



Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim



Effect of crate height during short-term confinement on the welfare and behaviour of turkeys

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ARTICLE INFO

Article history:

Accepted 8 June 2010

Keywords:

Behaviour
Muscle physiology
Stress
Transport
Turkeys
Welfare

ABSTRACT

During transport from the farm to the slaughter house birds are often confined in crates with limited space. In this study we investigated how the confinement of male turkeys in crates of 40, 55 or 90 cm height for 6 h, affected the turkeys welfare. We used both behavioural observations and physiological measures and the study was carried out under experimental conditions.

Thirty-six turkeys were placed singly in stationary crates for 6 h and during this time their behaviour was observed. The confinement for each bird was carried out twice on two separate occasions with around 1 week between confinements. The mean (\pm SE) weight of the birds in the first confinement period was 15.9 ± 0.2 kg and on the second occasion 17.3 ± 0.2 kg. Blood-samples were taken after the behaviour observations were finished and analyses of activities of creatine kinase (CK), aspartate aminotransferase (ASAT) and lactate were carried out. The heterophil:lymphocyte ratio (H:L) was also determined.

The behaviour observations revealed that birds in the 40 cm crates did not perform any standing (standing with straight legs), whereas birds in 55 and 90 cm crates spent 35.4 ± 4.3 and $42.2 \pm 5.8\%$ of the time, respectively, in this position. Conversely, birds in the 40 cm crates spent significantly more time in a low standing position (standing with the legs bent) than birds in the 55 and 90 cm crates. More stepping, turning and preening was performed in the 55 and 90 cm compared to the 40 cm crates, whereas more rising attempts were made in the 40 cm crates. Crate height had no effect on the activity of ASAT or CK activity or H:L ratio. There was a significant effect of crate height on the lactate with birds in the 55 cm crates having significantly lower lactate concentrations than birds in 40 cm crates, but there was no significant difference in lactate concentration between 55 and 90 cm or between the 40 and 90 cm crates. This may indicate that there was a difference between treatments on the anaerobic activity, although the effect of sampling procedure cannot be completely excluded.

Thus the degree of confinement in the crates had little influence on the physiological measures taken, although there was a large effect on the birds' behaviour. The 40 cm crates decreased the birds possibility to move and change their positions, whereas the 55 cm crates allowed the birds to stand up and move around almost as much as if kept in free height, even if they were not able to stretch their necks while standing.

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1. Introduction

Transporting animals are always a potentially stressful event. Among other stressors the space available is greatly restricted. The design of transport crates for poultry has mainly been based on practical and economical incentives but there is little knowledge of how the height of these crates affects the birds' welfare (European Food Safety Authority, 2004). According to the European convention for the protection of animals during transport (2004) "sufficient height which is appropriate for the size and intended journey should be provided when transporting animals". A common height of commercial crates is around 35 cm (Prescott et al., 2000) and for most poultry this height does not allow the birds to stand in an upright position which leads to the question if these crates can be considered to provide sufficient height.

Most turkeys are in transit for less than 5 h though transit times over 10 h have been reported (Warris and Brown, 1996). The common view is that poultry will lie down during transport (Broom and Fraser, 2007), so it is possible that they do not need more space than that which allows them to lie comfortably. However, since we do not know how it affects the birds to be forced to lie down for a prolonged period it can be speculated that it might be beneficial for the birds to have a higher crate, with possibility to stand up, when the crate will be kept stationary, for example during lairage. After the transport of broilers a correlation between breast bruises and transport time was found (Carlyle et al., 1997). The authors suggested that this was due to restriction to sternal recumbency in transport containers for prolonged periods. In addition, birds restrained in a small crate might experience psychological stress. In one study cockerels which were restrained for 10 min had increased concentrations of plasma corticosterone (Heiblum et al., 2000). Investigations of long-term housing of laying hens show that crated hens have a strong preference for cages which have a large vertical space allowance (Dawkins, 1985) and more hens worked to get access to a higher cage than a lower cage (Albentosa and Cooper, 2005).

The aim of the study was to investigate the effects of short-term restriction in crates of three different heights on the welfare and behaviour of male turkeys. The lowest height used was 40 cm, which is a commercially used height in the Finnish turkey industry and similar to commercial systems used in other countries. The height of 55 cm was chosen as a commercially feasible alternative where birds are provided with some additional space which might enable the birds to stand to some extent (but not to raise their heads while standing). As a control height, 90 cm was chosen since here the birds had free height and could stand up with their necks stretched. To single out the effect of the crate height from other transportation variables affecting the birds welfare, the animals were tested singly in stationary crates under controlled conditions. We estimated the birds response to the confinement by observing their activity, body postures and comfort behaviour in the crate, and analysed physiological variables of stress and muscle strain with blood-samples.

2. Materials and methods

2.1. Animals and housing

Forty-six white male turkeys of the Nicolas 300 strain were bought from a commercial farm and brought to the experimental facilities at 17 weeks of age. The mean weight of the birds at the farm was around 14 kg and birds weighing between 13.5 and 14.5 kg were selected for the experiment. During the transport from the farm to the experimental facilities, which took 3 h, the birds were kept loose on wood-shavings in two groups.

On arrival all turkeys were put together in a 29-square-meter-pen. The pen floor was covered with wood-shavings and cleaned regularly. The birds had *ad libitum* access to commercial turkey feed and water. Lights were on from 7:00 until 23:00 and the light intensity in the pen varied between 3 and 0.5 lux depending on the distance to the light sources. Temperature was kept between 17 and 19 °C. Birds were individually marked with a numbered leg ring.

All the birds were weighed on the day after arrival and the birds that were to be confined in the crates the following day were weighed the evening before. The study was approved by the Finnish animal research ethics committee.

2.2. Experimental crates

Each crate was 0.70 m × 0.52 m and heights were set at: 40, 55 or 90 cm. All crates had an adjustable roof, and the height for each crate was rotated between test days. The crates were placed in a row along a wall opposite to the turkeys home pen to allow visual contact to the main flock. In addition, the crated turkeys had limited visibility of the turkey in the adjacent crates. The front side of the crates facing towards the room were made of a 2 mm plastic-covered wire-grid (45 cm²), and could be opened on one side for the turkeys to easily walk in and out of the crates. The roof of each crate was of the same type of grid as the front, whereas the other three sides were made of a thicker 3 mm plastic grid (12 cm²). The experimental crates were rebuilt commercial crates so the floor was the same as in these, made of hard plastic and perforated by 9 mm diameter holes. The light intensity where the crates were placed was 0.5 lux and the temperature was maintained between 17 and 19 °C.

2.3. Confinement in the crates

Confinement in the crates began 10 days after arrival at the facility when the birds were 18 weeks old. The experimental period lasted for 2 weeks using 36 birds which were randomly allocated to six groups with six birds in each group. One group was confined in the crates per day for 6 h with one bird in each crate. In order to investigate effect of body weight each of the groups was confined twice to the crates and the two confinement occasions were carried out between 5 and 8 days apart. Six of the remaining birds were used as controls and four were kept as reserve birds.

The six birds, which were to be confined in the crates the following day, were separated from the main flock in the evening and kept in a 6.5 m² section of their original

Table 1
Description of the different behaviours observed of the turkeys while in the crates.

Behaviour	Description
Body postures	
Lying	Lying with the body in contact with the floor
Sitting	Sternum above the floor with at least one hock on the floor
Low standing	Sternum above the floor, legs above the floor but not fully straightened and the frontal part of the body (breast) is positioned lower than the rear part of the body
Standing	Sternum above the floor in a horizontal position or with the front part of the body being in a higher position than the rear part. Legs are straight or just a little bit bent
Other behaviours	
Preening	Beak moved in contact with the plumage
Stretching	One leg and often also one wing is lifted out or/and in a rearwards direction from the bird while it is standing
Stepping	Lifts one leg
Turning	Direction of body changes with at least 45° (includes stepping even if this is not recorded as such when turning is recorded)
Rising attempt	Bird move from the lying position to either a standing, sitting or low standing position

home pen overnight. This temporary enclosure was located in close proximity to the experimental crates (Fig. 1) and the reason for separating the birds the evening before was to minimize handling prior to testing. Confinement in the crates began at 8:00 h. The first time the birds were confined in a crate they were randomly selected for that particular crate and height. Two birds were allocated to 40 cm, two to 55 cm and two to 90 cm. The second time the birds were put in the crates the group consisted of the same individual birds as were used the first time of confinement. However, the treatment was semi-randomized to prevent the same individual bird being assigned the same crate height twice, i.e. if a bird had been in 40 cm the previous time it was now confined in either 55 or 90 cm. The birds were gently coaxed to walk into their crates. When inside the crate, the roof was placed to the allocated height. Video recording started as soon as all the birds were in their crates, which was approximately 10 min after the first bird entered its crate.

To control for the effect of increasing weight/age and prior experience of confinement in a crate, the six control birds were exposed only once to a 90 cm crate in the middle of the second confinement period and their behaviour was compared with the birds which spent their second confinement in a 90 cm crate.

2.4. Behaviour in crates

Birds were filmed from the front of the crates with a time lapse function and three infrared light cameras (TS

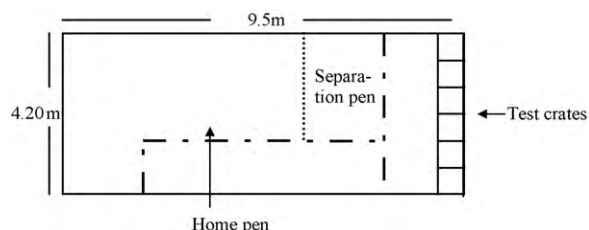


Fig. 1. Layout of the facilities. The thick dashed line indicates see-through metal bar barriers and the light dotted lines the temporary enclosure (separation pen) where the next group to be confined in the crates were kept overnight. When birds were inside the crates no birds had access to the separation pen.

6030PSC IR). The cameras were connected to a VCR (Panasonic VHS AG-6040) via a multiplexer (Robot MV16p). From the video recordings continuous behavioural observations of the whole observation period were carried out using the Observer 5.0 software (Noldus Information Technology B.V., Wageningen, The Netherlands). The different body postures (Table 1) were recorded as states and their total duration were determined over the 6-h confinement. Other behaviours (Table 1) were recorded as events, i.e. the total frequency that each of the behaviours occurred.

2.5. Physiological measures

After the confinement inside the crate, a blood sample was taken from half of the groups after the first confinement period and the other groups were blood sampled after the second confinement. The birds were moved out of the crate one at a time in the same order as they had been put inside the crate. The bird's head and most of its body were covered by a light linen blanket and the bird was held on its side. Blood-samples were taken from the wing vein using a 0.8 mm needle and an EDTA coated 3 mL vacutainer. After the blood sample was taken the bird was released and allowed to walk back to the home pen.

From the blood sample the heterophil to lymphocyte ratio (H:L), lactate concentration, creatine kinase (CK) activity and aspartate aminotransferase (ASAT) activity was measured.

Blood smears of the whole blood were made out within half an hour and air dried within an hour. Later the smears were fixed in methanol (100%) for 15 min, and stained with concentrated May-Grünwald solution for 5 min, then with Giemsa for 10 min, and rinsed in buffered water twice for 1.5 min. To determine differential leukocyte counts a minimum of 200 cells per smear were examined under a light microscope. H:L ratios were calculated by dividing the percentage of heterophils by the percentage of lymphocytes. Total leukocyte counts were carried out by diluting and staining the blood in a Natt and Herrick (1952) solution for more than 3 min but less than 1 h. Then the numbers of heterophils were counted in a Bürker counting chamber. All blood counts were carried out by the same person.

Table 2

Mean (\pm SE) percentage of time (*standing, sitting, low standing and lying*) and number of times (*rising attempts, stepping, turning, stretching and preening*) during the 6 h the birds performed these behaviours during (a), the first confinement period and (b), the second confinement period. The *P*-values of the comparison between treatments (Mann–Whitney test) is also presented. If $P > 0.10$ it is displayed as ns (non-significant).

Behaviour	Mean \pm SE			P-value		
	40 cm N = 12	55 cm N = 12	90 cm N = 12	40 cm vs. 55 cm	40 cm vs. 90 cm	55 cm vs. 90 cm
(a)						
Standing	0	45.8 \pm 6.1	45.8 \pm 7.4	0.000	0.000	ns
Sitting	2.2 \pm 1.5	0.2 \pm 0.1	0.6 \pm 0.6	ns	ns	ns
Low standing	0.7 \pm 0.2	0.01 \pm 0.01	0.16 \pm 0.15	0.000	0.000	ns
Lying	97.1 \pm 1.7	54.0 \pm 6.1	53.4 \pm 7.5	0.000	0.000	ns
Rising attempts	15.2 \pm 2.1	9.6 \pm 1.6	9.7 \pm 1.3	0.078	0.068	ns
Stepping	1.3 \pm 0.4	47.7 \pm 10.1	61.5 \pm 10.3	0.000	0.000	ns
Turning	2.2 \pm 0.7	49.9 \pm 23.3	72.6 \pm 24	0.000	0.000	ns
Stretching	0	0.6 \pm 0.2	3.3 \pm 0.9	0.089	0.001	0.014
Preening	5.4 \pm 1.4	18.6 \pm 2.1	30.3 \pm 4.9	0.000	0.000	0.068
(b)						
Standing	0	25.1 \pm 4.5	38.6 \pm 9.2	0.000	0.000	ns
Sitting	1.0 \pm 0.4	2.0 \pm 0.9	0.1 \pm 0.03	ns	0.001	0.033
Low standing	0.7 \pm 0.4	0.5 \pm 0.5	0.03 \pm 0.02	0.010	0.000	ns
Lying	98.3 \pm 0.8	72.5 \pm 4.6	61.3 \pm 9.2	0.000	0.000	ns
Rising attempts	15.3 \pm 1.9	9.5 \pm 0.8	8.0 \pm 1.3	0.012	0.002	ns
Stepping	0.08 \pm 0.08	29.5 \pm 4.8	58.0 \pm 20.0	0.000	0.000	ns
Turning	1.3 \pm 0.5	14.6 \pm 4.0	47.3 \pm 35.1	0.001	0.000	ns
Stretching	0	0.4 \pm 0.3	1.1 \pm 0.5	ns	0.089	ns
Preening	4.8 \pm 1.7	12.3 \pm 2.0	22.6 \pm 3.9	0.008	0.000	0.052

Plasma was obtained by centrifuging the blood at 2100 \times g for 10 min. Assays for CK and ASAT were carried out on the fresh plasma sample 1 h after the blood-sampling with an automatic serum chemical analyser (Konelab 30i Clinical Chemical Analyzer, ThermoFisher Scientific, Vantaa, Finland) and standardized IFCC methods.

The remaining plasma was stored at -20°C and later used for lactate analyses. Plasma lactate was analysed using a lactate analyser (YSI 2300 STAT Plus, YSI Inc., Yellow Springs, OH, USA).

2.6. Statistical analyses

Comparisons of the behaviour in the crates between heights were analysed with Mann–Whitney tests separately for the two confinement periods.

The Mann–Whitney test was also used for comparing the behaviour between the two confinement periods and between the control birds and birds exposed to a 90 cm crate during their second confinement.

A Kruskal–Wallis test with crate height as a factor was used to analyse H:L ratio, CK, ASAT and lactate. Both confinement periods were included in the same analysis since each bird had only been blood sampled after one of the confinement periods. In the event of a significant effect of treatment the Mann–Whitney test was used for pairwise comparisons. Correlations between CK, ASAT, lactate and H:L ratio and the weight of the bird were analysed using the Spearman correlation. Spearman correlation was also used to analyse for correlation between CK and ASAT activities.

Data were analysed using the SPSS 15.0 for windows (SPSS Inc., Chicago, USA).

3. Results

3.1. Effect of crate height on behaviour

Overall the turkeys spent 73% of the time in a lying position. There were large differences between the behaviour of the birds in the 40 cm crates compared to birds in the 55 and 90 cm crates which behaved more similar (see Table 2a for results of the first confinement period and Table 2b for the second confinement period).

3.2. Comparison of behaviour between first and second confinement period

The birds in the 55 and 90 cm crates spent more time *standing* in the first than in the second confinement period (45.8 \pm 4.7% vs. 31.8 \pm 5.2%; Mann–Whitney test; $P = 0.013$). More *turning* was performed by birds of all treatments in the first compared to the second confinement (41.6 \pm 11.9 vs. 21.0 \pm 11.9; $P = 0.03$). There was no significant difference in the amount of any of the other behaviours between the two confinement periods ($P > 0.1$ for all).

There were no significant differences ($P > 0.1$ for all) in any of the recorded behaviours or in *body weight* between the control birds and the twice-confined birds crated in the 90 cm crates during the second confinement period.

3.3. Physiological measures

Mean (\pm SE) CK activity was 25450 \pm 1733 IU/L, ASAT 625 \pm 24 IU/L, lactate 3.6 \pm 0.29 mmol/L and H:L ratio 0.98 \pm 0.09. Crate height did not influence the level of CK (Kruskal–Wallis test; $P = 0.86$), but there was a significant correlation between bodyweight and CK activity

($R_s = 0.54$, $P = 0.001$, $N = 36$) with increasing CK activity with increasing bodyweight. There was a significant effect of crate height on lactate concentration ($P = 0.007$; 40 cm (4.3 ± 0.7), 55 cm (2.7 ± 0.1) and 90 cm (3.6 ± 0.4)). Post hoc analysis revealed that birds in the 55 cm crates had significantly lower lactate concentrations than birds in the 40 cm crates ($P = 0.001$). Lactate concentration also tended to differ between the 55 cm and 90 cm crates ($P = 0.053$) with higher lactate concentration in the 90 cm crates. However, there was no difference between the 40 and 90 cm treatments ($P = 0.49$). There was no effect of treatment on ASAT ($P = 0.68$) and H:L ratio ($P = 0.62$). A positive correlation was found between CK and ASAT values ($R_s = 0.77$, $P < 0.001$, $N = 36$) and there was a tendency for a positive correlation between body weight and ASAT ($R_s = 0.30$, $P = 0.079$, $N = 36$). There was a negative correlation between H:L ratio and body weight ($R_s = -0.39$, $P = 0.02$, $N = 36$). No correlation was found between lactate concentration and body weight ($R_s = 0.02$, $P = 0.91$, $N = 36$).

4. Discussion

The male turkeys were not able to stand up in the 40 cm high stationary crates which led to reduced turning, stepping and preening, and an increased number of rising attempts. Thus, confinement in the 40 cm crates may have a negative impact on the birds welfare. As the birds gained weight, they were less active in the 55 and 90 cm crates and heavier birds also had higher creatine kinase (CK) activity, indicating muscle cell damage.

The low standing position, defined here as birds standing with hocks above the floor but knees bent, has been suggested by the Finnish animal welfare authorities (MMM, 2006) to be an alternative behaviour for normal standing for birds. However, our results suggest that low standing and sitting are not substitutes for standing. Low standing is rather a part of the standing sequence as it was only observed in birds in 55 and 90 cm crates when they were about to stand up or lie down completely. In addition the birds in the 40 cm crates attempted to rise more often than birds in the higher crates suggesting that they had a motivation to stand up more. Compared with the birds in the 40 cm crates, fewer differences in behaviour between the 55 and 90 cm crates were found. This is probably due to the birds being able to stand up in the 55 cm crates even if there was not enough space to have their heads and necks in a fully upright position.

Preening and stretching are often categorized as comfort behaviours. Since the amount of preening decreased with decreasing crate height and no stretching was observed in the 40 cm crates this might indicate a reduced welfare condition for these birds. However, as there was such a lack of space for the birds to move in the 40 cm crates it is impossible to say whether the decrease was due to the birds being mainly psychologically or physically inhibited from preening. Stretching has been observed to decrease with decreased crate height in laying hens (Dawkins, 1985; Nicol, 1987), although, the birds in those studies were not as physically restricted as our birds, in relative terms.

The second confinement period when the birds had increased in weight they stood less in the 55 and 90 cm

crates and all birds turned less. These differences in behaviour between the two confinement periods could be due to the birds having increased in age/weight or, when being confined a second time, they were affected by their previous experience. However, there was no difference in behaviour between the control birds confined only once at the heavier weight in the 90 cm crates and the other birds confined a second time in a 90 cm crate. This implies that being confined in the crates more than once had smaller impact on the birds behaviour than increased age or weight. The decrease in activity and preening together with the increase in CK imply a negative effect of confinement and increased weight.

CK activity was quite high in all birds. High activity of CK in the serum indicates muscle damage as the enzyme leaks out from the damaged muscle tissue (Broom, 2000). There was also a positive correlation between CK and ASAT activities. ASAT is a marker of soft tissue damage and thus also of muscle damage. Whether high CK activities are also indicative of an experience of pain in birds is unknown, but is a question of concern for welfare. In humans exercise-induced skeletal muscle damage lead to both reported muscle soreness and of a measurable increase in CK (Skurvydas et al., 2006).

We found the highest lactate concentration in the turkeys confined in the 40 cm crates which might have been due to these birds not being able to stand up for 6 h. However, the lactate concentration in birds from the 40 cm crates was not significantly higher than for the birds in the 90 cm crates which make the results difficult to interpret. One reason for the similar levels in birds from the 90 cm crates but not from 55 cm crates might be due to these birds being slightly more active in the crates. For example lactate concentration in transported horses increased with increased transport time (Stull, 1999). In contrast to this the lactate concentration decreased in pheasants in connection with transport (Suchy et al., 2007). In our study the mean lactate concentration, after 6-h crate confinement was near the 4 mmol/L limit, often referred to as the anaerobic threshold (Stegmann and Kindermann, 1982). In penguins 1.7 mmol/L (Ponganis et al., 1997) and in geese 2.0–2.6 mmol/L (Le Maho et al., 1981) concentrations were measured and regarded as baseline concentrations, whereas in broilers concentrations as high as 7.6 mmol/L have been reported (Bedanova et al., 2007).

It has been suggested that H:L ratios of 0.8 characterize high level of stress in broilers (Gross and Siegel, 1983). The mean heterophil:lymphocyte ratio in our study was 0.98 ± 0.09 , which may thus be interpreted as the turkeys were stressed. Whether the fairly high H:L ratio found in our birds was due to the stress of the experimental procedure or their general condition is impossible to say, particularly as the height of the crates had no effect.

5. Conclusion

The 55 cm crates allowed the birds to stand and resulted in the birds having a similar behaviour pattern to birds with free height, even if the birds could not stand up with stretched necks. In the 40 cm crates the birds ability to move was greatly restricted. However, physiological

measurements used in this study showed only minor differences. The results indicate a lower welfare level for birds confined in stationary 40 cm crates than those in 55 or 90 cm crates which calls for further studies of the effect of crate height on turkey welfare in a real transport situation.

Acknowledgements

The authors thank The Finnish Ministry of Agriculture and Forestry for funding the project. We also thank Länsi-Kalkkuna Company for funding and support with the animals. We are grateful to Satu Sankari for her advice with the blood sample analyses and to Niina Saarikangas and Virve Korhonen for help with the behaviour observations.

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