0.73	1.73±0.63	0.931
Interacting-kits[4]	3.02±0.84	4.75±0.86	3.45±0.97	2.17±1.86	3.17±1.29	0.291
Gnawing	9.22±3.28	11.08±4.42	8.78±4.69	13.1±4.34	10.52±2.77	0.289
G[3]	15.8±6.0	21.3±7.9	14.3±8.4	22.9±7.7*	18.6±4.9	0.032
L[4]	2.60±0.91	0.72±1.55	3.32±0.48	3.45±0.54	2.45±1.78	0.905
Paw scraping[3]	10.2±7.0	13.1±10.3	10.7±7.4	15.3±9.3	10.2±7.2	0.388

*, **, *** means differ significantly compared to reference method, $P<0.05$; $P<0.01$; $P<0.001$, respectively.

[1]Methods: R-2.4: regular short; R-8: regular long; I6: irregular short; I8: irregular long.

[2]Interactions method×type-of-cage were not significant.

[3]Only gestating does were considered.

[4]Only lactating does were considered.

[5]Interaction method×physiological stage was not significant.

[6]Effect of observation method. When the interaction of method with physiological state was significant also the effect of the method is shown considering gestating or lactating does independently.

Functional behaviours

The effect of observation methods and the comparison of the simplified sampling methods with the reference one on the time spent by does performing different functional behaviours are shown in Table 4.

The effect of observation methods on functional behaviours was always independent of the housing system. For resting, grooming, eating and gnawing, the effect of observation methods varied with the physiological stage, as differences between some simplified sampling methods with the reference method were observed in gestating but not in lactating does. In gestating does, irregular methods, I6 and I8, underestimated by 4.7 and 3.9% ($P<0.001$ and $P<0.01$) resting behaviour and overestimated by 20.0 and 22.5% ($P<0.001$ and $P<0.01$) grooming behaviour, respectively, compared to the reference method. The irregular method I6 also led to higher gnawing values in gestating does (by 44.9%, $P<0.05$) than the reference method. For the eating behaviour, the regular short method in gestating does led to lower values than the reference method (115±9 *vs.* 96±11 min/d; $P<0.01$).

Estimations of time spent on the other functional behaviours analysed such as drinking, sniffing, nursing, interacting with neighbour and kits and paw scraping did not differ from values obtained with the reference method. Average values registered for these behaviours in the reference and simplified sampling methods were on av. 1.65 *vs.* 1.69, 0.05 *vs.* 0.06, 0.48 *vs.* 0.51, 0.12 *vs.*0.14, 0.21 *vs.* 0.24 and 0.71 *vs.* 0.86% of the day, respectively.

DISCUSSION

The wide range of behaviours that can be analysed in an animal study with their duration and frequency during the day and even at different animal physiological stages evidenced the need to validate simplified observation methods to assure the quality of these studies. In the present study, the accuracy of simplified methods varied depending on the physiological stage of does for behaviours such as lying, sitting, resting, grooming and gnawing. These findings show that when animals change position or functional behaviour more frequently, as gestating does do when their parturition day approaches, some of the simplified methods, mainly the irregular ones, are not suitable to estimate behaviours performed throughout the whole day. However, regular methods (R2.4 and R8) were accurate for measuring behaviours of long length such as lying, sitting, resting and grooming, and the R2.4 method seems to be the most suitable one from a practical point of view. Therefore, this result shows that methods with short observation periods provide a useful estimate of animal behaviour if the duration of the activity is long enough, as Broom and Fraser (2007) asserted. Other methods similar to the regular short one used in this work, such as the scan sampling method, which describes animal behaviour at a fixed time interval, have already been validated in other species. Mitlöhner *et al.* (2001), in a study for feedlot cattle, showed that scan sampling methods can provide an unbiased estimate of percentage of behaviour time when the interval between scans is short enough relative to the duration of the behaviour, intervals of 60 min being too long for most of the behaviours studied. To this end, scan sampling techniques have been used in the study of rabbit behaviour and some of them with long intervals between observations, regardless of the behaviour. Morisse and Maurice (1997), in a study of rabbit behaviour, used sequences of 1 min every 15 min to evaluate different behavioural groups; however, Morisse *et al.* (1999), analysing the same group of behaviours lengthened the interval between sequences up to 60 min.

In the current work, when studying behaviours performed for a short time during the day (hyperactivity, eating, drinking, nursing or social interactions), high estimation errors were observed, mainly when using regular methods, the irregular long method (I8) being the most accurate for these traits (Figure 2). This result might be explained by the longer observation time from dusk until dawn, when rabbits are more active and performed these kind of behaviours with higher frequency, as observed by Alfonso-Carrillo *et al.* (2014) in a behaviour pattern study of rabbit does throughout 24 h. Even so, the irregular long method was not accurate enough for other short and infrequent behaviours such as standing or sniffing due to their high variation. An increase in the number of replicates would be advisable to gather more reliable information on this type of behaviours.

CONCLUSIONS

Simplified behavioural sampling methods can be used to reduce the total observation time required to assess different types of behaviours. However, depending on the behaviour studied, some methods are more accurate than others. From a practical point of view, the regular short method (R2.4) is the best option to study long duration behaviours. For shorter length behaviours and due to the nocturnal activity of rabbits, methods with longer recording time during the dark period would be preferable. Because of the high coefficient of variation in these latter behaviours mentioned, an increase in the number of animals observed would be recommended.

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