The effects of water deprivation on the behavior of laying hens

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ABSTRACT Freedom from thirst is one of the most undeniable welfare requirements. Nevertheless, the welfare implication of water deprivation because of a particular situation (e.g., transport) or as an involuntary consequence (e.g., sick, injured, or subordinate animals) remains unclear. This experiment investigated the behavioral changes in laying hens following various durations of water deprivation by using a motivation test based on passing through a narrow, vertical gap to access water. Twenty laying hens were subjected to water deprivation for various durations (0, 12, 18, 24, or 32 h) and the cost of access was varied by changing the width of the vertical gap (150, 135, 120, or 100 mm) to access the water side of the testing cage. An incomplete randomized block design was used with two tests per hen per wk for 5 wk. The testing apparatus was identical to their home cage but with two cages connected through an adjustable vertical gap and a drinker on the other side. Hens spent more time in the control side rather than the water side at 100 mm compared to 120 mm ($P = 0.03$). The hens' willingness to pass through a narrow vertical gap in order to access water did not vary according to the duration of water deprivation. Nonetheless, water-deprivation duration had a marked effect on the hens' location and behavior. Hens spent more time in the vicinity of the drinker at 18, 24, and 32 h compared to 0 and 12 h ($P < 0.05$). Hens spent more time drinking at 24 h and 32 h, followed by 18, 12, and finally 0 h ($P < 0.05$). Drinking latency and frequency were higher for all water-deprivation durations as compared to the 0 h control ($P < 0.05$). Water deprivation can be characterized by behavioral changes such as drinking duration, reaching a plateau at 24 h. Complementary physiological data are warranted to fully assess the impact of water deprivation on hen welfare.

Key words: dehydration, Five Freedoms, spent hens, thirst, welfare

INTRODUCTION

Freedom from hunger and thirst is the first of the Five Freedoms concept (FAWC, 1979) that have been widely adopted as guiding principles of animal welfare. Nevertheless, there is a lack of understanding of the welfare implication when an animal cannot access feed or water, and particularly of the length of time after which welfare can be considered compromised. Although this situation is obviously undesirable, investigating the welfare implications of feed and water deprivation is relevant to situations such as transport and lairage, but also to cases of lame, injured, or subordinate animals that may experience difficulty accessing these resources (Butterworth et al., 2002).

Transport journeys in large countries often exceed a few hours, sometimes extending up to 32 h in Australia, typically with no provision of feed and water due to the practical difficulty in supplying these. Furthermore, in Australia for instance, loss of value of 'spent' laying hens for meat consumption is resulting in longer distances to slaughter due to the reduced availability of slaughter facilities. Many codes of practice or welfare laws have limits on the maximum duration that animals should be without water, particularly during transport. Australian poultry transport standards state that the maximum time off water for poultry should not exceed 24 h (item SB10.1, Animal Health Australia, 2012). However, there is little scientific evidence to indicate the suitability of this duration of water deprivation in terms of hen welfare.

The welfare implication of water-deprivation duration for poultry has mostly been studied using physiological changes, with studies up to 48 h of water deprivation or feed and water deprivation. Various physiological indicators of dehydration (osmolality, packed cell volume, plasma electrolytes), metabolic status (glucose and lactate concentrations) and stress physiology (corticosterone and vasotocin concentrations) have been measured with inconsistent outcomes resulting from differences in strain, gender, age, production stage, and environmental conditions between studies (Koike et al., 1977, 1983; Arad et al., 1985; Stallone

and Braun, 1986; Knowles et al., 1995; Saito and Grossmann, 1998; Zhou et al., 1999; Iheukwumere and Herbert, 2003). Induced molt has been extensively studied, but with a focus on feed rather than water deprivation due to the impact of energy restriction (Webster, 2003). Furthermore, although broody hens can sustain extended periods of time without feed and water, their behavior and physiology change to allow such adaptation (Mrosovsky and Sherry, 1980; Sharp et al., 1984), making it an unsuitable model for hens in production.

Although physiological changes are informative, there is a crucial need to assess the discomfort or pain that may be experienced by hens during water deprivation. Behavior represents one of the most robust outputs of an animal's perception of challenges, which, coupled with physiology, provides a powerful approach to study animal welfare. Haskell et al. (2004), using water deprivation in an operant conditioning test, showed that 2 h of water deprivation induced redirected aggression toward a subordinate hen. Sprenger et al. (2009) used drinking behavior as an indicator of thirst in broiler chickens and showed a linear increase in water consumption between 0 and 24 h of deprivation, but broilers differ dramatically from layers in terms of metabolism. A behavioral approach has not been used to assess the welfare implication of the duration of water deprivation for laying hens.

Because water deprivation ultimately results in dehydration, hens should show an increased motivation to access water resources as time progresses. Hence behavioral demand tests, also called 'motivation tests', could provide useful information regarding the perceived need by the hen to drink (Kirkden and Pajor, 2006). Motivation tests typically use measures of the amount of work that an animal will perform to obtain the resource, with the performance of high workloads interpreted as a strong need for that resource in thwarting situations. The use of a narrow vertical gap of variable width has previously been validated to assess the welfare implication of offering a nest to laying hens (Cooper and Appleby, 1996). These researchers found that hens with an average width of 117 mm generally can squeeze through a 95-mm-wide vertical gap, with some effort, when highly motivated to access a nest prior to oviposition. This method also presents the advantage of requiring minimal training.

As a first step toward investigating the welfare implications of transport for spent laying hens, this study focused on the behavioral changes following water deprivation per se as its main current concern without considering other factors (e.g., handling, weather conditions) that could have concurrent and confounding effects. Because the demand for water is inelastic in most animals (Murphy et al., 1985), we hypothesized that increased duration of water deprivation should lead to increased willingness to access water with concurrent behavioral changes.
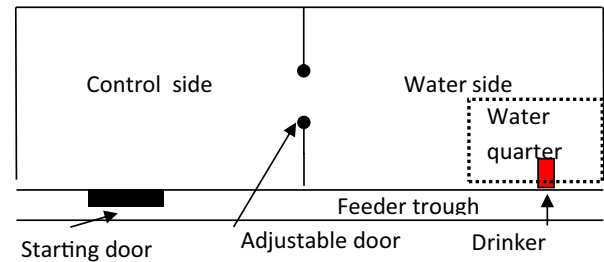

Figure 1. Testing apparatus.

MATERIALS AND METHODS

The project was approved by the University of Melbourne Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Housing

Twenty 39-week-old Hy-Line brown laying hens previously housed in conventional cages were obtained from a commercial farm and transported to the research facility. Mid-lay aged hens were preferred to end-of-lay, spent hens to ensure that they could sustain the repeated water-deprivation treatment. Hens were selected at the farm based on a relatively uniformly shaped trimmed beak as this could influence their ability to drink during the test. Hens were housed individually in cages (L × W × H: 61 × 50 × 45 cm) to control their water intake, with visual contact with their neighbors, and allowed 4 wk to acclimatize to their new environment prior to the start of the tests. They were kept on a 16 h light schedule (0500 to 2100 h) and fed ad libitum a formulated layer diet (formulated and mixed by the source farm, 15% crude protein) with ad libitum access to water through a cup drinker. Room temperature ranged between 18 and 24°C with a relative humidity of 50 to 60%.

Testing Apparatus

The testing apparatus was placed in a room adjacent to the home cages and consisted of four testing cages placed at each corner of the room and visually separated from each other. Each testing cage consisted of two conventional cages (122 × 50 × 45 cm), each identical to the home cage, and connected by a middle doorway (Figure 1). The width of the middle doorway was adjustable. The hen was placed in the control side, whereas the water side was identical except for the presence of a cup drinker, identical in type and location to the one present in their home cage. A camera was placed in front of each cage and videos were recorded on a computer. The testing cage was divided in three zones: the "control side", the "water side", and the "water quarter" within the water side and defined as a body width (approximately 13 cm) from the drinker. An empty feed trough was present in front of

the cage, and the front side consisted of horizontal bars, whereas the three other sides were solid-sided. Light intensity, temperature and humidity in the testing cages were maintained at similar levels as in the home cages.

Treatments

Vertical Gap In order to adapt the methodology from Cooper and Appleby (1996) to our hens' phenotype, the width of each hen at the widest point of the shoulders between the external sides of the wings were measured three times, three d apart, prior to the start of the tests. The hens were similar in width, varying from 122.7 mm to 132.7 mm. Using our average hens' width of 127 ± 0.7 mm (mean $\pm$ SEM), we chose 100-, 120-, 135-, and 150-mm doorway widths, proportionally equivalent to four of the five widths used by Cooper and Appleby (1996).

Water Deprivation Each hen was individually tested with access to water ad libitum (Control; 0 h) or after 12, 18, 24, or 32 h of water deprivation. We decided to test 12 h as the lower limit because it was difficult to study continuous behavioral changes in the motivation paradigm chosen, and based on one of our previous studies that showed only minor physiological changes 12 h after feed and water deprivation (Edwards et al., unpublished). Water was removed at various times of day according to the duration of water deprivation (0200, 1000, 1600, or 2200 h) and all hens were tested between 0845 and 1115 h (i.e., within $\pm$ 1 h of their water-deprivation treatment) the following day in a random order. Feed was always available in the home cage for all treatments but not in the testing cage.

Observations of the normal drinking behavior of hens during the acclimatization period made on four hens on different days revealed that laying hens drink at a regular frequency during the day, 7.64 ± 0.35 times per hour (means $\pm$ SE) during the daylight phase, but relatively little at night, 0.06 ± 0.05 times per hour (means $\pm$ SE) during the dark phase. This could have included a bias in the data because hens were tested in the morning and therefore various water-deprivation durations had different proportional amount of daylight vs. dark hours. However, 32 and 24 h both had similar proportion (21L:11D and 16L:8D), whereas 18 and 12 h had proportionally higher amounts of dark hours (10L:8D and 7L:5D). Nevertheless, testing during daylight hours ensured that hens were tested at a time when they were motivated to drink, and the times chosen were representative of field situations, as spent laying hens are most likely to be transported overnight and slaughtered during the day, hence similar to our testing time.

Tests

Each hen was given an acclimatization period by being placed individually in the testing apparatus for 15 min twice weekly over the 4 wk of acclimatization to explore and learn the location of the drinker, using the largest gap width of 150 mm. The drinker was identical in type and location to the one present in their home cage and contained 1 L of water, and all hens drank from it at least once.

In order to minimize the frequency of water deprivation on each hen, the hens were tested using an incomplete randomized block design by subjecting each hen to 10 test sessions out of the total of 20 possible combinations (5 water-deprivation durations × 4 gap widths). All hens experienced each water-deprivation duration twice, but gap widths were randomized across hens. This ensured that each possible combination was tested 10 times across all hens. The incomplete randomized block design also controlled for individual variability and sequence of testing effects. A minimum period of 62 h was given between each test and deemed sufficient on the basis of physiological return to baseline (Koike et al., 1983; Arad et al., 1985), allowing for two tests per wk for a total of five wk. The test started as soon as the experimenter closed the door of the testing cage and lasted for 30 min, after which the hens were returned to their home cage with ad libitum access to water. Hens were monitored for the next 2 h after testing to ensure that they resumed drinking and did not show signs of distress (panting, vocalizations or a lethargic state).

Data Collection

Videos were analyzed with the Observer software (version XT 8.0, Noldus, The Netherlands) with a continuous recording method using an ethogram to record behavior (Table 1) and location (control side, water side or water quarter) based on the feet of the hens. The main measures derived were the latency to attempt passage through the vertical gap from the control side to the water side, numbers of successful and failed crossing attempts, latency to reach the drinker for the first successful passage to the water side, time spent in the various locations of the testing cage, and the duration and frequency of drinking, exploratory and comfort behaviors. All observations were conducted by a single observer who was blind to water-deprivation duration, and testing days were randomly analyzed. All hens were in lay and the eggs were individually collected and weighed after each testing day around 1400 h.

Statistical Analysis

All data met the criteria for normality and homogeneity of variance. Data were analyzed using a mixed model (Proc Mixed, SAS Institute Inc., Cary, NC), which always included the fixed effects of water-deprivation duration and gap width, the random effect of day of testing; and included the interaction of water-deprivation duration with gap width, testing cage

Table 1. Ethogram used for behavioral observations.[1]

Drink	Beak in contact with the drinker in the water side.
Preen	Straightening of the feathers with the beak.
Walk	Any locomotory movement.
Stand	Stationary position in the cage and up on its two legs.
Crouch	Body lower than standing position, in contact with bottom of the cage.
Peck at feeder	Beak in contact with the feeder.
Peck at wall	Beak in contact with the walls or floor.
Head Poke	Head is positioned through the horizontal bars at the front of the cage.
Escape attempt[2]	Active attempt to escape with both neck and feet on or through the front bars of the cage.
Body Shake[2]	Entire body shakes from side to side in a rapid motion, fluff feathers.
Wing Flap[2]	Flap of the wings away from the body.
Head flick[2]	Head move in a short, sharp motion to the side.
Successful Crossing[2]	Move through gap from the control side to the water side.
Failed Crossing Attempt[2]	Attempt to move through the gap from the control to the water side by physically touching the door but remaining in the control side.

[1]An interruption of more than 5 s was considered a new bout.

[2]These behaviors were only recorded as events due to their short duration.

location, order of testing within a daily testing session, and hen width if significant. The model also included hen as a random effect and accounted for repeated measures over days with hens as subjects. The interaction of water-deprivation duration × gap width was never significant for any of the variables and therefore was removed from the model. When significant differences ($P < 0.05$) were detected, Tukey-Kramer tests were used for pairwise comparisons between all treatments.

RESULTS

Crossing the Vertical Gap

The numbers of successful crossings, failed crossing attempts, and the latencies to the first crossing attempt and to the first successful crossing did not differ according to water-deprivation duration (means ± SE: 8.1 ± 1.4 times, 0.7 ± 0.2 times, 65.1 ± 36.1 s, and 135.4 ± 63.3 s, respectively).

The effects of vertical gap width on behavioral variables are shown in Table 2. The number of failed crossing attempts differed according to gap width ($P = 0.03$), with more failed crossing attempts at the narrower gap of 100 mm compared to 150 mm ($P = 0.04$). However, failed crossing attempts were relatively few compared to successful crossings. The number of successful crossings also differed according to gap width ($P < 0.001$), with a preference for crossing 135 mm compared to other widths (all $P < 0.001$). The latency to the first crossing attempt dif-

fered according to gap width ($P = 0.02$), with hens taking longer for the first crossing attempt at 150 mm than 120 mm ($P = 0.02$), but not different from 135 mm or 100 mm ($P = 0.11$ and $P = 0.12$, respectively). However, the latency to the first successful crossing did not differ according to gap width ($P = 0.11$). The width of the hens had a significant effect on the latency to the first crossing attempt and the latency to the first successful crossing ($P = 0.002$ and $P = 0.005$, respectively).

Location

Location of hens differed according to water-deprivation duration (all $P < 0.01$; Figure 2), with the hens spending more time in the water quarter at 18, 24, and 32 h compared to 0 and 12 h (all $P < 0.001$ apart from 12 h vs. 18 h, $P = 0.04$).

The time hens spent in the control side of the testing apparatus differed according to gap width ($P = 0.03$), with the hens spending more time in the control side with 100 mm as compared to 120 mm (734 ± 67 s vs. 474 ± 67 s, $P = 0.03$), but no difference with other gap widths (135 mm: 628 ± 55 s, 150 mm: 517 ± 71 s).

Drinking Behavior

The duration of drinking increased according to water-deprivation duration ($P < 0.001$, Figure 3), with the hens spending more time drinking at 32 and 24 h compared to 18 h ($P = 0.05$ and $P = 0.02$,

Table 2. Effects of vertical gap width on behavioral variables.

Variables	100 mm	120 mm	135 mm	150 mm	P-value
Latency for the first crossing attempt (s)	49 ± 32[a,b]	17 ± 32[a]	45 ± 32[a,b]	148 ± 32[b]	0.02
Failed crossing attempts (number)	± 0.2[a]	0.6 ± 0.2[a,b]	0.5 ± 0.2[a,b]	0.4 ± 0.2[b]	0.03
Successful crossings (number)	6.4 ± 1.2[a]	6.0 ± 1.2[a]	13.3 ± 1.2[b]	6.2 ± 1.2[a]	0.001
Latency to the first successful crossing (s)	189.9 ± 56.6	89.3 ± 56.7	95.3 ± 56.6	167.2 ± 56.7	0.11

[a,b]Means in a row not sharing a common superscript differ ($P < 0.05$).
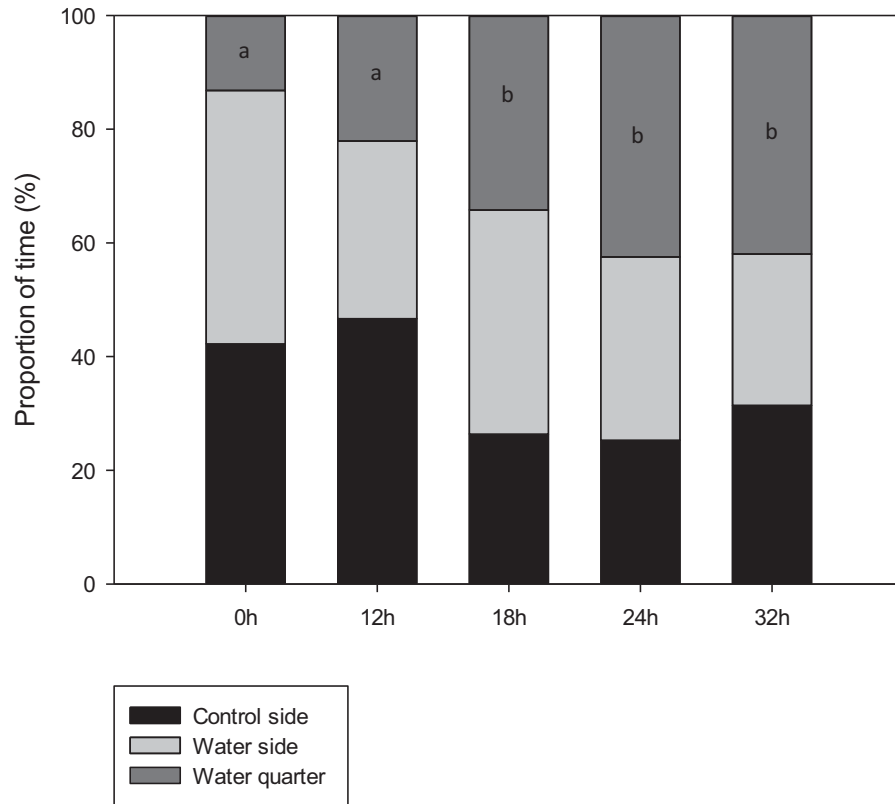
Figure 2. Proportion of time spent in the three locations of the testing apparatus (LS-means) by water deprivation. Means sharing any common letters within a category (Control side, water side or water quarter) are not significantly different ($P > 0.05$).
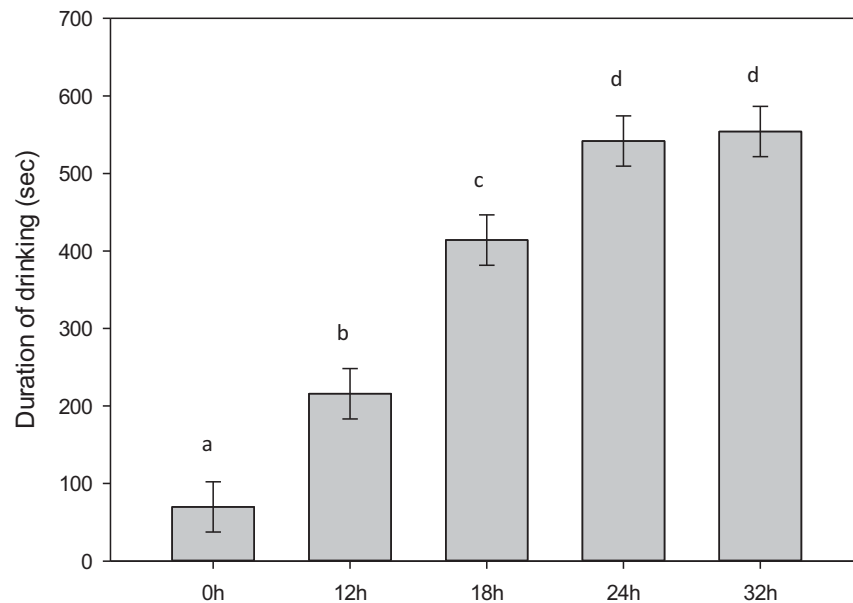
Figure 3. Duration of drinking (LS-means $\pm$ SEM) by water deprivation. Means sharing any common letters are not significantly different ($P > 0.05$).

respectively), 18 h spending more time drinking than 12 h ($P = 0.002$), and 12 h spending more time drinking than the 0 h, non-deprived hens ($P = 0.01$). The effects of duration of water deprivation on behavioral variables are shown in Table 3. The frequency of drinking also differed according to water-deprivation duration ($P = 0.002$), with hens at 0 h

drinking less frequently than hens submitted to all water-deprivation durations (all $P < 0.05$). Similarly, the latency to drink differed according to water-deprivation duration ($P = 0.004$) with hens at 0 h taking longer to reach the drinker than hens submitted to all water-deprivation durations ($P < 0.001$). Latency to drink also differed according to the day of testing

Table 3. Effects of duration of water deprivation on behavioral variables.

Variables	0 h	12 h	18 h	24 h	32 h	P-value
Drinking						
Drink Frequency (number)	1.9 ± 0.2^a	4.4 ± 0.5^b	3.7 ± 0.3^b	4.4 ± 0.4^b	3.7 ± 0.4^b	0.002
Latency to Drink (s)	494 ± 116^a	150 ± 68^b	112 ± 57^b	88 ± 38^b	154 ± 70^b	0.004
Exploration						
Walk Duration (s)	450 ± 34^a	458 ± 31^a	$372 \pm 30^{a,b}$	346 ± 31^b	313 ± 32^b	0.006
Stand Duration (s)	612 ± 41^a	$472 \pm 36^{a,b}$	$393 \pm 28^{b,c}$	314 ± 31^c	256 ± 26^c	<0.001
Comfort behaviors						
Preen Frequency (number)	1.2 ± 0.2^a	$0.9 \pm 0.2^{a,b}$	$0.6 \pm 0.2^{a,b}$	0.4 ± 0.1^b	$0.6 \pm 0.1^{a,b}$	0.01
Body Shake Frequency (number)	1.5 ± 0.2^a	$1.4 \pm 0.2^{a,b}$	$1.0 \pm 0.1^{b,c}$	0.8 ± 0.1^c	$1.1 \pm 0.1^{a-c}$	0.02
Wing Flap Frequency (number)	1.5 ± 0.2^a	$1.0 \pm 0.2^{a,b}$	$0.6 \pm 0.1^{b,c}$	0.2 ± 0.1^c	0.4 ± 0.1^c	<0.001

[a-c]Means in a row not sharing a common superscript differ ($P < 0.05$).

($P < 0.001$), with the hens taking longer to drink on the first day compared to all other days (all $P < 0.01$), apart from d 4 ($P = 0.12$).

Drinking duration, frequency, and latency did not differ according to gap width.

Exploratory Behaviors

The time spent standing differed according to water-deprivation duration ($P < 0.001$; Table 3), with the hens at 24 and 32 h standing less than hens at 0 and 12 h (all $P < 0.02$) and hens at 18 h standing less than hens at 0 h ($P < 0.001$). The time spent walking also differed according to water-deprivation duration ($P = 0.006$), with hens at 24 and 32 h walking less than hens at 0 and 12 h (all $P < 0.03$). Pecking at the empty feeder differed according to water-deprivation duration ($P = 0.003$, Figure 4), with hens at 32 h spending more time pecking at the feeder than hens at 0, 12, and 18 h ($P = 0.002$, $P = 0.03$ and $P = 0.04$, respectively).

The time spent walking differed according to gap width ($P = 0.04$), with hens spending more time walking at 135 mm (459 ± 34 s) compared to 100 mm or 150 mm (371 ± 30 s, $P = 0.04$; 341 ± 28 s, $P = 0.006$, respectively) but not different from 120 mm (380 ± 34 s, $P = 0.06$).

Standing or pecking at the feeder did not differ according to gap width. Pecking at the walls and floor (overall mean $\pm$ SEM: 191 ± 30 s), head poke (overall mean $\pm$ SEM: 222 ± 23 s), escape attempts (overall mean $\pm$ SEM: 23 ± 8 s), crouch (overall mean $\pm$ SEM: 30 ± 19 s), and the frequency of head flick behavior (overall mean $\pm$ SEM: 3.1 ± 0.4 times) did not differ according to water-deprivation duration or gap width.

Comfort Behaviors

The frequency of preening bouts differed according to water-deprivation duration ($P = 0.01$; Table 3) with fewer preening bouts in hens at 24 h compared to 0 h ($P = 0.01$). Similarly, the frequency of body shake differed according to water-deprivation duration ($P = 0.02$), with less body shake in hens at 24 h compared to 0 h ($P = 0.02$). Wing flap also differed according

to water-deprivation duration ($P < 0.001$), with more wing flaps seen in hens at 0 h compared to 18, 24, or 32 h ($P = 0.002$, $P < 0.001$ and $P < 0.001$, respectively).

Preening bouts, wing flapping, and body shaking frequencies did not differ according to gap width.

Egg Production and Weight

Hen-day egg production was 96% in average over the study. The eggs for d 1 were not weighed; however eggs from the remaining 9 d did not differ according to water-deprivation duration or day of testing (overall mean $\pm$ SEM: 63.6 ± 0.4 g; $P = 0.84$ and $P = 0.25$).

DISCUSSION

The hens' willingness to pass through a narrow vertical gap in order to access water did not vary according to the duration of water deprivation. Nonetheless, the duration of water deprivation affected a number of behavioral variables, with 24 and 32 h off water leading to changes on most behaviors (e.g., drinking duration), whereas changes were seen in some behaviors at 18 h (e.g., location of the hen close to the drinker, reduced standing).

The willingness to cross a vertical gap of variable width, which we used for the purpose of assessing motivation to access water, did not vary according to water-deprivation duration in the range of widths studied. Hens started having more difficulties at crossing a vertical gap of 100 mm compared to 150 mm, with more failed attempts and more time spent on the control, starting side. Nevertheless, crossing even the narrowest gap of 100 mm was not uncomfortable or impossible because the hens always accessed the water side, with no significant difference in the latency to successfully cross in less than about 3 min. It is possible that the gap was not narrow enough. Alternatively, it supports the hypothesis that the demand for water is rather inelastic (Murphy et al., 1985), and that hens were motivated to drink as early as after 12 h of water deprivation and willing to squeeze through even the narrowest gap to access water. There is no obvious explanation for the preference to cross the 135 mm gap, and the
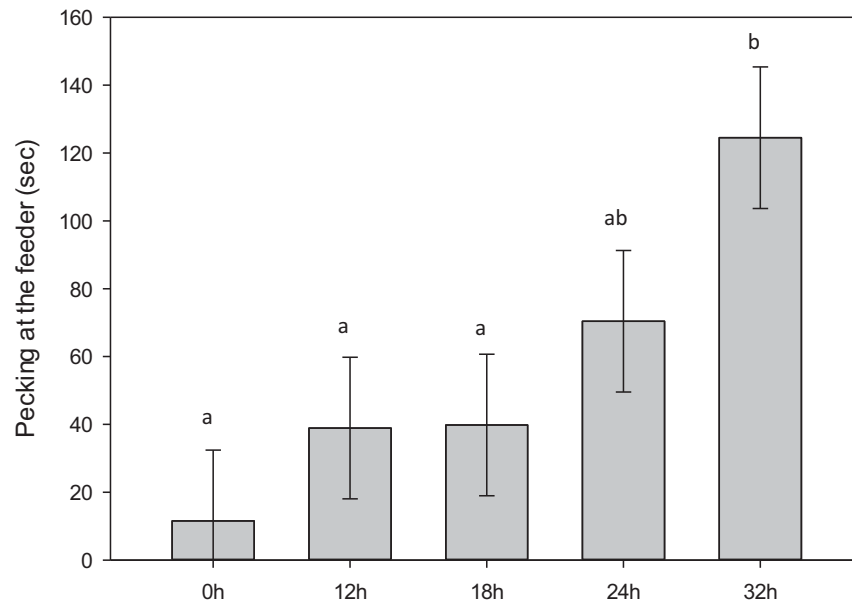
Figure 4. Duration of pecking at the feeder (LS-means ± SEM) by water deprivation. Means sharing any common letters are not significantly different ($P > 0.05$).

resulting higher locomotion, a finding not reported by Cooper and Appleby (1996). It may be that a slight ruffling or scratching of their feathers had a rewarding effect, because the hens were 127 mm wide on average, hence just 8 mm less than the 135 mm gap. Other behavioral demand tests may yield clearer results, such as operant conditioning through pecking operant keys (Haskell et al., 2004) or pushing through a weighted door (Duncan and Kite, 1987), despite requiring more training or being of less behavioral relevance (Fraser and Matthews, 1997).

The latency to drink decreased in all water-deprivation durations as compared to the control non-deprived treatment, indicating that hens were motivated to reach the water drinker when deprived of water for 12 h or more. Haskell et al. (2004) showed that as little as 2 h of water deprivation induced redirected aggression in hens, and therefore it questions when behavioral change indicative of thwarting or conflict conditions commence. As deprivation duration increased, hens drank for longer, but drinking duration reached a plateau at 24 h. This is in agreement with findings on broilers (Sprenger et al., 2009), in which water consumption increased linearly between 6 and 24 h of water deprivation. The fact that drinking duration reached a plateau between 24 and 32 h may be due to the fact that hens are physically restricted to ingest more than this amount of water in such a short period of time, although this hypothesis of a limited physical capacity for water intake should be investigated. Unfortunately, water consumption in terms of volume of water intake by the hens during the tests was not measured. The frequency of drinking bouts did not change, indicating that the hens adjusted their drinking behavior by drinking for longer periods of time rather than more often. The shorter time spent

walking and standing likely reflected the longer time dedicated to drinking.

Hens after 32 h of water deprivation also spent a considerable amount of time pecking at the empty feeder, despite the fact that hens were never feed deprived before testing. Feeding and drinking are often simultaneous acts (Savory, 1978), and birds under water restriction have been shown to voluntarily reduce their feed intake (Ross et al., 1981), probably explaining this concurrent increase in pecking at the (empty) feeder once water was available. However, feed intake during water deprivation was not measured. Abnormal behaviors such as redirected behaviors and displacement activities can appear if conflict or thwarting conditions persist, and aggression, pacing, excessive preening, or redirected pecking have been reported in hens that are feed or water deprived (Duncan and Wood-Gush, 1972; Haskell et al., 2000). However, pecking at the walls and floor did not change, which support that pecking at the feeder after 32 h of water deprivation was somehow related to the search for water or a reduced feed intake during water deprivation, rather than an abnormal behavior.

In terms of development of the behavioral changes, both the location of the hens close to the drinker in the 'water quarter' and drinking behavior linearly increased as time off water increased, reaching a plateau at 24 h with no differences between 24 and 32 h in most behaviors. However, 18 h was intermediate across most behavioral variables and consequently behavioral changes were seen earlier than 24 h after water deprivation.

Comfort behaviors during the test (i.e., once water intake was possible) were reduced, although inconsistently, with less preening and body shaking at 24 h but not 32 h, and less wing flapping at 18, 24 and 32 h

compared to the non-deprived treatment. Comfort behaviors, such as preening, decrease when a bird is stressed (Duncan and Wood-Gush, 1972), or possibly in our case because other behaviors such as drinking took priority in this context. Nonetheless, comfort behaviors are generally infrequent or of short duration as we found here. Interestingly, the decrease in the number of preening bouts corresponds to anecdotal veterinary knowledge that removing water for 24 h decreases feather pecking and cannibalism in commercial flocks (Peter Scott, personal communication).

Although each hen was submitted to the test 10 times, with different combinations each time, there was very little day effect, apart from the longer latency to drink on the first day of test when they were first water deprived and exposed to narrower vertical gaps. However, learning occurred quickly as all other days were comparable. Notwithstanding learning, the willingness to reach the water drinker and consequently drink for an extended time (10 min out of the 30 min test after 24 and 32 h of water deprivation) persisted across the series of 10 tests, which highlights the priority of drinking for hens in a state of negative water balance. Nevertheless, water deprivation or the cumulative effects from the water deprivation did not affect egg weight or egg production, as found by Savory (1978).

Although behavioral changes occur as early as the first time point of 12 h in this experiment, behavioral changes do not necessary equate strictly to a state of compromised welfare as behavior is primarily a coping strategy to adapt to change. Nevertheless, there are no clearly defined thresholds indicative of acceptable and unacceptable welfare in the measured responses. When relying on behavioral, physiological, and fitness measures to determine welfare risks, a value-based judgment is made about what degree of change in these indicators is likely to indicate compromised animal welfare. Furthermore, this experiment was conducted under favorable handling and climatic conditions. It should be recognized that factors other than water deprivation are likely to influence hen behavior and welfare for practices such as transport: feed deprivation, health status of the hens prior to loading (mid-lay aged hens were used here rather than spent hens), body condition, handling stress, social stress, transport duration, weather conditions and time in lairage. For instance, both deprivation of food for 23 h daily or water for 6 h result in an increase in frustration-induced aggression in laying hens (Haskell et al., 2000), and therefore the social environment may also impact further on this situation. Further research is required to determine and quantify the influence of those factors on the behavior and welfare of laying hens.

CONCLUSIONS

The hens' willingness to pass through a narrow vertical gap in order to access water did not vary according to the duration of water deprivation. Nonetheless, hens changed their behavior according to the duration of water deprivation. Hens changed their behavior as early as 12 h after water deprivation, the first time point. Behavioral changes by 24 h were similar to 32 h, suggesting that a plateau was reached in terms of behavioral adaptation. Nevertheless, behavioral changes do not necessary equate strictly to a state of compromised welfare, as behavior is primarily a coping strategy to adapt to change. Therefore complementary physiological data are warranted to fully assess the welfare implication of water deprivation.

ACKNOWLEDGMENTS

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