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The effects of interval of testing and quality of resource on the choice behaviour of laying hens in a Y-maze test.

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Executive Summary

Preference tests, in which the preferences of animals are studied, may tell us what is important to an animal and thus provide an indication of what is required to optimise its welfare. Animal preference tests conducted in a Y-maze apparatus, where an animal makes a choice between two resources, may appear to be relatively straightforward. However, aspects of the design of the Y-maze preference test may have the potential to influence animal motivation and thus choice behaviour leading to spurious results that are not reflective of the animal's true preferences.

This project examined two factors in the design of Y-maze preference test methodology on the motivation and choice behaviour of laying hens; interval of testing and type of dust substrate.

This experiment was a 2 x 3 factorial design comparing peat moss or sawdust and intervals of testing (every day, alternate days or every third day). It was conducted over two periods with each hen (n=12) allocated to a different interval treatment in each period. Only birds that had had exposure to peat moss were allocated to peat moss treatments and likewise for sawdust. Following Y-maze familiarisation and training, hens were tested for their choice between social contact (a familiar, subordinate hen) or dust (either peat moss or sawdust) over 13 days, with testing conducted on days corresponding to the hens' interval treatment (i.e. either daily, alternate days or every third day).

Results revealed that hens preferred dust to social contact, regardless of interval or dust treatment. While hens tested daily had a tendency to leave the start box of the Y-maze slower than hens tested on every second or third day, no differences were observed between interval or dust treatments in the time to choice (time from leaving start box to entering a Y-maze arm). There were no differences observed in the behaviour hens displayed when dust was selected (dustbathing or foraging), however, this was likely due to the large individual variation in dust how the hens interacted with the dust.

Overall, the results indicate that neither the interval of testing nor type of dustbathing substrate influenced hen motivation or choice behaviour. These results warrant further research to determine whether lack of observed differences were due to the relatively low number of experimental animals. Furthermore, even though the interval and type of resource had no effect on the choice between social contact and dust, incorporating another, more attractive resource may yield different results.

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Introduction

Preference tests, in which the preferences of animals are studied, may tell us what is important to an animal and thus provide an indication of what is required to optimise its welfare. Animal preference tests conducted in a Y-maze apparatus, where an animal makes a choice between two resources, may appear to be relatively straightforward. However, aspects of the design of the Y-maze preference test may have the potential to influence animal motivation and thus choice behaviour, leading to spurious results that are not reflective of the animal's true preferences.

One such aspect of the test, the quantity of reward, has previously been found to impact on laying hen motivation (Laine *et al.*, 2009). The quantity of reward in a Y-maze preference test often refers to the amount of time with which the animal has contact with its chosen resource. Laine *et al.* (2009) tested hens in a Y-maze for their choice between social contact and peat moss. Hens were allocated into one of three treatments which differed in the quantity of peat moss reward; 'short' (2 minutes), 'intermediate' (20 minutes) and 'long' (45 minutes). The quantity of social contact reward remained at 5 minutes for all hens. Results indicated that hens in the intermediate treatment appeared to have reduced motivation for peat moss, as demonstrated by their increased time to make a choice and a tendency to select peat moss less often when compared to the other treatments. Thus, one factor in the design of Y-maze preference methodology may influence preference. The present experiment examined the effect of two other aspects of the design of Y-maze preference tests; the interval of testing and quality of resource.

The interval of testing may alter the motivation of an animal. In most Y-maze studies, animals are deprived of the resources of interest in their home cage and only have access to the resource in the test. Therefore, the interval of testing determines the duration of resource deprivation. The period of deprivation may influence animal motivation. For example, hens deprived of food exhibit more exploratory behaviour (Nicol and Guilford, 1991) and are faster to run an alleyway (Petherick *et al.*, 1992) and a Y-maze (Laine *et al.*, 2007) to a food reward, compared to hens not deprived. This indicates that the deprivation of feed increases hen motivation for feed. Likewise, Nicol and Guilford (1991) also reported that hens deprived of peat moss showed increased exploratory behaviour compared to those not deprived, indicating deprived hens were more motivated to access the peat moss. Therefore, if the period of deprivation impacts the motivation of animals, the interval of testing may be a potentially important factor in the design of animal preference tests.

The interval of testing may be a particularly important factor if behavioural patterns related to the resources under investigation has known temporal rhythms of occurrence. One such example is dustbathing behaviour. Dustbathing consists of a sequence of behavioural elements including scratching, bill raking, wing shaking and rubbing (van Liere, 1992). The motivation to dustbathe appears to build up over time and laying hens perform, on average, one, 27-minute dustbathing bout every second day, with peak dustbathing activity around midday to early afternoon (Vestergaard, 1982; Vestergaard *et al.*, 1990; Hogan and van Boxel, 1993). This is suggestive of an endogenous circadian rhythm of motivation. In addition, following a period of dustbathing deprivation, hens have been found to reduce the latency to dustbathe and increase the dustbathing bout duration when dustbathing is next allowed (Vestergaard, 1982), conforming to the Lorenzian 'psychohydraulic' model of motivation (Hogan and van Boxel, 1993).

Like the interval of testing, the quality of a resource on offer may also affect animal motivation. The term quality refers to 'characteristics with respect to excellence' (Heinemann Australian Dictionary, 1995). With respect to dustbathing substrates, quality may relate to the effectiveness of the material for its function. The function of dustbathing is to regulate and distribute feather lipids, thus maintaining plumage (van Liere and Bokma, 1987). Research has found that substrates that are composed of smaller particles, such as sand or peat moss are more efficient at reaching the proximal (plumulous) part of the feathers and thus provide a more effective dustbathing subatrate in terms of removing excess feather lipids compared to substrates composed of larger particles, such as wood shavings (van Liere and Siard, 1991).

This experiment incorporated two dustbathing substrates, sawdust and peat moss. These substrates differed in particle size (see Figure 1) and therefore likely quality as a dustbathing substrate. Furthermore, laying hens have been found to prefer specific dust substrates in which to dustbathe. Research has found that peat moss

and sand are preferred by hens for dustbathing compared to sawdust or shavings (Petherick and Duncan, 1989; van Liere *et al.*, 1990; Shields *et al.*, 2004; de Jong *et al.*, 2007). This preference is possibly due to the effectiveness of peat moss and sand for dustbathing compared to sawdust or shavings.

There may also be differences in the quality of social contact. The 'effectiveness' of social contact of a particular hen to another hen may be dependant on the relationship between the two hens. Hens prefer to be closer to familiar hens rather than unfamiliar, and subordinate hens to dominant hens (Dawkins, 1982; Bradshaw, 1992; Grigor *et al.*, 1995). Therefore, a familiar and/or subordinate hen would be more effective at providing social contact compared to an unfamiliar and/or dominant, as it is more likely that the hens will be in closer contact and/or interact with one another.

Social contact is presumably important for laying hens. When isolated, domestic chicks have been found to show behavioural and physiological indicators of stress, however, these stress indictors may be reduced when the test chick was placed in the same environment with a conspecific (Jones and Merry, 1988). Moreover, Jones (1984) found that the fear-related responses of a chick in an open field were reduced when the conspecific was a familiar chick compared to an unfamiliar chick. In adult laying hens several behaviours have been found to be socially facilitated with many individuals performing the behaviour in synchrony. Examples include feeding (Hughes, 1971; Webster and Hurnik, 1994), preening (Webster and Hurnik, 1994) and possibly dustbathing (Duncan *et al.*, 1998). Furthermore, Mills and Faure (1989) reported that when hens were isolated from familiar conspecifics, the isolated hen showed reduced feeding and increased behaviours that may be related to frustration (movement and preening), implying that social isolation may cause frustration. These findings all indicate that social contact is important for laying hens.

The hypothesis tested in this experiment was that differences in the interval of testing and quality of dustbathing substrate presented in the Y-maze would impact on the motivation, and potentially, the choice behaviour of laying hens. To test this, hens were preference tested in a Y-maze for their choice between social contact and a dustbathing substrate with testing conducted at three intervals.

Objectives

The objective of this study was to examine the effects of interval of Y-maze testing and quality of a dustbathing substrate on the motivation and choice behaviour of laying hens.

Methodology

Animals and housing

Selection of experimental animals

Experimental hens were selected from a flock of 80 beak-trimmed hens (Brown Hy-line laying strain) sourced from a commercial farm at approximately 22 weeks of age. The hens were transported to a pre-experimental facility where they were housed in 10 cages, each cage (1.14 m x 0.50 m x 0.45 m) housing eight birds. Each cage contained an external feed trough at the front of the cage, three nipple drinkers at the rear and a plastic tray (0.46 m x 0.22 m x 0.03 m). The 10 cages were located in two adjacent rooms, with five cages in each room. The rooms differed in the dust substrate provided in the cages; it was important that the hens experienced only the substrate with which they were to be tested and allocation to different rooms was the best method to ensure there was no cross contamination of substrates. The trays in one of the rooms were filled daily with peat moss (Canadian TE-EM sphagnum peat moss, particle size < 1 mm in width), while the cages in the other room were filled daily with sawdust (Pollard's Sawdust Supplies sawdust and shavings, particle size approximately 3 – 8 mm in width), (refer to Figure 1). The rooms were otherwise maintained in an identical manner. Peat moss and sawdust were chosen as the two substrates as numerous

studies have found that peat moss is highly preferred over sawdust for dustbathing (Petherick and Duncan, 1989; van Liere and Siard, 1991; de Jong *et al.*, 2007).

Figure 1. Photograph of dust substrates provided in the experiment. Sawdust is above the ruler and peat moss is below. Ruler measurements are in millimetres.

Birds were selected for the experiment at 32 weeks of age on their assessed social status, defined as either 'dominant' or 'subordinate'. Hens were assessed during 5-minute direct observations of each cage of hens when feed (Barastoc Golden Yolk layer pellets) access was re-allowed following overnight feed deprivation (mean of 17 hours deprivation, range of 16.25 to 17.75 hours). Feed deprivation was carried out by covering each cage's feed trough with three metal covers. When each cage was re-allowed feed access, only one metal cover was removed thus reducing the space of the feed trough (to approximately 0.28 m of feed trough length), which did not provide enough space for all birds in the cage to feed simultaneously. Observations were made each morning, commencing at 1030 hours, over six consecutive days. The order of cage observations was randomised each day. Social interactions associated with the presentation of feed were recorded to assist the assessment of dominant (defined as the hen that pecked another individual at the feed trough or easily regained access to feed) and subordinate (hen was the recipient of a peck at the feed trough, could not easily regain access to feed and/or paced or vocalised while attempting to gain access) behaviour in hens. Individual hens were identified via coloured leg bands. Each time a hen was observed displaying a behaviour designated as either dominant or subordinate, the event was recorded and totals determined. At the end of the observation period, individuals from the same cage that were consistently recorded as dominant and subordinate were chosen as a pair. Twelve pairs of hens (six pairs from each room) were chosen as experimental hens, while a further three pairs were chosen as spares.

Experimental hen housing

Selected hens were transported to the experimental facility and housed individually in cages (0.57 m x 0.50 m x 0.48 m). Each cage contained an external feed trough at the front of the cage and 1-2 nipple drinkers at the rear. Hens had *ad libitum* feed and water in their home cage throughout the experiment. Each cage also contained a plastic tray identical to the tray that was in the original group cages. The dustbath was filled daily with either sawdust or peat moss, depending on which dust substrate they were provided with in their previous cages. The experimental facility consisted of three tiers, with five pairs of cages on each tier. Figure 2 shows the housing set-up for the experiment. As it was undesirable to have the hens housed in different rooms (because of the possible effect of room) an attempt to eliminate cross contamination of substrates was made by housing the sawdust hens on the top tier, with the remaining hen pair in the cage on one end of the second tier. Peat moss hens were housed on the bottom tier with the remaining hen pair housed in the cages on the other end of the second tier. Spare hens were housed in the centre, middle tier cages. Each hen pair (i.e. the dominant and subordinate hens chosen from the same group-housed cage) was randomly allocated into a pair of adjoining cages. Pairs of cages were separated by metal mesh, which allowed limited tactile contact between neighbours. Each pair of cages was separated from other pairs by solid metal walls. Dominant hens were chosen to be test subjects for the experiment while the subordinate neighbour of each dominant hen was designated to be the 'social option' in the Y-maze for the dominant individual during testing. This pairing was determined as to ensure the 'social option' in Y-maze testing was attractive and non-threatening to the test bird, as it has previously been demonstrated that hens will avoid unfamiliar hens or familiar hens that are dominant to them (Dawkins, 1982; Bradshaw, 1992; Grigor *et al.*, 1995). From here onwards, dominant hens will be referred to as test hens and subordinate hens will be referred to as non-test hens. Hens were checked, fed and dustbaths refilled at approximately 1030 h each morning. Egg production for each test hen was recorded daily. The hens were kept in a constant environmental temperature of about 21^{o}C and a light:dark cycle of 16:8 h, with lights coming on at 0500 h.

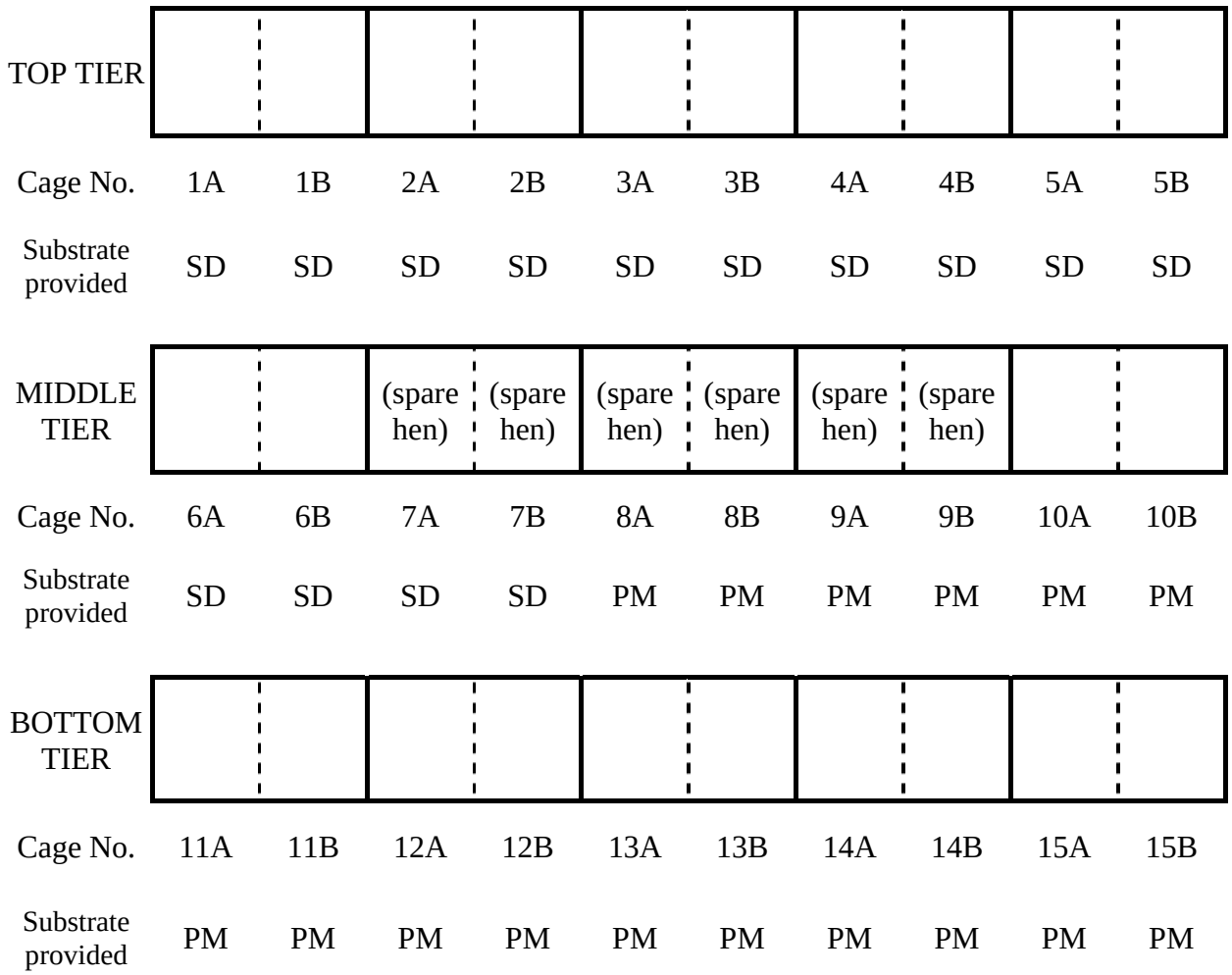

Figure 2. Diagram of experimental hen housing. Substrates provided were sawdust (SD) and peat moss (PM). Solid lines represent solid walls/floors/roofs, dashed lines represent mesh dividers between pairs of cages.

Pre-experimental handling

To ensure hens were accustomed to being handled, all hens (including non-test birds) were given 1 minute of handling twice a day for 7 days. Initially the cage door was opened and the hen was touched. Over the week, the intensity of the handling increased such that, by the end of the week, each bird was picked up and held outside their home cage before being returned. Pre-experimental handling was conducted in the week immediately prior to the commencement of preference testing.

Preference testing

Experimental design

This experiment was a 2 x 3 factorial design comparing peat moss or sawdust and intervals of testing (every day, alternate days or every third day) (Table 1). It was conducted over two periods with each hen allocated to a different interval treatment in each period. Only birds that had had exposure to peat moss were allocated to peat moss treatments and likewise for sawdust.

Table 1. Description of Y-maze treatments

Dust substrate	Interval of testing		
	Every day (1)	Every second day (2)	Every third day (3)
Peat moss (PM)	PM1 (n=2 per period)	PM2 (n=2 per period)	PM3 (n=2 per period)
Sawdust (SD)	SD1 (n=2 per period)	SD2 (n=2 per period)	SD3 (n=2 per period)

Y-maze apparatus

The Y-maze was a purpose-built apparatus constructed from galvanised steel. The floor and removable roof pieces were constructed from mesh, while the walls (0.49 m high) were constructed from a solid metal sheet (Figure 3). The start box had a mesh gate and walls, thereby providing birds with a view of the contents of each arm prior to being released from the start box. The gates between the choice area and each arm were solid metal. The mesh flooring of the maze was identical to the flooring of the hens' home cage, while the wall and roof piece were a smaller mesh. All the gates in the Y-maze were fixed to cords and run through pulleys so that all gates could be opened and closed by one operator standing behind the start box.

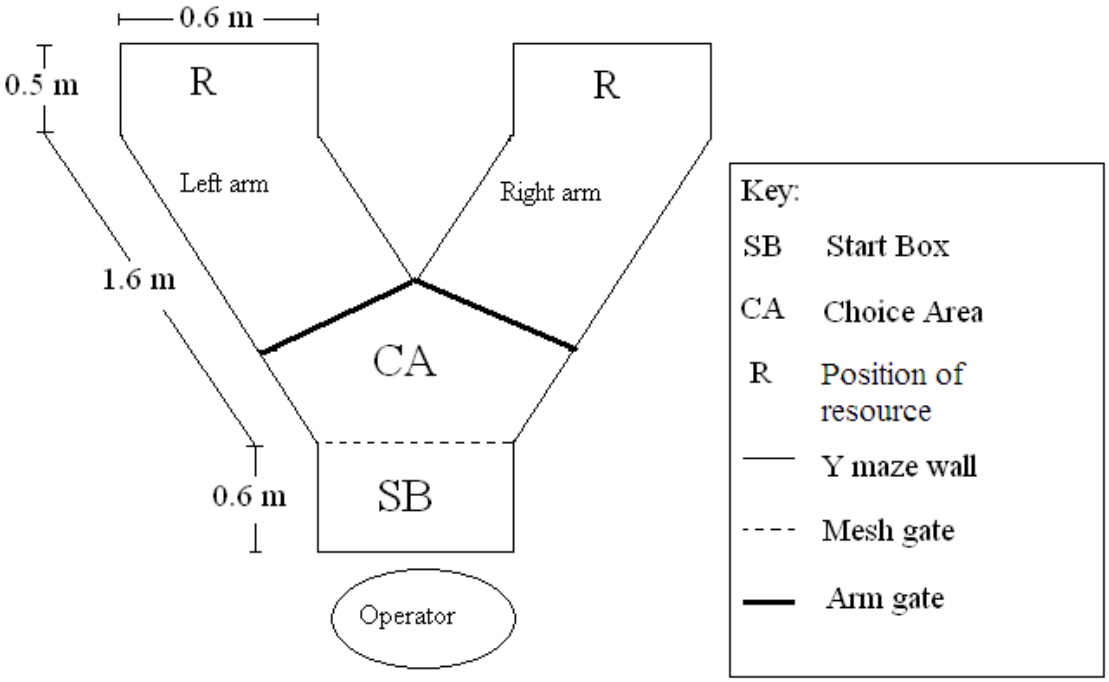

Figure 3. Diagram of Y-maze apparatus.

Y-maze familiarisation

Test hens were familiarised to the Y-maze once per day over four consecutive days, commencing at 1100 h. In a randomly determined order, each hen was placed in the start box (SB) of the Y-maze with both arm gates open and no resources present. After 10 s the SB gate was opened and the hen was allowed to leave. If the hen had not left within 30 s she was gently pushed forward (with a flat hand on the tail). The SB gate was closed once she had left the SB. Each hen was given a total of 5 min in the Y-maze once she had left the SB to freely explore the apparatus.

Y-maze training

Throughout Y-maze training and testing, hens were socially isolated in their home cage by placing opaque rubber partitions between pairs of cages and dustbaths were removed from hens' home cages.

Each hen was randomly allocated one Y-maze arm (left or right) in which the dustbath was always placed. The dustbath was identical to the tray the birds previously had in their home cage and was refilled with either peat moss or sawdust (depending on which substrate the individual hen was allocated) between hens after every training/testing trial. The other arm contained the test hen's neighbour, which was confined in a mesh-fronted box at the end of the Y-maze arm. If the Y-maze arm in which the social option was placed had previously contained the dustbath, the arm was cleaned as to ensure no peat moss or sawdust remained in the arm.

Hens were randomly allocated to an interval treatment (n=2 per dust/interval treatment per period, refer Table 1). All birds were trained individually, in a randomly determined order for five training sessions per bird. Hens were trained on days that corresponded to the interval of testing treatment. This meant that the commencement of training was staggered so as to ensure all treatments could commence testing on the same day (Table 2). The training sessions aimed to ensure that each hen had equal contact with each Y-maze arm so that she learnt what each contained. To accomplish this, on each training day each hen received two consecutive trials in the Y-maze, in which only one Y-maze arm was open at a time. The arm in which the bird was first allowed access was determined randomly for each bird each training day and was subsequently altered each training session.

Each hen was placed in the SB for 10 s before the SB gate was opened. The hen was allowed 30 s to move out of the SB, if she had not, she was gently pushed forward into the choice area. The SB gate was closed after the hen had moved out. The hen was then given another 30 s to move into the open arm, if she had not, she was gently pushed towards the arm. This continued until the hen had entered the arm. The hen remained in the Y-maze for 2 minutes before either being returned to the SB for the second consecutive training trial or being returned to her home cage. Two minutes in the arm was chosen based on results from previous work examining the effects of quantity of reward (Laine *et al.*, 2009).

Table 2. Example timeline for training and testing hens on different interval treatments for one period. Recovery referred to the period between training and testing periods in which hens had *ad libitum* access to dust and social contact in the home cage.

	Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
	Day number							-14
Treatment	Daily (1)							
	Alternate days (2)							
	Third day (3)							Train

	Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
	Day number	-13	-12	-11	-10	-9	-8	-7
Treatment	Daily (1)							
	Alternate days (2)					Train		Train
	Third day (3)			Train			Train	

	Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
	Day number	-6	-5	-4	-3	-2	-1	0
Treatment	Daily (1)			Train	Train	Train	Train	Train
	Alternate days (2)		Train		Train		Train	
	Third day (3)		Train			Train		

	Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
	Day number	1	2	3	4	5	6	7
Treatment	Daily (1)	Test	Test	Test	Test	Test	Test	Test
	Alternate days (2)	Test		Test		Test		Test
	Third day (3)	Test			Test			Test

	Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
	Day number	8	9	10	11	12	13	14
Treatment	Daily (1)	Test	Test	Test	Test	Test	Test	RECOVERY
	Alternate days (2)		Test		Test		Test	RECOVERY
	Third day (3)			Test			Test	RECOVERY

	Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
	Day number	15	16	17	18	19	20	21
Treatment	Daily (1)	RECOVERY						
	Alternate days (2)	RECOVERY						
	Third day (3)	RECOVERY						

	Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
	Day number	22	23	24	25	26	27	28
Treatment	Daily (1)	RECOVERY						
	Alternate days (2)	RECOVERY						
	Third day (3)	RECOVERY						

Y-maze testing

The Y-maze testing procedure was identical to that for training, but both Y-maze arm gates were open when the hen was in the SB. When the hen moved out of the SB and voluntarily entered one of the Y-maze arms, the gate of the arm not chosen was closed and the hen remained in the maze for 2 minutes.

Y-maze testing occurred over a period of 13 days (Table 2). This meant that birds on treatment 1 had 13 test trials, birds on treatment 2 had 7 test trials and birds on treatment 3 had 5 test trials. Although this meant that birds on different interval treatments had unequal numbers of test trials, this format of testing allowed birds to be tested over the same period, so as to minimise any bias that may have occurred over time. At the completion of the 13 day testing period, hens were allowed 15 days 'recovery' (Table 2) in which hens had *ad libitum* access to dustbathing substrate and social contact in their home cage.

Each Y-maze test was digitally recorded in real-time by three cameras (black and white digital CCD) mounted above the Y-maze. The arm and resource choice was recorded as well as the time to leave the SB (defined as the time from when the SB gate was opened until the hen moved into the choice area) and time to choice (defined as the time from when the hen left the SB to when she entered one of the Y-maze arms). In addition, if dust was chosen, the nature of the interaction was also recorded (no interaction, foraging behaviour or dustbathing). Foraging was defined as a hen pecking and/or scratching the dust and a dustbathing bout was defined to commence when the hen performed the first vertical wing shake.

Results

Statistical analysis

One hen was excluded from the study as she did not lay from approximately mid-way through the first testing period through to the end of the experiment. This hen was a peat moss hen and was allocated into third day and second day interval treatments for periods 1 and 2 respectively.

For mean time measurements the main effect of interval treatment was examined using a residual maximum likelihood (REML) mixed model analysis of the logarithm of the mean measurement with a fixed effect for each combination of dust (sawdust and peat moss) and period, a fixed effect of interval treatment and a random effect of bird. The effect of interval treatment was examined using an approximate F test (Kenward and Rogers, 1997), after adjusting for other terms in the model. In analyses where the variance of the bird effect was estimated to be negative this was allowed to stand, by analogy to the standard approach in analysis of variance of balanced experiments. The relationship of the number of trials contributing to a mean and the residual variation was examined graphically, but no pattern was apparent.

A similar approach was used for testing whether interval treatment differed with dust treatment (i.e. the presence of a dust by interval interaction), except that the model included an extra dust by interval interaction term.

The effect of interval on the proportion of trials that dust was chosen was analysed as a binomial logistic general linear model with fixed effects for each combination of dust and period, the effect of each bird and the effect of interval treatment. This analysis included an over-dispersion parameter and the binomial total was taken as the number of tests. Predicted values of treatment were calculated on the logistic scale, after adjusting for other terms in the model, with equal weighting to all birds that had some trials in which social was chosen. The effect of the dust by interval interaction was tested by adding this interaction to the model.

The effects of interval treatment and dust by interval interaction on the proportion of dust-chosen trials where dustbathing occurred were tested in a similar way, with the exceptions of dispersion being fixed at 1 due to

the estimated dispersion being less than 1, and side of dust was used as a covariate. It was not possible to estimate interval effects on the logistic scale because all hens had at least one period in which they either dustbathed in every dust-chosen trial or did not dustbathe on any dust-chosen trial.

The situation for the proportion of dust-chosen trials where foraging occurred was similar, except that side of dust was not an effective covariate. Only one hen did not have at least one period with foraging occurring in all dust-chosen trials, or with no foraging occurring in any dust-chosen trials. This implied that estimates on the treatment effects could not be made reliably on the logistic scale.

Choice behaviour and time to choice

The interval of testing treatment had no significant effect on the choice behaviour for dustbathing substrate or social contact (P = 0.52). There was a tendency for hens in the 'everyday' treatment to take longer to leave the start box compared to the other interval treatments (P = 0.060). However, the time to choice did not significantly differ between interval treatments in both overall trials (P = 0.17) and when dust (P = 0.39) and social contact (P = 0.21) were examined separately (Table 3).

Overall, there were no significant differences between sawdust and peat moss birds in terms of choice behaviour and time to choice (Table 3).

Table 3. Effect of interval of testing treatment on the time to choice and choice behaviour of laying hens.

		Log transformed (Back transformed)				P value	
	Trans-formation	Daily	Alternate	Third	sed	Interval of testing treatment	Interval treatment differs with dust
Mean time to leave starting box (s)	Log_{10}	0.29 (1.9)	-0.27 (0.5)	-0.08 (0.8)	0.210-0.222	0.060	0.73
Mean time to choice (s)	Log_{10}	0.23 (1.7)	-0.05 (0.9)	0.08 (1.2)	0.138-0.145	0.17	0.65
Mean time to dust choice (s)	Log_{10}	0.19 (1.5)	-0.04 (0.9)	0.10 (1.2)	0.161-0.169	0.39	0.41
Mean time to social choice (s)	Log_{10}	0.27 (1.9)	-0.08 (0.8)	0.15 (1.4)	0.168	0.21	Not estimable
Proportion of trials dust chosen	Logistic	1.52 (0.82) *0.87**	2.1 (0.89) *0.92**	2.6 (0.93) *0.95**	0.88-1.00	0.52	0.47
Proportion of dust-chosen trials where dustbathing commenced	Logistic (used side of bath covariate)	Not possible to estimate on the logistic scale because all hens had at least one period in which they either dustbathed in every dust-chosen trial or did not dustbathe on any dust-chosen trial				1.00	1.00
Proportion of dust-chosen trials where foraging occurred	Logistic	Not possible to estimate reliably because there was only one hen that did not have at least one period in which foraging occurred on all dust-chosen trials, or foraging did not occur on any dust-chosen trials				0.24	1.00

* Back transformed values in the proportion of trials dust chosen are given for those hens that selected did not select dust in every test trial (which meant excluding three birds which selected dust on all trials). Values in italics are corrected by including a value for 1 (i.e. dust chosen on 100% of trials) for the excluded three birds.

Behaviour in the Y-maze

The foraging and dustbathing behaviour for each interval and dust treatment are shown in Figure 4. Although there was no significant statistical difference between the occurrence of dustbathing and foraging behaviours between either interval or dust treatments (refer Table 3), this is most likely due to individual variation. The individual hen variation in dustbathing and foraging behaviour in the Y-maze is presented in Table 4.

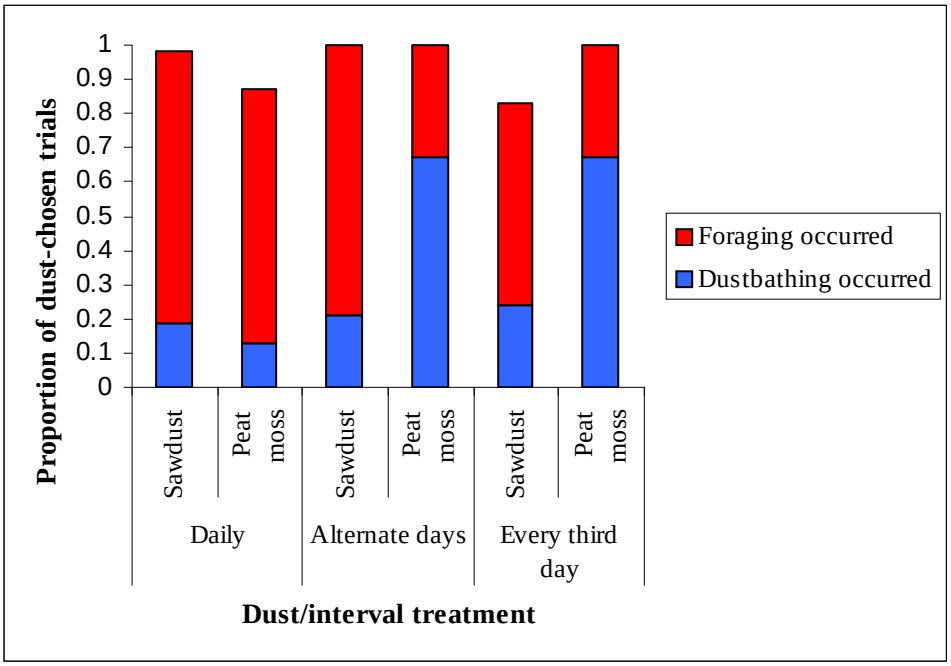

Figure 4. Proportion of dust-chosen trials in which dustbathing or foraging behaviour occurred for each dust and interval treatment (raw data presented).

Table 4. Individual variation in dustbathing and foraging behaviour in the Y-maze. Data arranged from least to most foraging behaviour.

Bird number	Dust type	Proportion dust-chosen Y-maze trials	
		Foraging	Dustbathing
11	Peat moss	0	1
2	Sawdust	0.08	0.92
13	Peat moss	0.11	0.44
4	Sawdust	0.27	0.45
10	Peat moss	0.5	0.5
14	Peat moss	0.94	0.06
1	Sawdust	1	0
3	Sawdust	1	0
5	Sawdust	1	0
6	Sawdust	1	0
12	Peat moss	1	0

Discussion

The present results revealed that the choice behaviour of hens was unaffected by interval and dust treatments. All hens preferred dust to social contact. While the hens tested daily had a tendency to be slower to leave the start box than the other interval treatments, the time to choice was not affected by either interval or dust treatments.

The choice behaviour of hens revealed that the dust substrate provided in the Y-maze was a very attractive resource for all birds, as opposed to social contact. Interestingly, the type of dust substrate did not affect choice for dust, which is contradictory to expectations. If hens are given the choice between an unfamiliar and a familiar dustbathing substrate, they may initially show a preference for the familiar substrate, but will eventually select the substrate of a higher quality (van Liere and Siard, 1991). However, all hens in this experiment only had experience with the dust substrate with which they were presented in the Y-maze. Therefore, the dust presented was familiar to each hen.

Although sawdust is a less preferred dustbathing substrate compared to peat moss (Petherick and Duncan, 1989; de Jong *et al.*, 2007), it may not be a lower quality foraging substrate. Petherick and Duncan (1989) found that hens performed more foraging on peat moss and sawdust compared to sand and wood shavings. Using an operant conditioning task, de Jong *et al.* (2007) reported that hens showed no clear preference for a particular material in which to forage (when offered sand, peat moss, wood shavings or wire floor). Furthermore, hens prefer to perform foraging behaviour in materials that are loose and manipulable (Moffat, 2000). Both peat moss and sawdust are loose and manipulable substrates. Therefore, in contrast to their use as a dustbathing substrate, peat moss and sawdust may be equally attractive as a foraging substrate.

The behaviour of hens with respect to their use of dust in the Y-maze was not significantly different between interval or dust treatments (Table 3). However, looking at the behaviour of each individual in the Y-maze (Table 4) revealed that all but one peat moss hen performed dustbathing on at least one dust-chosen trial. Conversely, only two sawdust hens performed dustbathing, while the other four sawdust hens performed foraging behaviour on all dust-chosen trials. Individual variation in dust substrate use in a Y-maze has been observed previously (Petherick *et al.*, 1991; Laine *et al.*, in prep.). Petherick *et al.* (1991), who utilised peat moss as the substrate, suggested that peat moss may "switch on" dustbathing behaviour in some hens, while this is not the case for other hens. Laine *et al.* (in prep.) found that individual variation in dust usage was not just in the Y-maze, but was consistent when hen behaviour was observed in the home cage when their dustbath was refilled; 'dustbathers' had a shorter latency to commence dustbathing and performed dustbathing on more days compared to 'non-dustbathers' (i.e. hens that foraged rather than dustbathed).

Another reason for why the preference for dust was so high compared to social contact could be due to the extent of social contact available in the home cages, as restriction was only visual; hens still had auditory and olfactory contact with the flock. Furthermore, when in the start box and choice area of the Y-maze, hens had (brief) visual contact with the stimulus hen in the Y-maze. Perhaps this brief visual contact (perceiving that there was a hen in the vicinity), coupled with the sound and smell of other hens when in the home cage was sufficient social contact for hens, leading to the low preference for social contact.

Speed of movement may be used as an indicator of motivation (e.g. Petherick *et al.*, 1992). Although hens tested daily had a tendency to leave the start box slower compared to those tested on every second or third day, once in the Y-maze the time to choice did not differ between interval or dust treatments. Even though dustbathing behaviour typically occurs every second day (Vestergaard, 1982), the results indicate that all hens had a similar level of motivation to obtain a resource in the Y-maze.

The results obtained in the study warrant further research to better understand methodological issues with preference testing that may affect choice behaviour. These methodological issues may influence the results obtained from preference tests, thus leading to spurious results that are not reflective of the animal's preferences. A similar but larger scale experiment in the future, utilising a resource that may be more competitive with dust (e.g. feed), may help in determining whether lack of differences in the present study was due to the relatively low number of experimental animals.

The results from this experiment indicate that neither the interval of testing nor the quality of dustbathing substrate presented affected choice behaviour when social contact and dust was offered as rewards. All hens showed a clear preference for dust to social contact regardless of the type of dust substrate or interval of testing. However further research on these aspects, utilising a resource that may be more competitive with dust (e.g. feed), is necessary to comprehensively examine the effects of interval and quality of the resource on choice behaviour.

Implications

For the design of preference tests utilising similar resources, the findings from this study indicate that the interval of testing and quality of dustbathing substrate may not be significant factors affecting choice behaviour. However, further research examining features of Y-maze preference test design that may affect choice behaviour, such as the social context of the test, genetic strain and age of the test animal, time of day of testing and experience with the resources on offer is needed to ensure that the results obtained from preference tests reflect the animal's true preferences for the resources under study.

Recommendations

The preferences of animals may indicate what is important for their welfare. To assess animal preferences, we must be assured that that the methodology is rigorous to avoid spurious results. Therefore, further research examining design features of Y-maze preference tests should be conducted to ensure design features of the tests are not unintentionally influencing animal motivation and thus choice behaviour.

All hens in the present study showed a clear preference for dust over social contact regardless of the type of dust substrate or interval of testing. However further research on these aspects, utilising a resource that may be more competitive with dust (e.g. feed), is recommended to comprehensively study the effects of interval of testing and quality of the resource on choice behaviour. Furthermore, research into other preference test design features, such the social context of the test at the time of testing, may assist in improving the rigor of Y-maze preference tests to study animal preferences.

It is also of interest that the present results suggest differences between individual hens in their use of the dust substrate: some hens may be consistent dustbathers while others may be consistent foragers when presented with a dust substrate. Further research is clearly required, but one interpretation, for example, is that hens may differ in how they use a dust substrate, how important a dust substrate is for them, and thus they may also differ in their welfare requirements. If this difference in use of a dust substrate is a real effect, it also raises some interesting questions about its genetic and/or experiential basis.

References

Bradshaw, R. H., 1992. Conspecific discrimination and social preference in the laying hen. *Applied Animal Behaviour Science*, 33, 69-75

Dawkins, M. S., 1982. Elusive concept of preferred group size in domestic hens. *Applied Animal Ethology*, 8, 365-375

de Jong, I. C., Wolthuis-Fillerup, M., Van Reenen, C. G., 2007. Strength of preference for dustbathing and foraging substrates in laying hens. *Applied Animal Behaviour Science*, 104, 24-36

Duncan, I. J. H., Widowski, T. M., Malleau, A. E., Lindberg, A. C., Petherick, J. C., 1998. External factors and causation of dustbathing in domestic hens. *Behavioural Processes*, 43, 219-228

Grigor, P. N., Hughes, B. O., Appleby, M. C., 1995. Social inhibition of movement in domestic hens. *Animal Behaviour*, 49, 1381-1388

Heinemann Australian Dictionary, 1995. Holland, M (Ed), n.p., 898

Hogan, J. A., Van Boxel, F., 1993. Causal factors controlling dustbathing in Burmese red junglefowl: some results and a model. *Animal Behaviour*, 46, 627-635

Hughes, B. O., 1971. Allelomimetic feeding in the domestic fowl. *British Poultry Science*, 12, 359-366

Jones, R. B., 1984. Open-field reponses of domestic chicks in the presence of a cagemate or a strange chick. *IRCS Medical Science*, 12, 482-483

Jones, R. B., Merry, B. J., 1988. Individual or paired exposure of domestic chicks to an open field: some behavioural and adrenocortical consequences. *Behavioural Processes*, 16, 75-86

Kenward, M. G., Rogers, J. H. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, 53, 983–997

Laine, S., Arnold, N. A., Hemsworth, P. H., 2007. Choice behaviour of laying hens: effects of deprivation of feed and dustbath substrate. *Proceedings of the Australian Poultry Science Symposium*, 19, 28-31

Laine, S.M., Cronin, G.M., Petherick, J.C., Hemsworth, P.H. 2009. Does the quantity of reward in a Y-maze preference test affect hen choice and motivation? *Proceedings of the Australian Poultry Science Symposium*, 20,153-156

Mills, A. D., Faure, J. M., 1989. Social attraction and the feeding behaviour of domestic hens. *Behavioural Processes*, 18, 71-85

Moffat, L., 2000. An investigation into the importance of foraging behaviour to laying hens. Masters Thesis, University of Guelph, Canada

Nicol, C. J., Guilford, T., 1991. Exploratory activity as a measure of motivation in deprived hens. *Animal Behaviour*, 41, 333-341

Petherick, J. C., Duncan, I. J. H., 1989. Behaviour of young domestic fowl directed towards different substrates. *British Poultry Science*, 30, 229-238

Petherick, J. C., Waddington, D., Duncan, I. J. H., 1991. Learning to gain access to a foraging and dustbathing substrate by domestic fowl: is 'out of sight out of mind'? *Behavioural Processes*, 22, 213-226

Petherick, J. C., Sutherland, R. H., Waddington, D., Rutter, S. M., 1992. Measuring the motivation of domestic fowl in response to a positive and a negative reinforcer. *Applied Animal Behaviour Science*, 33, 357-366

Shields, S. J., Garner, J. P., Mench, J. A., 2004. Dustbathing by broiler chickens: a comparison of preference for four different substrates. *Applied Animal Behaviour Science*, 87, 69-82

van Liere, D.W., 1992. The significance of fowls' bathing in dust. *Animal Welfare* 1, 187-202

van Liere, D.W., Bokma, S., 1987. Short-term feather maintenance as a function of dust-bathing in laying hens. *Applied Animal Behaviour Science* 18, 197-204

van Liere, D.W., Siard, N. 1991 The experience with litter and subsequent selection of bathing substrates in laying hens. In: D.W. van Liere, Function and organization of dustbathing in laying hens, PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands

van Liere, D. W., Kooijman, J., Wiepkema, P. R., 1990. Dustbathing behaviour of laying hens as related to quality of dustbathing material. *Applied Animal Behaviour Science*, 26, 127-141

Vestergaard, K., 1982. Dust-bathing in the domestic fowl - diurnal rhythm and dust deprivation. *Applied Animal Ethology*, 8, 487-495

Vestergaard, K., Hogan, J. A., Kruijt, J. P., 1990. The development of a behavior system: Dustbathing in the Burmese red junglefowl I. The influence of the rearing environment on the organization of dustbathing. *Behaviour*, 112, 99-116

Webster, A. B., Hurnik, J. F., 1994. Synchronization of behaviour among laying hens in battery cages. *Applied Animal Behaviour Science*, 40, 153-165

Plain English Compendium Summary

Project Title:	The effects of interval of testing and quality of resource on the choice behaviour of laying hens
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Objectives	To determine the effects of the interval of testing and quality of resource on the choice behaviour of laying hens in a Y-maze preference test.
Background	The preferences of an animal may indicate what is important for an animal's welfare. Preference tests carried out in a Y-maze offer animals the choice between two resources. Entering a Y-maze arm of one of the resources (making a choice) is considered to reflect the animal's preference. There may be factors in the design of preference tests that may influence an animal's motivation to make a choice (i.e. the resource it chooses). This experiment examined the effects of two design factors; the interval between tests of preference and the type of resource offered.
Research	The hens were offered one dust substrate, either peat moss or sawdust, and a familiar hen (social contact) in a Y-maze and their choice behaviour was studied. The interval of testing was also varied: daily, alternative days, every third day and the research was conducted over two periods with each of the 12 hens allocated to a different treatment in each period. Hens were reared with access to only the substrate with which they were tested. In their home cages, hens were visually isolated from other hens and had no dust access. Prior to testing, hens underwent familiarisation and training sessions in the Y-maze. Hens were then preference tested for their choice between social (the familiar hen) and dust substrate (either sawdust or peat moss). Tests were conducted over 13 days per period, so that hens tested daily received 13 trials, those tested on alternate days received 7 trials and those tested every third day received 5 trials.
Outcomes	The results of this experiment indicate that neither the interval of testing nor the type of the dustbathing substrate affected choice behaviour when social contact and dust was offered as rewards. All hens showed a clear preference for dust to social contact regardless of the type of dust substrate or interval of testing. However, there was surprising variation between hens in their use of the dust substrate: most hens offered peat moss predominantly dustbathed, while most hens offered sawdust predominantly foraged.
Implications	When designing preference tests in the future utilising similar resources, the findings from this study indicate that the interval of testing and quality of the dustbathing substrate may not be significant factors affecting choice behaviour. However, further research examining features of Y-maze preference test design that may affect choice behaviour, such the social context of the test, genetic strain or age of the test animal, time of day of testing and experience with the resources on offer is needed to ensure that the results obtained from preference tests reflect the animal's true preferences for the resources under study.
Publications	