Satiety splits drinking behavior into bouts: the organization of drinking in birds[1]

J. Rusakovica,*[2] T. Plötz,† V. Kremer,‡ P. Glover,‡ I. Kyriazakis*

*School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, Tyne and Wear, NE1 7RU, UK;

†Open Lab, School of Computing Science, Newcastle University;

‡Aviagen Turkeys Ltd, Tattenhall, Cheshire, CH3 9GA, UK;

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[2] Corresponding author: julija.rusakovica@ncl.ac.uk

ABSTRACT: The regulation of the drinking behavior of animals is usually overlooked and the traits associated with it are not well-defined. We used a unique data set to develop ideas about the analysis and regulation of drinking behavior in birds. The data were generated by a custom-made equipment that measures automatically the individual drinking behavior of a large number of turkeys from different genetic lines. We hypothesized that there is a biologically significant unit by which drinking behavior can be expressed and understood. We developed a novel method, based on mixture distribution models, to allow clustering of drinking events and splitting behavior into bouts. Drinking behavior was found to be predicated on the same principles of satiety that underlie feeding behavior. Within bouts, drinking was interrupted by short non-drinking intervals, whereas bouts were separated by long non-drinking intervals, indicative of bird physiological need. Based on this methodology, a number of drinking behavior traits were identified that revealed differences in the organization of drinking behavior between the turkey genetic lines. Similarly, time accumulation patterns of drinking behavior traits within a day differed within and between genetic lines, suggesting that variation in drinking behavior exists and birds use different behavioral strategies to meet their water intake requirements. However, evolution of drinking behavior traits over time was similar between the lines, suggesting conservation of this behavioral organization. As well as providing ideas about the regulation of drinking behavior, the developed behavioral traits may be of practical relevance, as water utilisation, along with feed efficiency, is part of overall biological efficiency. The methodology should be applicable for the definition of drinking traits in other livestock species, and be used for the identification of deviations from 'normal' drinking behavior.

Key words: drinking behavior, modelling behavior, satiety, turkey, water intake.

INTRODUCTION

Water intake serves several physiological functions, and for some animals drinking may also fulfil behavioral needs. There are also instances where water consumption exceeds physiological needs (McKinley et. al., 2004). Animals of several species do not drink continuously in time, but do so in bouts. Within such bouts actual drinking may be interrupted by non-drinking intervals. The question is whether this behavior of drinking in bouts is underlined by any physiological principles, or occurs randomly in time. There are several advantages in understanding the basis of 'normal' drinking behaviors in animals. Feeding behavior, for example, is understood on the basis of the physiological state of satiety. Deviations from the 'normal' patterns of feeding behavior may then be indicative of health and welfare problems. However, it has been suggested that the same principle of satiety (substituted by the term of thirst) cannot be applied or understood in animals, presumably because of both the physiological and non-physiological functions of drinking behaviour (Rolls and Rolls, 1991). The above arguments have not been helped by the lack of equipment able to record continuously drinking of animals in a social context. As a consequence, the methodology of measuring and analysing drinking behavior in animals has received significantly little attention. In this paper we exploit a novel system to measure individual drinking behavior in birds, kept in commercial groups. Our hypothesis is that their drinking behavior will be predicated on the same principles of satiety that underline feeding behavior. Our expectation is that, within bouts, actual drinking may be interrupted by short non-drinking intervals, while bouts will be separated by long non-drinking intervals indicative of bird physiological need. We subsequently develop traits related to drinking behavior and its regulation, and investigate how these traits may be affected by factors such as bird genotype and age.

MATERIALS AND METHODS

Ethical note

The data used in this study was derived from animals in the primary turkey pedigree breeding programme of Aviagen Turkeys Ltd. Our study was mainly observational and used data routinely recorded on the pedigree farm; birds were individually identified with RFID (Radio-Frequency Identification) tags. Individual identification is the basis of genetic selection on pedigree farms. We have previously shown that these RFID do not cause any adverse effects on behaviour (Howie et. al., 2009a). The water intake recording equipment and the cameras, when used, were installed before the arrival of the turkeys, so no disturbance was caused. A small number of birds were handled to identify individually through video observations. The handling of the birds was done by professional staff to minimise disturbance and subsequent observations suggested that this handling and marking had no effect on their behavior. The latter procedure was approved by the Newcastle University Animal Welfare Ethics Review Board.

Birds, Housing and Water Intake recording equipment

Records of visits to electronic drinkers were obtained for three turkey breeding lines: (1) line A (n = 954 777 events) from 4627 turkeys from 6-9 weeks of age, (2) line B (n = 770 984 events) from 2351 turkeys from 10-13 weeks of age and (3) line C (n = 146 170 events) from 291 turkeys from 10-13 weeks of age (Table 1).

Birds were male turkeys. Birds from line A were from a paternal line, selected with an emphasis on feed efficiency, breast meat yield and growth, whereas lines B and C represented maternal lines, with an emphasis on reproductive performance and feed efficiency. All three lines were selected for leg health and fitness traits. They were part of routine genetic evaluation at Aviagen Turkeys. Testing of lines B and C at the same age allowed us to test whether the drinking behavior of birds differed between genetic lines.

Birds from each genetic line were routinely hatched every week. Different hatches were placed, grown and reared in different sheds. Testing for water intake took place in pens equipped with water stations. The test pen for a double station measured 14.8m x 6.1m, which corresponds to a maximum of 52 kg/m2 and 2.5 birds/m2 at the end of rearing, for the heaviest line. The pen was equipped with conventional group feeders hanging on feeding lines distributed throughout the shed, and 16 electronic drinkers in double stations (8 drinkers in single stations), placed as a line on one side of the pen. Prime quality wood shavings were used as litter. This resulted in ≈ 19 birds per drinker with mean drinker occupancy (i.e. percentage of time during which a bird was using a particular drinker) during the experiment of 19.9%, 13.6% and 14.8% for lines A, B and C, respectively. This allowed birds to drink without competition.

Birds were placed in the experimental shed one week before the recording started to allow them to adapt to the drinking system. During this period lighting and temperature were maintained in line with commercial husbandry practises, i.e., 14 hours of light at minimum 30 lux and 10 hours of dark and 19-23^{o}C. Birds were fed a standard turkey grower diet. An in-house developed electronic drinking system using transponder-based data capture was used to record bird individual drinking behavior. Access to each drinker was regulated by a set of transparent plastic dividers, which were adjusted to bird size as birds grew to ensure that only one bird could use the drinker at a time. Each of the drinkers contained a water bowl connected to weighing scales. Each bird was fitted on their lower leg with a small passive RFID transponder, bearing a unique identification code which was recorded when a bird entered a drinker. The RFID transponders enabled a drinking event (visit) to be ascribed to a specific bird. Extensive bird observations suggested that the leg transponder did not affect bird normal behaviour (Howie et. al., 2010). A visit started when the system detected bird presence in the drinker and finished when the bird left the drinker. Each visit was recorded only when water consumption occurred. The automated system recorded start and end time of each visit, visit duration, water intake per visit and bird identification (ID). Start and stop times were recorded to the nearest second, and water consumed was recorded to the nearest ml. Also recorded for each visit were the date and the identification codes for the hatch, pen and drinker. Intervals between visits to the water station were estimated as intervals between subsequent visits by the same bird.

Video Observations

Video observations were set up to (1) validate data recorded by the electronic drinkers, and (2) record feeding events associated with drinking episodes to determine any associations between feed and water intake. Four colour CCTV cameras (Hikvision DS-2CD2132-I) were positioned approximately three meters above the pen area, capturing both electronic drinkers and feeders described above. The clocks on the cameras and the computer collecting data from the electronic drinkers were synchronized at the start of the experiment. Continuous video recording was conducted between 06:00 - 20:00 when lights were on, during which times the majority of all visits occurred (91%, 89% and 98% for lines A, B and C, respectively). Ten birds were randomly selected and colour-marked with black spray in different shapes in order to make them individually identifiable on the video. The other birds in the pen were sprayed with food colouring using non-specific patterns, to avoid too much attention from the rest of the group on the birds of interest.

The video analysis was conducted on five days of continuous video recordings. Both feeding and drinking events were measured by frequency and duration. For each visit to the drinker or feeder, the observer recorded bird ID and start and stop times of each visit. The drinking visit was defined as "bird standing on the RFID tag reader (antenna pad) and ingesting water". The start of a visit was when the bird stood with both feet on the tag reader or ingested water. The end of the visit was when the bird left the drinker. Subsequently, visits recorded by the automated system were compared with measures from the video analysis and recorded feeding events were associated with the corresponding interval length between visits to the water station. These data were used to estimate reliability of the electronic water station system by calculating predictability (likelihood that a bird detected by the electronic water station is detected as present at the drinker by video observations) and sensitivity (likelihood that a bird present at the drinker is detected present by the electronic water system).

Electronic Data Screening

Data screening involved several steps, including elimination of system errors, outlier detection and data flooring. Any visits that were not correctly recorded by the system were removed from the analysis. Visits were classified as outliers based on the water usage per visit to visit duration ratio; any visits with a ratio above or below two standard deviations from the mean were not included in the analysis. This allowed identifying long visits with low water usage and similarly, short visits with abnormally high water records. As there were occasions when birds remained in the drinker after drinking activity took place, such as resting or sleeping, data flooring was performed on the remaining data to include only the visits during which birds were drinking water. This involved limiting the maximum length of a visit to the longest visit length observed during the video analysis. In total, this resulted in elimination of < 1% of visits for lines A and C, and < 3% of visits for line B. The processed data set contained: line A – 948 045 visits, line B – 767 950 visits, line C – 144 109 visits.

Bout Analysis

Because of the clear diurnal rhythm that birds showed, it was decided to use data recorded during the hours when lights were on. We wanted to identify drinking bout criteria consistent with our hypothesis for satiety underlying drinking behavior. A drinking bout criterion was defined as the shortest interval between visits to the drinker that was considered to be part of a bout and was estimated by fitting a mixture model (MM) to the natural log-transformed intervals between visits (Celeux, 2007). According to the estimated criterion, intervals between drinking events could be assigned to either within bout intervals or between bout intervals.

Bouts were characterised by duration and frequency, and were defined as time intervals spent in drinking activity. In MMs data records are modelled as separate subpopulations with the overall population being a mixture of the latter, resulting in a model with a finite number of subpopulations. Hence, the first step was to find the number of subpopulations in the data.

During video observation it was identified that bird visits to drinkers with short interval length between visits were the result of system oversensitivity to bird movements inside the drinker, which resulted in some visits being fragmented. To reduce this error, an appropriate merging criterion was necessary. This was selected using a Receiver Operating Characteristic (ROC) curve (Hanley and McNeil, 1982), which was used to estimate and visualise true positive and false positive rates for different merging criteria using data from the electronic system and video observations. In total, 60 merging criteria were tested, ranging from 1 to 60 seconds. For each merging criterion, a number of visually observed intervals between drinking episodes was compared with RFID records. If an interval was detected by both methods, it was considered as true positive, while intervals detected only by the RFID system were considered as false positives. Plotted merged visit data showed a clear bimodal distribution, suggesting an initial number of groups. Based on this finding, various combinations of two and three process models, including Gamma, Exponential, Weibull and Normal, were investigated to determine which of them can give both biologically and statistically good description of the data. It was found that a model comprising a truncated log-normal for within bout intervals and a log-normal distribution for between bout intervals gave consistent results for all genetic lines (Equation 1).

$$pdf = p\left(\frac{1}{\oint\left(\frac{1.39-\mu_1}{\sigma_1}\right)}\right)\left(\frac{1}{\sigma_1\sqrt{2\pi}}\right) exp\left(\frac{-[x-\mu_1]^2}{2\sigma_1^2}\right) + (1-p)\left(\frac{1}{\sigma_2\sqrt{2\pi}}\right) exp\left(\frac{-[x-\mu_2]^2}{2\sigma_2^2}\right) \quad (1)$$

where pdf is a probability density function for a Normal mixture model, μ_1,μ_2 and σ_1 ,σ_2 are means and standard deviation of the truncated log-normal and log-normal distributions, p is the proportion of intervals in the first distribution, $\oint$ is a correction factor for a truncated distribution, and x is natural log-transformed interval length between visits.

The model was fitted for each genetic line and a bout criterion was estimated at the intersection point between the two distributions. Based on the estimated bout criterion, drinking behavior traits were estimated for each line. All estimated drinking behavior traits were tested for statistical significance in statistical software R (R Dev. Core Team, 2014) using a non-parametric Kruskal-Wallis test for the three lines of birds with post-hoc analysis using the package pgirmess as proposed by Siegel & Castellan (1988).

To investigate whether drinking visits were distributed randomly across time or were guided by physiological principles, such as thirst and satiety, the starting probability (Pstart) of a bird starting to drink within 30 minutes versus the time since the last visit (t) was calculated for each line. This criterion was chosen to reduce variation in estimated probability, which happened at smaller values. The probabilities were calculated from the data in the following way: number of intervals $> t$ and $\leq t+30$ minutes divided by the number of intervals $\geq t$ minutes. In addition, an empirical cumulative probability distribution function (ecdf) was computed as another method that can be used to compare probabilities of starting the next visit to a drinker within n seconds or less from the last visit. Opposite to the previous approach, this method calculates cumulative probability and can be used to indicate which intervals between drinking episodes are most likely to occur.

Patterns of Drinking Accumulation Time

We tested whether birds accumulate their drinking activity throughout the day by having time-related traits with same length intervals or a combination of different length intervals. We would expect birds to have variation in the interval length, however the extent of this variation is uncertain both within and between the lines. To test for this expectation we used Lorenz curves (Gastwirth, 1972), which were obtained by plotting the cumulative contribution of the different interval lengths to the total time for four time-related drinking behavior traits: 1) distribution of intervals between visits, 2) bout duration, 3) drinking time within a bout and 4) non-drinking time within a bout. The approach was used to compare patterns of time accumulation between the three genetic lines, which was done by comparing time intervals from the shortest to the longest for each trait expressed in percentages from the overall time length. The advantage of the method is that Lorenz curves can be expressed as a single parameter called the GINI (G) index, which is a standard statistic for comparing patterns of accumulation using this approach. A G index ranges from 0 to 1, where G = 1 would indicate that distribution of time intervals is highly unequal in length, with large differences between shortest and longest intervals for a given trait and relatively high proportion of short intervals contributing to the total time. G = 0 would indicate that all intervals have similar length and contribute equally to the total time. The higher the G index, the larger the inequality in the distribution of interval length would be. G indices were estimated for each bird within each line for the three genetic lines and tested using Kruskal-Wallis test to estimate variation in the accumulation patterns between the lines with post-hoc analysis using the package pgirmess as proposed by Siegel & Castellan (1988).

Evolution of Drinking Behavior

Estimated drinking behavior traits were used to investigate the evolution of drinking behavior during the experiment and the effect of genetic line on bird drinking behavior. One of the objectives was to determine whether birds from different lines have similar underlying structural changes to drinking behavior over time. Principal Component Analysis (PCA) (Jolliffe, 2002) was used to examine inter-relationships of drinking traits within and between genetic lines using R software (R Dev. Core Team, 2014). Separate PCA was performed on overall means of the experiment for each line using the correlation matrix of sample data of the seven drinking behavior traits defined above: five daily traits: 1) bout frequency, 2) bout duration, 3) drinking time, 4) non-drinking time, 5) water usage, and two traits estimated per bout: 6) drinking time per bout, 7) water usage per bout. Principal components that accounted for at least 95% of variability were kept in the analysis. To facilitate comparison of changes in drinking behavior between days of the experiment, estimated principal component loadings from the overall means were multiplied by standardised data computed for each day of the experiment in order to convert daily scores to the same scale. As the length of the experiment differed for some hatches within genetic lines, the number of days for which the analysis was performed was limited to the shortest duration for a hatch within each line (Table 1). First and last days of the experiment were excluded from the analysis, as they did not contain data for the full days.

To determine changes in behavior over time, Pearson correlation coefficients were estimated between daily component scores for each component for each line relative to the start of the experiment. To visualise data, correlations computed between daily component scores were added together for each day and compared between days for each line using the mixed-effect model (McCulloch and Neuhaus, 2001), with observation day as a fixed effect and animal as a random effect. Additionally, drinking behavior traits were estimated for each day of the experiment for each line. All comparisons between the genetic lines were made using the linear mixed-effect model, with observation day and genetic line as fixed effect, and animal as a random effect. Subsequently, Tukey post hoc test was used for pairwise comparison of genetic lines.

RESULTS

Video Observations

During the five days of video observations the electronic system recorded 50% more visits to the water station compared to the manual observations. However, 47.4% of the visits recorded by the automated system occurred within the visit time detected by direct video observations, implying that either the video observations did not distinguish visits with small interval length between visits or more likely the electronic system was giving false breaks within visits. Closer inspection of these intervals revealed that they occurred when a bird was inside the drinker and were the result of bird movement, which included leg, head and body movements while being present inside the drinker. Because of this, birds showed non-uniformity concerning the definition of a drinking visit, as for some birds the system detected each water sip as a separate visit, resulting in a visit being fragmented into many short visits with small between visit intervals, while other birds were more consistent in their drinking behavior. This resulted in different distributions of visit durations and interval length between visits for the electronic and observed data (Figure 1 a, b).

Based on data from the video validation, it was decided to combine such intervals together to reduce the error in the analysis. Figure 2 shows the ROC curve with true positive and false positive rates for each tested merging criterion. The merging criterion was chosen at three seconds as the false positive rate at this criterion was zero. When this criterion was applied to the video data set, it resulted in a 58.4% merging rate and a significant reduction of non-uniformity in the data set.

The overall reliability of the electronic system was estimated from video observations and resulted in a predictability of 98.8% and sensitivity of 98.6%. In general, the system did not correctly record visits due to bird-drinker interactions: this included situations when the ID tag was not placed correctly on the tag reader, while the bird being inside the drinker, or when two birds were in the drinker at the same time, resulting in two IDs being ascribed to a single visit.

Bout Analysis

Table 2 shows daily recorded measures made by the electronic drinkers and the estimated water usage rate. All estimates were statistically different between the lines ($p < 0.01$), apart from the daily water usage between lines A and B and mean visit duration between lines B and C (p >

0.05). Figure 3 shows histograms of the interval length between visits on a logarithmic scale for hours of the day when light was present with the fitted MM for each genetic line. A two-process model gave statistically consistent estimates between the lines. All three genetic lines demonstrated two distinct modes in data distribution with different proportions of interval length between the modes. The fitted probability MM contained one truncated log-normal distribution at four seconds for within bout intervals, as shorter intervals were considered to be the result of bird movement inside the electronic drinker. The second distribution was log-normal for between bout intervals.

Drinking behavior traits were estimated using a bout criterion (Table 3). The bout criterion was estimated at the intersection point between the two distributions and resulted in 665 s for line A, 672 s for line B and 602 s for line C. Number of visits per bout varied between 1.11 and 1.70, for lines A and C respectively. Water usage per bout, drinking time per bout and number of visits per bout were significantly different between the three lines ($p < 0.01$), while bout duration was not significantly different between lines A and C, and non-drinking time per bout and bout frequency were not significant between lines B and C ($p > 0.05$). According to the video observations, 99% of feeding events occurred between rather than within drinking bouts. The calculated Pstart of a next visit to the drinking station within 30 minutes after the last visit shows that birds from line A initiated their next visit after a shorter period of time, compared to the two other lines, as their probability curve grew faster than for two other lines (Figure 4 a). All three lines demonstrated an initial rapid decrease in Pstart up to approximately 10 minutes, as these intervals were associated with drinking occurring within a bout. Afterwards, Pstart started to increase over time reaching a marked peak after 1 - 2 hours since the last visit for birds from line A. For lines B and C the peak was not clearly defined, suggesting that birds from these two lines showed more variation in drinking behavior. Cumulative distribution of the interval length between visits (ecdf) showed that birds from line C had a much higher probability of starting the next visit shortly after the previous visit (Figure 4 b). However the probability curves for all three lines converged at around 3 hours, when the majority of visits occurred, constituting 95.7%, 93.5% and 96.8% of the total number of visits for lines A, B and C, respectively. This indicates both that birds usually do not spend more than 3 hours between drinking events and that variation in drinking behavior between the lines is accounted for by short between drinking intervals.

Patterns of Drinking Accumulation Time

Figure 5 presents the Lorenz curves plotted for each genetic line estimated for four drinking behavior traits during the hours of the day when light was present. The percentage of total time spent in drinking visits shows that the distribution of interval length between drinking visits differs between the genetic lines (Figure 5 a). Fifty percent of the shortest intervals between visits contributed 70% to the total between interval time for birds of line A, whereas for line B and line C they contributed 80% and 90%, respectively. Similarly, distribution of non-drinking time is highly unequal in its accumulation pattern, with a G index close to one, implying high prevalence of short non-drinking intervals within a bout (Figure 5 d). In contrast, accumulation patterns of drinking time and bout duration show greater equality in the contribution of interval length to the accumulation pattern (Figure 5 b, c).

Calculated G indices per individual bird for the drinking behavior characteristics are significantly different between the lines ($p < 0.01$), suggesting that there is variation in the distribution of birds with different time accumulation preferences (Table 4). While birds from lines B and C are more similar in the accumulation time over the four characteristics, birds from line A have a more equal distribution across four characteristics, meaning less variation in drinking behavior for this line.

Evolution of Drinking Behavior

The analysis was carried using four principal components, as they accounted for 95% variability in the data. Bird drinking behavior evolved during the experiment; however this change was similar between the lines (Figure 6), as all three lines showed a downward shift in the estimated correlations between the daily scores of the four principle components identified by the PCA. Performing statistical tests on added daily correlation coefficients resulted in significant differences in bird behavior relative to the start of the experiment (line A: $t = -4.242$, $p < 0.01$, line B: $t = -5.357$, $p < 0.001$, line C: $t = -2.592$, $p < 0.05$). Similarly, there was a difference in the absolute correlations between lines A and C ($z = 10.48$, $p < 0.01$), and between B and C ($z = 12.51$, $p < 0.01$) throughout the experiment. However, no significant difference was found between lines A and B ($z = -2.03$, $p > 0.1$).

Distribution of drinking behavior characteristics over the experiment for the three lines showed that bird daily bout frequency, bout duration and drinking time per bout decreased over the experiment, while daily water usage and water usage per bout increased (Figure 7). Comparisons of drinking behavior traits using the mixed-effect model showed that all traits were significantly different between the lines ($p < 0.001$), except for mean bout frequency between lines A and B ($z = -1.784$, $p > 0.1$), mean daily non-drinking time per bout between lines B and C ($z = -1.098$, $p > 0.1$) and mean water usage per bout between lines A and B ($z = -0.818$, $p > 0.1$), A and C ($z = 0.275$, $p > 0.1$), B and C ($z = 1.093$, $p > 0.1$). This suggests that differences in drinking behavior between the lines exist, but these differences are not consistent.

Analysis of inter-relationships between the drinking behavior traits showed that daily bout duration had similar loadings across principal component 1 for each line (Figure 8). Other relationships between drinking behavior traits differed between the lines: lines A and B had a more similar relationship between the traits, compared to line C, with similar loadings for daily bout frequency, daily nondrinking time within a bout, daily drinking time and mean water usage per bout across principal components. All three lines showed opposite loadings for bout frequency and water intake per bout over PC1, implying a negative correlation between these two traits, while bout frequency and daily water intake showed no significant correlation, indicating that birds use different strategies to attain the same amount of water.

DISCUSSION

We utilised a novel system that enables the recording of the drinking behavior of turkeys kept in large groups, to develop a modelling methodology for the analysis and interpretation of their drinking behavior. The system allows us to understand the basis of drinking behavior and its regulation in birds selected for different productive traits. Contrary to the measurement and analysis of feeding behaviour (Kyriazakis and Tolkamp, 2011), the analysis of drinking behavior has received significantly less attention, due in part to past limitations of measuring drinking behavior in a social context. The methodology for the analysis and interpretation of drinking behavior was developed on turkeys, but the ideas advanced should have implications for the drinking behavior in other animal species.

The study was conducted in a commercial setting, which allowed us to have access to large bird numbers from different genetic lines. The employed recording system has advantages over previous approaches used to record water intake (Maselyne et. al., 2015a), as it allowed to detect drinking events of individual birds continuously and on a large-scale in a group-based environment. This allowed us to extend the analysis beyond simple estimates of water intake, and additionally focus on traits closely related to drinking activity. The system measured total water removed from the drinker which would include both water consumed as well as water spilt (Manning et. al., 2007b). As it was not possible to discriminate between the two, irregular water spillage was monitored by farm staff.

Other potential limitations of the system are the actual drinker set up and its position in relation to the feeders. As the water station was designed to record individual drinking behavior and avoid cross-readings, once a bird was inside a drinker, it was separated by side plates from the rest of the flock. In addition, the feeders were located at some distance from the drinkers and this arrangement most likely had an effect on the natural sequence of drinking and feeding events. For example, (Bley and Bessei, 2008) found in a study on individual feeding behavior of group-housed pekin ducks that electronic systems influence the distribution of feeding events in time, as once a bird was inside an electronic feeder/drinker, it was less likely that it would alternate between feeders and drinkers.

A first step of the analysis involved the validation of the system used for recording drinking behavior. This revealed system oversensitivity when registering visits due to bird movement, thus fragmenting some of the visits. We introduced a criterion, based on statistical methodology, according to which visits to drinkers could be classified into fragmented visits. In general, data correction from automated recording systems is relatively common, but the methodology used for this purpose varies between studies due to different settings of the recording systems and is frequently based on arbitrary criteria (Brown-Brandl and Eigenberg, 2011; Casey et. al., 2005; Maselyne et. al., 2015b; Mendes et. al., 2001). Electronic system reliability showed high agreement with data obtained from the visual observations, with high scores for predictability (98.8%) and sensitivity (98.6%). We conclude that the system used in our study is suitable for recording animal drinking behavior. As in previous studies, most of the actual errors in our study occurred due to bird-drinker interactions, such as multiple birds being in one drinker at the same time. It has been shown that bird density influences system sensitivity and accuracy measures. While the number of such incidences in our study was small, a study on drinking behavior of group-housed pigs (Andersen et. al., 2014) showed that increased competition results in higher number of interrupted visits. This implies that in large scale studies conducted in commercial settings animal density should be taken into account to ensure both high performance of the recording equipment and sufficient access to the system for the animal.

Following system validation, four methods were applied to characterise bird drinking behavior. Firstly, a mixture model was used to classify interval length between drinking events into within and between bout intervals. Secondly, conditional and cumulative probability functions were calculated to identify if drinking behavior is random in time or has a physiological basis. Unlike feeding behavior, which has a physiological basis (Howie et. al., 2009b; Tolkamp et. al., 1998), water use may arise from other sources (Howard, 1975; Manning et. al., 2007a). The modelling method allowed identification of novel traits associated with drinking behavior, which could not be calculated without an appropriate bout criterion, and to compare these traits between the different genetic lines. Thirdly, time accumulation patterns were used to characterise bird preferences towards allocating their time to a particular drinking activity. Lastly, evolution of drinking behavior between the genetic lines was examined.

Distribution of interval length between visits showed a well-defined separation in the interval length between visits to drinkers for the three lines, and appeared to follow a similar pattern to the distribution of visits to a feeder observed in birds (Howie et. al., 2009b; Howie et. al., 2010). There was a population of short intervals which was considered to be intervals within a drinking bout and a population of intervals which was considered to represent intervals between drinking bouts. We applied several statistical distributions to model intervals between drinking visits and we investigated which combination of distributions could provide an appropriate description of data. Since we had left-truncated data, we fitted a truncated log-Normal distribution to describe intervals within a drinking bout. For the second distribution, we attempted to fit distributions such as Normal, Gamma and Weibull that have been previously applied to similar types of data (Lundy et. al., 2012; Yeates et. al., 2001). In particular, we were interested in the fit of the Weibull distribution, as it can apply on skewed data and appears to have some properties consistent with the concept of satiety (Yeates et. al., 2001). However, the application of the alternative functions to Normal distribution did not improve the description of the populations of longer intervals. The above mixture model enabled us to identify a bout criterion, which resulted in similar estimates of a bout criterion between the lines, i.e. a truncated normal and normal distributions. We consider this to be an important outcome of our study. Despite having substantial amounts of "free" time birds spent only small proportion of time in drinking behavior, which is in agreement with the concept of satiety.

Feeding was not considered to be within a drinking bout, but according to the analysis, occurred between drinking bouts. This is in contrast with the previous studies on feeding behavior, where drinking was considered to be within a feeding bout (Howie et. al., 2009a; Yeates et. al., 2001), and a bout included both feeding and drinking. In general, this can be explained by the difference in the length between feeding and drinking bout criteria. According to previous studies, feeding bout criteria may be longer than drinking bout criteria (Huzzey et. al., 2005; Tolkamp et. al., 2011), and as feeding in general is more frequent in time, it is more likely that drinking will occur within feeding bouts. However, this could also have been influenced by our system settings, as it took longer for birds to move between drinkers and feeders, thus limiting the probability that drinking would be associated with feeding.

We hypothesised that drinking behavior is predicated on the physiological principles of satiety (Fitzsimons, 1998; McKinley and Johnson, 2004). The separation of visits to the drinkers into two populations enabled us to calculate the conditional probability of initiating a visit immediately after the previous visit (Pstart). Pstart was initially low, but in general increased over time for all turkey lines with gradual decline for longer intervals. As it has been argued previously by Tolkamp and Kyriazakis (1999), physiological regulation predicts that the probability of an animal initiating a behavior of a visit to a feeder or drinker to be low after the completion of a previous one, but will increase over time. If drinking were not to follow physiological regulations, Pstart would have a uniform flat line shape, whereas the determined Pstart of drinking behavior was almost identical to the one for feeding behaviour (Howie et. al., 2009b; R Dev. Core Team, 2014) suggesting that both feeding and drinking behavior have a similar physiological basis, that of satiety.

The above methodology enabled us to identify a number of drinking behavior traits, such as bout frequency, duration of a bout, number of visits per bout, drinking time per bout, non-drinking time per bout and water usage per bout. Importantly, these traits were based on biological principles and are likely be of potential value (Kyriazakis and Tolkamp, 2011), and therefore, of potential interest to turkey breeders. For example, according to the European Food Safety Authority (EFSA), currently there is an increased interest to identify risk factors associated with the drinking behavior traits in poultry that could lead to increased health risks (EFSA, 2010). In particular, traits related to the efficient water use by birds are associated with decreased litter moisture and better gut health (Swalander et. al., 2013), while overall time spent in drinking activity, bout duration and non-drinking time per bout may be indicators of time spent in proximity to drinkers and be associated with the risk of health challenges associated with wet litter, such as food pad dermatitis.

Drinking bouts consisted of a small number of visits (maximum of 1.70 visits for line C). Most of the calculated traits were significantly different between the lines. Birds from line A exhibited different behavior compared to the other two lines, which can partly be accounted for by their younger age. However since we have two differences for line A, their age and genetics, we could not make any reliable conclusions when comparing this line to the other two. Differences in the traits, such as water usage per bout and drinking time per bout between lines B and C suggested that there is a true difference in the drinking behavior between these two lines, which were tested at similar ages. Birds from line B tended to have a higher drinking rate in comparison to birds from line C, which also explains their shorter bout duration. In contrast, non-drinking time per bout was shorter for line A, implying that birds from this line at the age considered do not split their water intake into many visits. Another trait that differed between the lines was the number of visits per bout, which was higher for line C compared to lines A and B. The difference in this trait between the lines was mostly due to higher proportion of short intervals between visits to the same drinker with a length of up to 13 seconds. Bird densities in the pens were similar making it unlikely that these intervals resulted from interrupted visits; the differences may be true behavioral differences between the lines.

Accumulation patterns of time-related traits were used to obtain information regarding drinking activities throughout the day and investigate whether birds of different ages or selected for different traits have similar organisation of drinking activity. Overall, the G indices were statistically different between lines for all traits considered. Birds from line A had smaller variation in estimated drinking behavior traits, compared to the two other lines, suggesting a more equal, on average, organisation of drinking behavior within this line. Lines B and C which consisted of same age birds were more similar in the distribution of drinking traits during the day, with line C having the highest variation of the estimated traits.

Bird drinking behavior had a tendency to change with time for the estimated traits. In general, birds showed a decrease in the number of bouts over time accompanied by an increase in the amount of water used per bout. Similarly drinking time and duration per bout in general decreased over time, the extent of which varied between the lines. This is consistent with the time related trends seen in the feeding behavior of turkeys (Howie et. al., 2010). One interesting finding is that daily bout frequency, daily total water usage and daily total drinking time were uncorrelated or weakly correlated in all three lines. As estimates of daily bout frequency differed between birds in each line, it suggested that birds have different strategies in meeting their water requirement. These results are supported by a similar study on bird feeding behavior, where it was found that birds vary in the distribution of bouts and intervals between them (Howie et. al., 2009b), and as this was heritable it is perhaps useable. However, overall change in drinking behavior was similar between the lines, suggesting that while difference between the lines in the organisation of drinking exists, the more fundamental aspects of behavior, such as regulation of drinking and drinking behavior evolution over time is conserved between the lines. This means that birds have the flexibility to adapt their behavior without compromising essential body functions and regulations.

In conclusion, we have developed a methodology that enables the analysis of drinking behavior in birds. The methodology suggests that drinking behavior is underlined by the principles of satiety, and for this reason may have applicability across different animal species. The methodology also enabled us to identify a number of drinking behavior traits that arise from biologically defined criteria of drinking bouts. There seemed to be differences in these traits between bird genotypes selected for different productive traits. Therefore, these drinking behavior traits may be of potential relevance to turkey breeders, as water utilisation, along with feed efficiency, is part of overall biological efficiency. Furthermore, drinking behavior could provide further insights on the link between water usage and health, and the environmental impact of turkey production systems.

LITERATURE CITED

1. Kyriazakis, I. and Tolkamp BJ. Hunger and Thirst. In: Appleby, M. C. and Mench, J. A. and Olsson, A. S. and Hughes BO, editor. Animal Welfare. Wallingford, Oxon: CABI; 2011. p. 44–63.
2. Siegel, S. & Castellan Jr NJ. Nonparametric Statistics for The Behavioral Sciences. 2nd ed. New York: McGraw-Hill; 1988.
3. Howie JA, Tolkamp BJ, Avendano S, Kyriazakis I. The structure of feeding behavior in commercial broiler lines selected for different growth rates. Poult Sci. 2009;88(6):1143–50.
4. Tolkamp BJ, Allcroft DJ, Austin EJ, Nielsen BL, Kyriazakis I. Satiety splits feeding behaviour into bouts. J Theor Biol [Internet]. 1998 Oct 21;194(2):235–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9778436
5. Lundy MG, Harrison a., Buckley DJ, Boston ES, Scott DD, Teeling EC, et al. Prey field switching based on preferential behaviour can induce Levy flights. J R Soc Interface. 2012;

6. Yeates MP, Tolkamp BJ, Allcroft DJ, Kyriazakis I. The use of mixed distribution models to determine bout criteria for analysis of animal behaviour. J Theor Biol [Internet]. 2001;213(3):413–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11735288

7. Howie JA, Tolkamp BJ, Avendano S, Kyriazakis I. A novel flexible method to split feeding behaviour into bouts. Appl Anim Behav Sci [Internet]. 2009;116(2-4):101–9. Available from: http://www.sciencedirect.com/science/article/B6T48-4TVHYY2-1/2/ec8688a7b39ba7d68dc66bc3c6c01ab6

8. Huzzey JM, von Keyserlingk M a G, Weary DM. Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. J Dairy Sci [Internet]. Elsevier; 2005 Jul [cited 2014 Mar 29];88(7):2454–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15956308

9. Tolkamp BJ, Allcroft DJ, Barrio JP, Bley TAG, Howie JA, Jacobsen TB, et al. The temporal structure of feeding behavior. 2011;378–93.

10. Tolkamp BJ, Kyriazakis I. To split behaviour into bouts, log-transform the intervals. Anim Behav Anim Behav. 1999;57:807–17.

11. Swalander LM, Glover PK, Kremer VD, Bailey RA. Driving Robustness and Gut Health for the European Turkey Industry. Proceedings of the 7th Turkey Science and Production Conference. 2013.

1. **Rolls BJ, Rolls ET** 1991 Thirst. Press Syndicate of the University of Cambridge,.

2. **Kyriazakis I, Tolkamp BJ.** 2011 Hunger and Thirst. In Animal Welfare, edited by BO Appleby, MC Mench, JA Olsson, AS Hughes.Wallingford, Oxon: CABI, p. 44–63.

3. **De Jong IC, Gunnink H, van Harn J.** 2014 Wet litter not only induces footpad dermatitis but also reduces overall welfare, technical performance, and carcass yield in broiler chickens. J. Appl. Poult. Res. 23, 51–58. (doi:10.3382/japr.2013-00803)

4. **Manning L, Chadd SA, Baines RN.** 2007 Key health and welfare indicators for broiler production. World’s Poultry Science Journal 63: 46–62. (doi:10.1079/WPS2005126)

5. **McKinley MJ, Johnson AK.** 2004 The physiological regulation of thirst and fluid intake. News Physiol. Sci. 19: 1–6. (doi: 10.1152/nips.01470.2003)

6. **Howie JA, Tolkamp BJ, Bley T, Kyriazakis I.** 2010 Short-term feeding behavior has a similar structure in broilers, turkeys and ducks. Brit. Poultry Sci. 51, 714–724. (doi: 10.1080/00071668.2010.528749)

7. **Celeux G.** 2007 Mixture models for classification. In: Advances in Data Analysis, edited by Decker R, Lenz HJ. Springer Berlin Heidelberg, p. 3–14.

8. **Hanley JA, McNeil BJ.** 1982 The Meaning and Use of the Area under a Receiver Operating Characteristic (ROC) Curve. Radiology 143, 29–36.

9. **R Development Core Team.** 2014 R: A Language nand Environment for Statistical

Computing. R Foundation for Statistical Computing,Vienna, Austria.

10. **Siegel S, Castellan Jr, NJ.** 1988 Nonparametric Statistics for The Behavioral Sciences (Second edition). New York: McGraw-Hill.

11. **Gastwirth JL.** 1972 The estimation of the Lorenz curve. The Review of Economics and Statistics 54: 306–316.

12. **Jolliffe I.** 2002 Principal Component Analysis (Second edition). Springer.

13. **McCulloch CE, Neuhaus JM.** 2001 Generalized linear mixed models. John Wiley & Sons, Ltd.

14. **Tolkamp BJ, Allcroft DJ, Austin EJ, Nielsen BL, Kyriazakis I.** 1998 Satiety splits feeding behavior into bouts. J. Theor. Biol. 194, 235–250. (doi:10.1006/jtbi.1998.0759)

15. **Maselyne J, Adriaens I, Huybrechts T, De Ketelaere B, Millet S, Vangeyte J, Saeys W.** 2015 Measuring the drinking behavior of individual pigs housed in group using radio frequency identification (RFID). Animal 11, 1–10. (doi:10.1017/S1751731115000774)

16. **Maselyne J, Saeys W, van Nuffel A.** 2015 Review: Quantifying animal feeding behavior with a focus on pigs. Physiol. Behav. 138, 37–51. (doi:10.1016/j.physbeh.2014.09.012)

17. **Howie JA, Tolkamp BJ, Avendano S, Kyriazakis I.** 2009 The structure of feeding behavior in commercial broiler lines selected for different growth rates. Poult. Sci. 88, 1143–1150. (doi:10.3382/ps.2008-00441)

18. **Yeates MP, Tolkamp BJ, Allcroft DJ, Kyriazakis I.** 2001 The use of mixed distribution models to determine bout criteria for analysis of animal behavior. J. Theor. Biol. 213, 413–425. (doi: 10.1006/jtbi.2001.2425)

19. **Howie JA, Tolkamp BJ, Avendano S, Kyriazakis I.** 2009 A novel flexible method to split feeding behavior into bouts. Appl. Anim. Behav. Sci. 116, 101–109. (doi:10.1016/j.applanim.2008.09.005)

20. **Huzzey JM, von Keyserlingk MAG, Weary DM.** 2005 Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. J. Dairy Sci. 88, 2454–2461. (doi:10.3168/jds.S0022-0302(05)72923-4)

21. **Tolkamp BJ, Allcroft DJ, Barrio JP, Bley TAG, Howie JA, Jacobsen, TB, Kyriazakis, I.** 2011 The temporal structure of feeding behavior. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301, 378–393. (doi:10.1152/ajpregu.00661.2010)

22. **Fitzsimons JT.** 1998 Angiotensin , Thirst , and Sodium Appetite. Physiol. Rev. 78: 583-687.

23. **McKinley MJ, Cairns MJ, Denton DA, Egan G, Mathai ML, Uschakov A, Oldfield BJ.** 2004 Physiological and pathophysiological influences on thirst. Physiol. Behav. 81, 795–803. (doi:10.1016/j.physbeh.2004.04.055)

24. **Tolkamp BJ, Kyriazakis I.** 1999 To split behavior into bouts, log-transform the intervals. Anim. Behav. 57, 807–817. (doi: 10.1006/anbe.1998.1022)

25. **EFSA (European Food Safety Authority).** 2010 Scientific Opinion on the influence of genetic parameters on the welfare and the resistance to stress of commercial broilers. Scientific Opinion 8, 1–82. (doi:10.2903/j.efsa.2010.1666)

BIBLIOGRAPHY

Andersen HML, Dybkjær L, Herskin MS. Growing pigs' drinking behaviour: number of visits, duration, water intake and diurnal variation. *Animal* 8: 1881–1888, 2014; doi:10.1017/S175173111400192X

Bley TAG, Bessei W. Recording of individual feed intake and feeding behavior of pekin ducks kept in groups. *Poultry Science* 87: 215–221, 2008; doi:10.3382/ps.2006-00446

Brown-Brandl TM, Eigenberg RA. Development of a livestock feeding behavior monitoring system. *American Society of Agricultural and Biological Engineers* 54: 1913–1920, 2011.

Casey DS, Stern HS, Dekkers JCM. Identification of errors and factors associated with errors in data from electronic swine feeders. *Journal of Animal Science* 83: 969–982, 2005.

Celeux G. Mixture models for classification. In: *Advances in Data Analysis*, edited by Decker R, Lenz HJ. Springer Berlin Heidelberg, 2007, p. 3–14.

EFSA (European Food Safety Authority). Scientific Opinion on the influence of genetic parameters on the welfare and the resistance to stress of commercial broilers. *Scientific Opinion* 8: 1–82, 2010; doi:10.2903/j.efsa.2010.1666.

Fitzsimons JT. Angiotensin , Thirst , and Sodium Appetite, *Physiological Reviews* 78: 583 687, 1998.

Gastwirth JL. The estimation of the Lorenz curve. *The Review of Economics and Statistics* 54: 306–316, 1972.

Hanley JA, McNeil BJ. The Meaning and Use of the Area under a Receiver Operating Characteristic (ROC) Curve. *Radiology* 143: 29–36, 1982.

Howard BR. Water balance of the hen during egg formation. *Poultry Science* 54: 1046–1053, 1975; doi:10.3382/ps.0541046

Howie JA, Tolkamp BJ, Avendano S, Kyriazakis I. The structure of feeding behavior in commercial broiler lines selected for different growth rates. *Poultry Science* 88: 1143–1150, 2009; doi:10.3382/ps.2008-00441

Howie JA, Tolkamp BJ, Avendano S, Kyriazakis I. A novel flexible method to split feeding behaviour into bouts. *Applied Animal Behaviour Science* 116: 101–109, 2009; doi:10.1016/j.applanim.2008.09.005

Howie JA, Tolkamp BJ, Bley T, Kyriazakis I. Short-term feeding behaviour has a similar structure in broilers, turkeys and ducks. *British Poultry Science* 51: 714–724, 2010; doi: 10.1080/00071668.2010.528749

Huzzey JM, von Keyserlingk MAG, Weary DM. Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. *Journal of Dairy Science* 88: 2454–2461, 2005; doi:10.3168/jds.S0022-0302(05)72923-4

Jolliffe I. *Principal Component Analysis* (Second edition). Springer, 2002.

Kyriazakis I, Tolkamp BJ. Hunger and Thirst. In *Animal Welfare*, edited by BO Appleby, MC Mench, JA Olsson, AS Hughes.Wallingford, Oxon: CABI, 2011, p. 44–63.

Lundy MG, Harrison A, Buckley DJ, Boston ES, Scott DD, Teeling EC, Houghton JDR. Prey field switching based on preferential behaviour can induce Levy flights. *Journal of The Royal Society Interface*, 2012; doi:10.1098/rsif.2012.0489

Manning L, Chadd SA, Baines RN. Water consumption in broiler chicken: a welfare indicator. *World's Poultry Science Journal* 63: 63–71, 2007; doi:10.1079/WPS2006127

Manning L, Chadd SA, Baines RN. Key health and welfare indicators for broiler production. *World's Poultry Science Journal* 63: 46–62, 2007; doi:10.1079/WPS2005126

Maselyne J, Adriaens I, Huybrechts T, De Ketelaere B, Millet S, Vangeyte J, Saeys W. Measuring the drinking behaviour of individual pigs housed in group using radio frequency identification (RFID). *Animal* 11: 1–10, 2015; doi:10.1017/S1751731115000774

Maselyne J, Saeys W, van Nuffel A. Review: Quantifying animal feeding behaviour with a focus on pigs. *Physiology & Behavior 138*: 37–51, 2015; doi:10.1016/j.physbeh.2014.09.012

McCulloch CE, Neuhaus JM. *Generalized linear mixed models*. John Wiley & Sons, Ltd, 2001.

McKinley MJ, Cairns MJ, Denton DA, Egan G, Mathai ML, Uschakov A, Oldfield BJ. Physiological and pathophysiological influences on thirst. *Physiology & Behavior* 81: 795–803, 2004; doi:10.1016/j.physbeh.2004.04.055

McKinley MJ, Johnson AK. The physiological regulation of thirst and fluid intake. *News in Physiological Sciences : An International Journal of Physiology Produced Jointly by the International Union of Physiological Sciences and the American Physiological Society* 19: 1–6, 2004; doi: 10.1152/nips.01470.2003

Mendes EDM, Carstens GE, Tedeschi LO, Pinchak WE, Friend TH. Validation of a system for monitoring feeding behavior in beef cattle. *Journal of Animal Science* 89: 2904–2910, 2011; doi:10.2527/jas.2010-3489

R Development Core Team. R: A Language nand Environment for Statistical Computing. *R Foundation for Statistical Computing*, 2014, Vienna, Austria.

Rolls BJ, Rolls ET. *Thirst*. Press Syndicate of the University of Cambridge, 1991.

Siegel S, Castellan Jr, NJ. *Nonparametric Statistics for The Behavioral Sciences* (Second edition). New York: McGraw-Hill, 1988.

Swalander LM, Glover PK, Kremer VD, Bailey RA. Driving Robustness and Gut Health for the European Turkey Industry. In *Proceedings of the 7th Turkey Science and Production Conference*, 2013.

Tolkamp BJ, Allcroft DJ, Austin EJ, Nielsen BL, Kyriazakis I. Satiety splits feeding behaviour into bouts. *Journal of Theoretical Biology* 194: 235–250, 1998; doi:10.1006/jtbi.1998.0759

Tolkamp BJ, Allcroft DJ, Barrio JP, Bley TAG, Howie JA, Jacobsen, TB, Kyriazakis, I. The temporal structure of feeding behavior. *American journal of physiology. Regulatory, integrative and comparative physiology* 301: 378–393, 2011; doi:10.1152/ajpregu.00661.2010.

Tolkamp BJ, Kyriazakis I. To split behaviour into bouts, log-transform the intervals. *Animal Behaviour* 57: 807–817, 1999; doi: 10.1006/anbe.1998.1022

Yeates MP, Tolkamp BJ, Allcroft DJ, Kyriazakis I. The use of mixed distribution models to determine bout criteria for analysis of animal behaviour. *Journal of Theoretical Biology* 213: 413–425, 2001; doi: 10.1006/jtbi.2001.2425

TABLES

Table 1. General overview of the data available for drinking behaviour analysis with number of hatches available for each line. Duration of the drinking recording experiment and number of birds per hatch are presented using minimum, maximum and median values to show variation in these characteristics within and between the lines.

Genetic Lines	Number of hatches	Experiment duration (days)			Number of birds per hatch		
		Min	Max	Median	Min	Max	Median
A	29	14	16	15	131	292	146
B	15	23	28	24	135	224	142
C	2	22	24	23	141	144	143

Table 2. Medians with interquartile ranges of the number of visits, water usage per visit and visit duration, and total drinking time and total water usage estimated per day per individual bird for the three genetic lines of turkeys.

Recorded parameters	Line A	Line B	Line C
Number of visits	11.17[a,b] (10, 13.7)	12.17[a,c] (10.21, 14.42)	15.32[b,c] (12, 20.45)
Water usage per visit (ml)	68.03[a,b] (57.02, 80.39)	64.96[a,c] (54.02, 77.67)	45.90[b,c] (34.24, 56.71)
Mean visit duration (s)	77.95[a,b] (66.37, 91.22)	51.82[a] (42.5, 61.84)	48.21[b] (34.47, 61.17)
Daily drinking time (s)	903.91[a,b] (777.87, 1054.99)	623.39[a,c] (542.96, 706.7)	699.68[b,c] (600.86, 821.95)
Daily water usage (ml)	790.29[b] (701, 890.45)	785.62[c] (725.61, 847.30)	687.29[b,c] (624.21, 760.81)
Water usage rate (ml/s)	0.88[a,b] (0.75, 1.02)	1.26[a,c] (1.11, 1.45)	0.99[b,c] (0.84, 1.16)

[a] $P < .01$ between the lines A and B.

[b] $P < .01$ between the lines A and C.

[c] $P < .01$ between the lines B and C.

Table 3. Medians with interquartile ranges of drinking behavior traits per day per individual bird for three turkey genetic lines A, B and C, estimated after grouping visits to the water station into bouts.

Traits	Line A	Line B	Line C
Bout criterion (s)	665	672	602
N of bouts	10.64[a,b]	10.22[a]	9.64[b]
	(9.25, 12.27)	(8.83, 11.65)	(8.19, 11.87)
N of visits per bout	1.08[a,b]	1.17[a,c]	1.49[b,c]
	(1.05, 1.13)	(1.11, 1.26)	(1.26, 1.95)
Bout duration (s)	93.89[a]	80.48[a,c]	94.7[c]
	(81.82, 107.84)	(70.51, 92.68)	(82.82, 106.97)
Drinking time per bout (s)	85.36[a,b]	61.78[a,c]	73.98[b,c]
	(73.11, 99.17)	(52.17, 72.32)	(61.18, 85.74)
Non-drinking time per bout (s)	7.24[a,b]	17.10[a]	18.58[b]
	(3.69, 12.25)	(10.73, 24.79)	(11.42, 28.23)
Water usage per bout (ml)	74.50[a,b]	77.15[a,c]	68.92[b,c]
	(63.52, 86.91)	(66.50, 89.86)	(57.75, 85.57)

[a] P < .01 between the lines A and B.

[b] P < .01 between the lines A and C.

[c] P < .01 between the lines B and C.

Table 4. GINI indices estimated per line, and medians with interquartile ranges measured per individual bird within each line (A, B and C) for intervals between visits, bout duration, non-drinking time and drinking time per bout for individual birds.

Traits	Line A	Line B	Line C
Intervals between visits	0.39	0.47	0.63
	0.36[a,b] (0.33,0.4)	0.44[a,c] (0.4, 0.49)	0.56[b,c] (0.46, 0.67)
Bout duration	0.33	0.45	0.42
	0.29[a,b] (0.25,0.33)	0.41[a,c] (0.36,0.47)	0.37[b,c] (0.33, 0.44)
Non-drinking time per bout	0.97	0.94	0.88
	0.97[a,b] (0.95,0.98)	0.94[a,c] (0.91, 0.96)	0.88[b,c] (0.82,0.93)
Drinking time per bout	0.28	0.33	0.37
	0.24[a,b] (0.22, 0.26)	0.30[a,c] (0.28, 0.33)	0.32[b,c] (0.29, 0.36)

[a] $P < .01$ between the lines A and B.

[b] $P < .01$ between the lines A and C.

[c] $P < .01$ between the lines B and C.

FIGURES

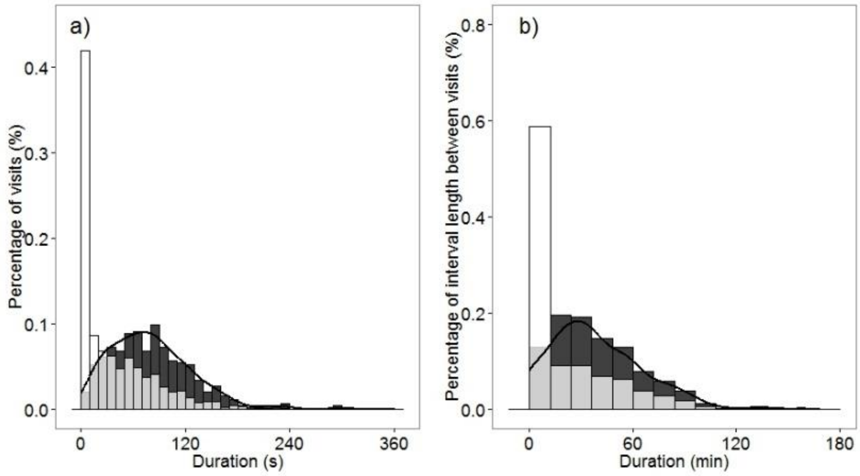

Figure 1. Data distribution following the validation step, where intervals less than 4 seconds have been excluded. (a) Frequency of visit durations to the water station with a bin size of 10 seconds and (b) frequency of interval length between visits to the water station with a bin size of 20 minutes, estimated by the electronic system (white bars) and manual observations (black bars), with the overlaid density line for the manual observations estimated from the corresponding histogram.

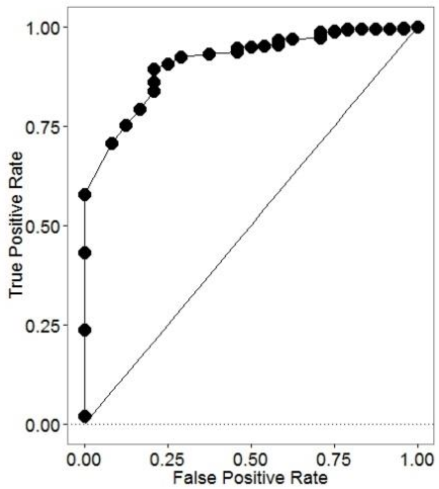

Figure 2. Receiver Operating Characteristics (ROC) curve showing true and false positive rates for each merging criterion tested (0 to 60 seconds) with markers from left to right, estimated from automated (via radio-frequency identification) versus manually recorded visits. The true positive rate measures the proportion of positives (visits recorded by the RFID system) that are confirmed through direct video observations. The false positive shows the proportion of positives detected by the RFID system that are rejected by video observations.

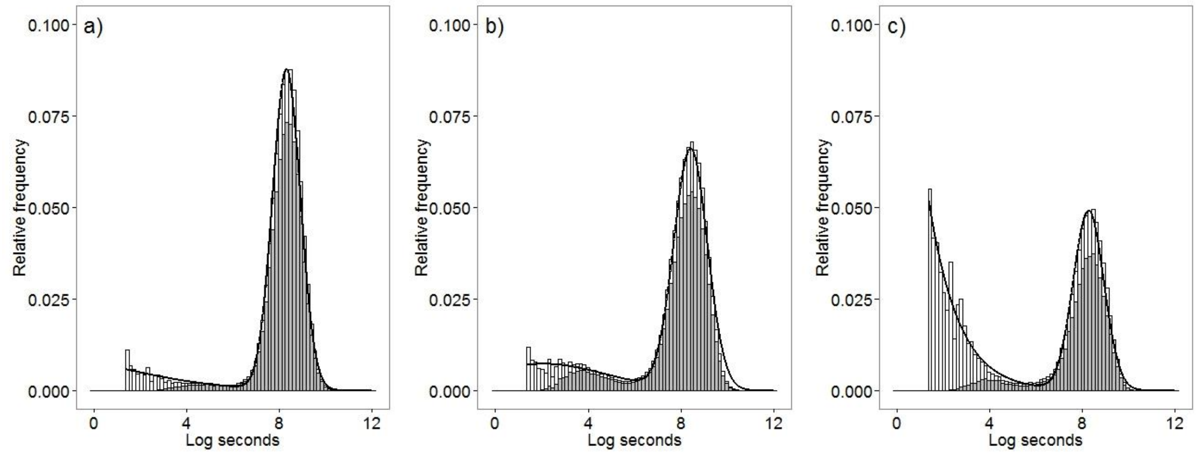

Figure 3. Frequency distribution of intervals between visits to the same drinker (white bars) and different drinkers (grey bars) whilst light was present in the turkey sheds (06:00-20:00). Intervals are expressed on a natural-log scale (bin size = 0.15 log units). The solid lines are fitted mixed models to the natural-log transformed interval length between visits; they contain a truncated log-normal distribution for within bout intervals and a log-normal distribution for between bout intervals for three turkey genetic lines A, B and C, graphs (a), (b) and (c) respectively.

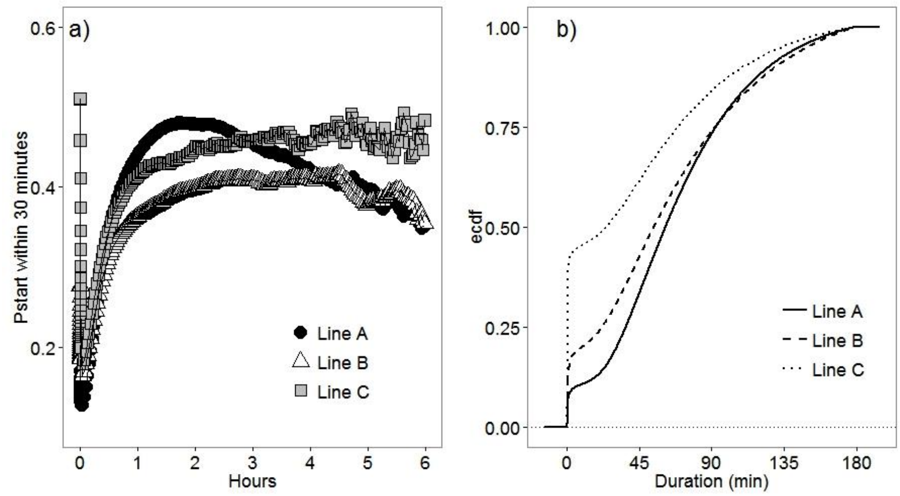

Figure 4. Probabilities of a next visit. (a) Probability of a bird starting a next visit to an electronic drinker (Pstart) within the next 30 minutes since the last visit and (b) empirical cumulative probability distribution function (ecdf) of interval length between visits to the electronic drinker for three genetic lines of turkeys. Graph (b) shows the cumulative probability of a next visit within n seconds or less for the three genetic lines.

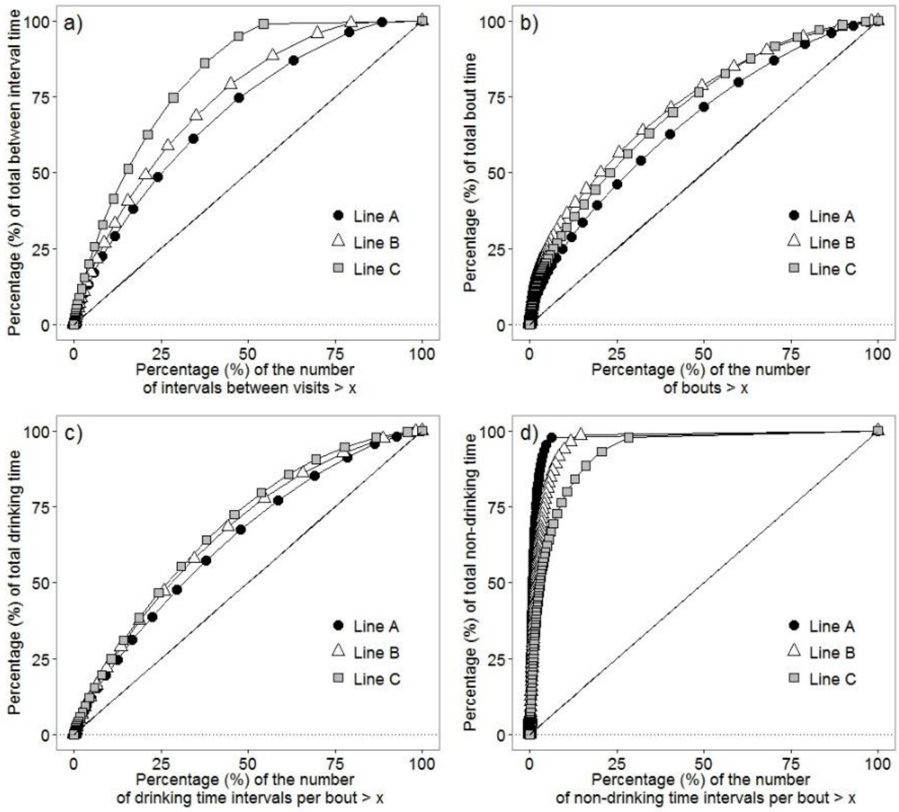

Figure 5. Accumulation patterns of four time-related drinking behavior traits for the turkey genetic lines A, B and C: (a) intervals between visits to the water station, (b) bout duration, (c) drinking time per bout and (d) non-drinking time per bout. The black line is an equality line (GINI = 0), where intervals of different length contribute equally to the accumulation pattern. Y-axis shows the cumulative percentage of total time for a given trait, while x-axis shows the cumulative percentage of unique interval length, where x = interval length for a given trait (ordered from the smallest to the largest).

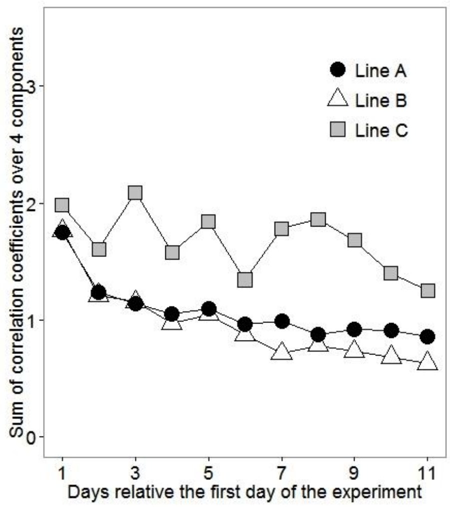

Figure 6. Pearson correlation coefficients relative to the first day of the experiment and added over four principal components for each day from Principal Component Analysis (PCA) that accounted for 95% of variability in the data, for lines A, B and C. The correlation coefficients were estimated for each principal component separately and added together for visualisation purposes.

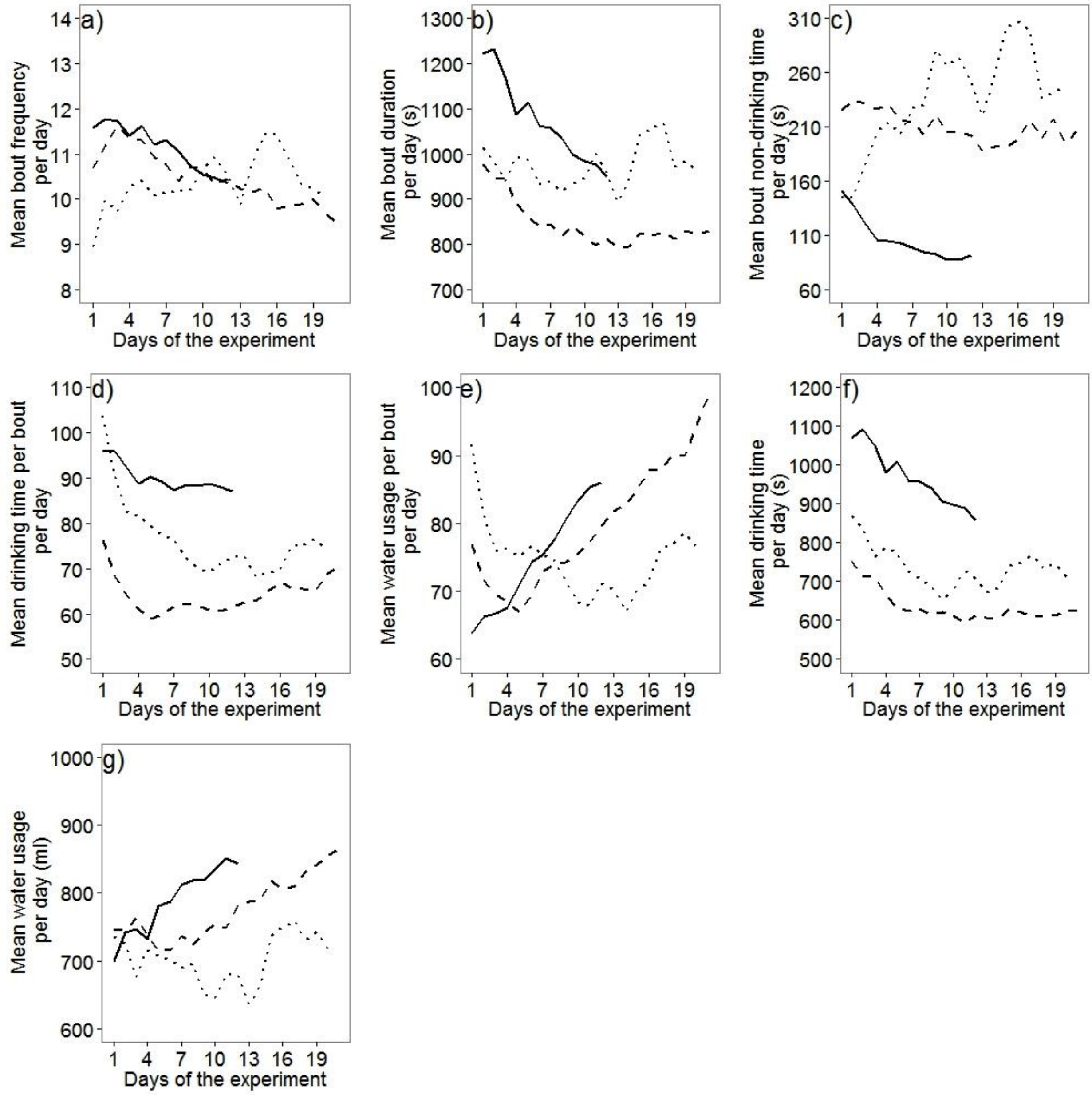

Figure 7. Distribution of bout and daily related drinking behavior characteristics during the experiment calculated per each day of the experiment for line A (solid line), line B (dashed line) and line C (dotted line): (a) mean bout frequency, (b) bout duration, (c) non-drinking time within a bout, (d) mean drinking time per bout, (e) mean water usage per bout, (f) drinking time, (g) water usage. The number of days was taken as a shortest experiment length for a given genetic line.

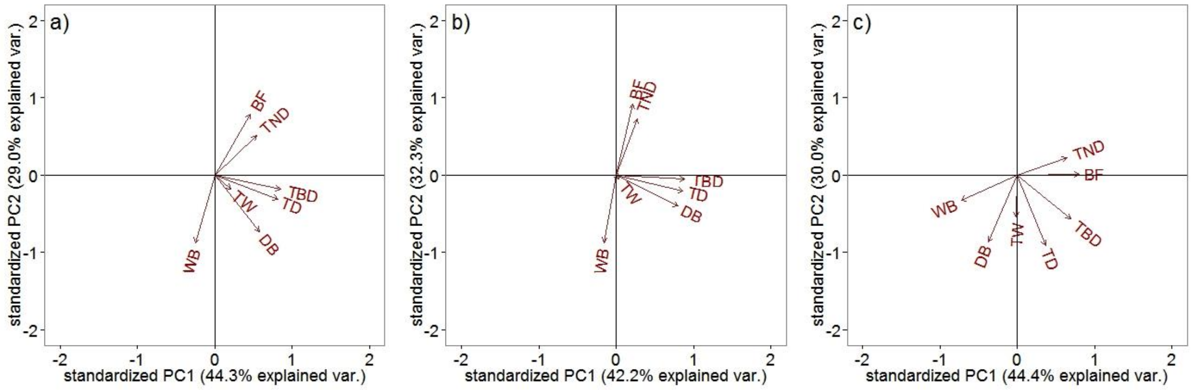

Figure 8. Principal component analysis showing inter-relationships between 7 drinking behavior traits over the first two principal components. Traits include: BF – daily bout frequency, TBD – daily bout duration, TD – daily drinking time, TND – daily nondrinking time within a bout, TW – daily water usage, DB – mean drinking time per bout, WB – mean water usage per bout, for genetic lines A (a), B (b) and C (c), respectively. The angle between the vectors representing traits indicates correlation between traits. For highly correlated traits vectors point in the same direction (or opposite directions for highly negatively correlated traits), whereas uncorrelated traits have a right angle between the vectors.