Dehydration indicators for broiler chickens at slaughter

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ABSTRACT Freedom of (prolonged) thirst is considered to be of paramount importance for animal welfare. This emotion normally results from dehydration, which can be measured using physiological indicators. Because no reliable physiological indicator for thirst was available for broilers, we aimed to identify such a measure in this study. This indicator would ideally be integrated into quality control systems in commercial slaughter plants. In the first experiment, water deprivation was manipulated systematically by withdrawing water for different durations (total water withdrawal for 0 (control), 24, 36, or 48 h, or a 10-d period with restricted access to water for 2 times 10 min per day). A significant decrease in drained blood content and BW occurred from 36 h of total water deprivation onward (both P = 0.03), whereas long-term restricted access tended to decrease drained blood content (P = 0.05). No effect of water deprivation or restriction on skin turgor was found. In the second experiment, water was withdrawn for 0 (control), 6, 12, 24, or 48 h. Plasma chloride concentration was increased after 6 h of water withdrawal, but did not rise further with longer withdrawal. If assessed at slaughter, chloride will thus mainly reflect the catching-to-slaughter interval. In contrast, plasma creatinine and hematocrit levels showed a numerical decrease after 6 h of water withdrawal, but rose again after prolonged withdrawal. Plasma creatinine values were significantly higher in 24-h-deprived birds than in 6-h-deprived birds (P < 0.01), allowing for discernment between water withdrawal during catching and transport from dehydration that had occurred on the farm. Blood sodium concentrations and plasma osmolality showed a steady increment between 0 and 24 h of water deprivation (P < 0.001 and P < 0.001 for both), and may thus be used to assess the combined effects of water deprivation on farm and during the catching-to-slaughter interval. These findings may form the basis of an on-farm or at-slaughter test that could be included in integrated animal welfare assessment schemes.

Key words: broiler, animal welfare, dehydration, water deprivation, assessment

INTRODUCTION

Freedom of (prolonged) thirst is generally considered to be of paramount importance for animal welfare (Brambell, 1965; Vanhonacker et al., 2008; Tuyttens et al., 2010). This state of discomfort cannot be assessed directly by physiological measures. However, water deprivation (i.e., a shortage of water intake compared with the physiological optimum) normally causes thirst, and physiological assessment of dehydration following water deprivation is thus likely to provide indirect information about thirst levels. In addition to its potential as a welfare indicator, water deprivation is also an indication of health problems (Manning et al., 2007) and decreased performance (Tabler, 2003; Viola et al., 2009). However, current methods for assessing water deprivation implemented within welfare monitoring schemes are not satisfactory (Sprenger et al., 2009). Such monitoring schemes usually evaluate absence of (prolonged) thirst by determining the number of birds per drinker place (e.g., Welfare Quality, 2009). Although such resource-based indicators can often be assessed reliably and quickly, they do not assess water deprivation accurately or precisely because birds that cannot reach or operate the drinkers will go unnoticed.

In an experimental setting, voluntary water uptake from an open drinker was shown to be a valid measure of thirst in broiler chickens, as it increased proportionally with the duration of water deprivation (Sprenger et al., 2009). This test may form the basis of an animal-based indicator of thirst that could be included into
on-farm welfare assessment schemes. However, such tests are quite time consuming and give no information about the individual’s level of thirst (Sprenger et al., 2009). Furthermore, measuring thirst on-farm will not give information about one of the most critical periods with regards to the risk of dehydration (i.e., during catching and transport to the slaughter plant). In commercial practice, water is usually withdrawn just before the first bird of a flock is caught and crated for transport to the slaughter plant. This depopulation process often takes several hours [crating rate of 1,000 to 1,300 broilers per hour per catcher (Delezie, 2006)]. Water deprivation continues as the birds are transported to the slaughter plant, a journey that may take several more hours. Upon arrival at the slaughter plant, a variable amount of time passes before the birds are slaughtered (EFSA Panel on Animal Health and Welfare, 2011).

The objective of the present study was to identify a reliable and fast animal-based indicator of water deprivation in the broiler rearing period that can be easily integrated into the quality control systems carried out at the slaughter plant. This indicator should be informative of on-farm water deprivation, as well as during the catching-to-slaughter interval. In literature, correlations between thirst/water deprivation and skin turgor (Laron, 1957; Laron and Crawford, 1957), capillary refill time (EFSA Panel on Animal Health and Welfare, 2011), plasma osmolality (Butterworth et al., 2002), blood sodium, plasma chloride, and creatinine concentrations (Knowles et al., 1995, 1996; Saito and Grossmann, 1998; Iheukwumere and Herbert, 2003), and blood volume and hematocrit levels (Zhou et al., 1998; Iheukwumere and Herbert, 2003) have been described. In this study, we investigate their accuracy as indicators of different periods of water deprivation and discuss the feasibility of measuring them in a commercial setting.

**MATERIALS AND METHODS**

All procedures were approved by the ethics committee for animal experiments at the Institute for Agricultural and Fisheries Research.

**Experiment 1**

**Birds, Housing, and Treatments.** This experiment had 4 rounds. Within each round, thirty 24-d-old Ross 308 broiler chicks (1:1 sex ratio) were housed in groups of 6 birds in littered floor pens of 2.2 m², except for the first round, which included only 18 birds. Ambient temperature varied between 18 and 22°C and an 18L:6D light schedule was used. A standard broiler diet was kept at 21°C, and a 20L:4D light schedule was applied. Water and standard broiler feed was available ad libitum until the start of the withdrawal treatments, water was also provided ad libitum.

Five treatments were applied. Chickens in the control treatment (0 h) had continuous ad libitum access to water. In 3 other treatments, water was withdrawn by removing the drinker either 24, 36, or 48 h before euthanasia. The fifth treatment consisted of long-term restricted access to water (10 d before euthanasia, the drinker was removed, and was returned for 10 min twice per day). This treatment was designed to simulate some of the farm-dependent deprivation problems birds can experience such as inefficient use of drinking nipples due to leg disorders, high stocking density, and disease (Houldcroft et al., 2008; SCAHAW, 2000). At approximately 1000 h on d 40, all chickens were euthanized by cutting the jugular vein and carotid artery on one side of the neck. The carcasses were suspended upside down and allowed to bleed out.

Within each of the rounds, each treatment was randomly allocated to 1 pen, which is the experimental unit in our design. The exceptions to this was the first round, which included only a control group, and a 24-h and a 48-h water withdrawal group (in other words, no 36-h water withdrawal or long-term water restriction group).

**Measurements.** Directly before euthanasia, BW, capillary refill time, and skin turgor were determined. Capillary refill time was measured by squeezing the wattle between the thumb and index finger for 10 s until the skin between the 2 fingers turned white, then recording the time needed for the color to return once pressure was released. To evaluate skin turgor, the skin of the birds’ left thigh was taken between the thumb and index finger and lifted to a height of approximately 1 cm, where it was kept for 10 s. Skin turgor time was recorded as the interval between releasing the skin and the re-establishment of the previous skin condition.

During euthanasia, blood drained from the vessels of the neck of the inverted broilers was captured until blood flow was greatly reduced (after approximately 1.5 min), and subsequently weighed. This is not the most accurate method to determine blood volume because an undetermined part of the blood remains in the body. However, birds are bled out in this manner during the slaughter process. This makes this measure easy and fast to perform in the slaughter plant, which would allow an increased number of individuals to be assessed. We refer to this indicator as drained blood content, and we will look at absolute and relative drained blood content (relative to BW).

**Experiment 2**

**Birds, Housing, and Treatments.** The second experiment had 2 rounds. Within each round, thirty 37-d-old Ross 308 broiler chickens (1:1 sex ratio) were housed individually in pens of 0.5 m². Ambient temperature was kept at 21°C, and a 20L:4D light schedule was applied. Water and standard broiler feed was available ad libitum until the respective starts of the experimental treatments. These broilers were randomly divided over 5 treatments: 0 (control), 6, 12, 24, and 48 h of water withdrawal; therefore, bird is the experimental unit in this design. All treatments ended on d 39 between 0000 and 1500 h, when the birds were euthanized using
a nonpenetration captive bolt device (CASH Poultry Killer, Abato, Loon Op Zand, the Netherlands).

Measurements. Directly before euthanasia, blood was taken from the wing vein with a 25-ga needle and 2-mL syringe and collected in lithium-heparinized tubes. Immediately after blood collection, blood was aspirated in a heparinized capillary tube (150 µL) and introduced into a blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Zaventem, Belgium) for the determination of hematocrit (%) and sodium (mmol/L) levels. The remaining blood was centrifuged for 10 min at 1,077 × g at 4°C, after which plasma was stored at −18°C for later analysis of osmolality, creatinine, and chloride. Plasma osmolality (mOsm/kg) was measured with a vapor pressure osmometer 5500 (Wescor 5500 XRS, Prosan nv, Merelbeke, Belgium). Creatinine (µmol/L) was measured in the first round only, according to the method of Helger et al. (1974). Chloride concentrations (mmol/L) were measured using a Quantichrom Chloride Assay Kit (DICL-250, Gentaur, Brussels, Belgium).

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS) version 9.3 for Windows (SAS Institute Inc., Cary, NC). Capillary refill, skin turgor time, and absolute and relative drained blood content (relative to BW) were analyzed using a MIXED model (PROC MIXED). Round and pen were included as random effects, and treatment, sex, and their interaction as categorical fixed effects. Observations on individual birds were used. Hematocrit, blood osmolality, blood sodium, and plasma chloride concentrations were analyzed using the same MIXED model, except that sex was omitted as a random effect (because broilers were housed individually). Plasma creatinine was measured in 1 round only; thus, round was omitted from the model for this indicator. Statistical significance was evaluated at a significance level of 0.05. Fixed effects were tested with the traditional F-tests, and degrees of freedom were predicted using the Satterthwaite formulas (Littell et al., 1996). Pairwise comparisons between treatments were tested at a total significance level of 0.05 using the Tukey-Kramer adjustment for multiple comparisons.

Receiver operating curves (ROC) were created for several indicators to show their sensitivity to 6 and 48 h of water withdrawal. We made ROC curves for plasma chloride and creatinine concentrations discerning between the control group and the 6-h-deprived group, and for blood sodium and plasma chloride and creatinine concentrations and hematocrit values discerning between the control group and the 48-h-deprived birds.

RESULTS

Interaction and Sex Effect, Experiments 1 and 2

No significant interactions between treatment and sex effects were found for any of the investigated indicators in either of the experiments.

In contrast, some sex effects were found (Table 1). In the first experiment, males had a higher BW and a higher drained blood content than females. In the second experiment, males had higher sodium concentrations, plasma osmolality, and hematocrit levels than females. The other indicators were not significantly affected by sex.

Treatment Effect, Experiment 1

Drained blood content decreased steadily with increasing water withdrawal duration (P = 0.009, Figure 1A). Significant pair-wise differences (P < 0.05) were found between control birds and birds deprived for 36 and 48 h. Birds deprived for 36 and 48 h tended (P < 0.1) to have less blood than 24-h-deprived birds. Long-term restricted access also led to a tendency for decreased blood content compared with the control birds (P < 0.1). In contrast to what was found for absolute drained blood content, no treatment effects on relative drained blood content were found (P = 0.585).

Body weight decreased steadily with increasing water withdrawal duration (P = 0.002, Figure 1B). Significant pair-wise differences (P < 0.05) were found

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<th>Table 1. Sex effect on the different physiological indicators investigated in experiments 1 and 2 as possible thirst indicators</th>
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<td><strong>Experiment 1</strong></td>
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<td>BW (g)</td>
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<td>Capillary refill (s)</td>
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between control birds and birds deprived for 36 ($P = 0.027$) and 48 h ($P = 0.003$) and with those with long-term restricted access ($P = 0.006$). The group of birds deprived of water for 24 h showed higher BW compared with the 48-h-deprived birds ($P = 0.027$) and those with restricted water access ($P = 0.044$).

Capillary refill time was influenced by the treatments ($P = 0.030$, Figure 1C). Capillary refill was significantly faster after long-term water restriction than after 36 h of deprivation ($P = 0.021$), with control birds having intermediate values. Capillary refill time tended to be faster in the broilers with long-term restricted access compared with birds subjected to 24 h deprivation ($P = 0.075$) and 48 h deprivation ($P = 0.069$).

Skin turgor (data not shown) was not affected significantly by the treatments ($P = 0.343$).

**Treatment Effect, Experiment 2**

Duration of water withdrawal affected blood sodium concentrations and hematocrit values, plasma chloride and creatinine concentrations, and plasma osmolality ($P = 0.001$, $P = 0.017$, $P = 0.001$, $P < 0.001$, and $P < 0.001$, respectively). Blood sodium concentration increased steadily with increasing duration of water withdrawal, becoming significantly higher than in the control birds after 24 h of deprivation (Figure 2A). Sodium concentration leveled off with longer water deprivation. Plasma chloride concentration increased significantly after 6 h of water withdrawal, but did not rise any further with longer withdrawal (Figure 2B). Plasma osmolality increased during the first 24 h of water withdrawal, at which point values were significantly higher than those of the controls and 6 and 12 h of water withdrawal (Figure 2C). However, after 48 h of withdrawal, osmolality was decreased again. Hematocrit levels showed a somewhat erratic pattern: 6 h of withdrawal led to numerically lower values than 48 h of withdrawal, with all other treatments resulting in intermediate values (Figure 2D). Plasma creatinine levels showed a nonsignificant decrease during the first 6 h, but afterward increased with longer water withdrawal periods. Creatinine levels were higher after the longest water withdrawal period than after the other periods.

**Figure 1.** Box plots showing the response of broilers’ drained blood content (A), living BW (B), and wattle capillary refill time (C) to different periods of complete water withdrawal and to 10-d-long 20 min/d water access. Least squares means without a common letter (a–c) differ significantly ($P < 0.05$).
Birds deprived for 24 h tended to have higher creatinine levels compared with the control ($P = 0.085$) and 6-h-deprived birds ($P = 0.004$; Figure 2E).

**ROC Curve Analysis**

Several potential parameters suggested by the analysis described above were evaluated for their ability to indicate water deprivation in broilers using ROC curves (Figure 3). In an ROC curve, the sensitivity (true positives) is plotted against the false positives ($1 - specificity$) at different cut-off values. The most optimal cut-off value is in the upper left corner (Bradley, 1997); all samples detected are true positives. The area under the curve gives the performance of the test: 1 is a perfect test (no false positives) and 0.5 is a worthless
test (same number of true positives as false positives). Figure 3A illustrates the sensitivity of plasma chloride and creatinine to 6 h of water deprivation. It shows that creatinine would be a bad indicator for a 6-h water withdrawal period, as it stays close to the bisector. Chloride, on the other hand, would be much better. It would also be a good indicator for a 48-h deprivation period (Figure 3B). If no false positives would be allowed, almost 60% of all 48-h dehydrated birds would be detected. Creatinine concentration, however, comes out best for the detection of a 48-h water withdrawal period. When no false positives would be allowed, 85% of the deprived birds would be detected. A sensitivity of 100% for dehydration can be obtained with a 15% false positive rate. Sodium concentration in broilers’ whole blood is presented third (Figure 3B). Around 50% of the dehydrated birds would be detected.

**DISCUSSION**

Very little previous research has been conducted on the development and validation of indicators of dehydration when end-of-life broiler chickens are moved from the farm, transported, and slaughtered. In this study, the validity of several potential water deprivation indicators was evaluated. Drained blood content, BW, capillary refill, sodium and chloride concentrations, plasma osmolality, and hematocrit and creatinine levels were found to respond to various stages of water deprivation.

The first category of indicators under discussion are those that responded to 6 h of water deprivation, but which did not show considerable changes after longer deprivation. Such indicators are of little value as at-slaughter indicators of water deprivation sustained on-farm. This is because the catching-to-slaughter interval (during which all birds are completely deprived of water) usually exceeds 6 h, thus making it impossible to discern between birds that are only dehydrated due to this interval, and those that already suffered from on-farm water deprivation. Nevertheless, these indicators could be used to measure short-term on-farm water deprivation. In this study, chloride fits best into this category of indicators. However, this finding contrasts with previous research in which it took 72 h for chloride concentrations to be significantly increased (Koike et al., 1983). This warrants further investigation. Furthermore, the sensitivity of chloride analysis as an indicator of short-term (6 h) water deprivation was limited, as our model showed that less than 60% of deprived birds would be detected if no false positives are allowed. In animal welfare assessment schemes, it is important to minimize the number of false positives (here, non-deprived chickens that are classified as water deprived) because erroneous penalization may make farmers more reluctant to resolve the problem.

The second category is formed by those indicators that showed opposite reactions to short (6 h) and medium or long water withdrawal (24, 36, or 48 h). Such indicators can discern between water deprivation sustained on-farm and caused by the catching-to-slaughter interval. Our study indicates that plasma creatinine values showed the greatest potential to do so. Increases in broilers’ creatinine levels caused by medium- and
long-term dehydration had not been evaluated before this study, but a study in pigeons (Lumeij, 1987) corroborates our findings. Also, a study from Ihenkwumere and Herbert (2003) observed higher creatinine levels in broilers given restricted access to water. In addition, the sensitivity of the creatinine test was high, detecting over 80% of 48-h dehydrated birds if no false positives are allowed, and all if 20% false positives were allowed. Hematocrit followed approximately the same pattern as creatinine during the first stages of water withdrawal, it showed no further increase when withdrawal exceeded 12 h. Also, the ROC curve of hematocrit was not good (low sensitivity when specificity was high). Therefore, creatinine analysis would be preferred to hematocrit analysis. The slightly erratic response of hematocrit to water withdrawal is in line with previous research (Zhou et al., 1998).

The third category consists of indicators that, when applied at-slaughter, would assess the combination of on-farm deprivation and catching-to-slaughter deprivation. Whole blood sodium concentration showed an approximately steady increase during the first 24 h of water withdrawal and remained stable afterward. This is supported by many other studies in layer and broiler chickens (Chamblee and Morgan, 1982; Koike et al., 1983; Arad et al., 1985; Chamblee et al., 1988; Robinson et al., 1990; Swayne and Radin, 1991; Knowles et al., 1995, 1996). Therefore, sodium concentration can be used to detect medium-term (24 h) water deprivation, although a nonsignificant increase occurred already after 6 h in our study. The sensitivity of this test is limited, however, because only 70% of dehydrated birds would be detected even when 10% false positives are allowed. Plasma osmolality also showed a clear increase after 24 h of water deprivation, in line with Koike et al. (1983). But plasma osmolality values decreased again between 24 and 48 h of water withdrawal. This may be because prolonged withdrawal causes decreased feed intake (Koike et al., 1983), which in turn results in decreased plasma osmolality (Knowles et al., 1995). The validity of the 48 h decrease found in our study can be questioned because it is not supported by previous studies using deprivation periods of 48 h (Koike et al., 1983; Arad et al., 1985) or longer (Swayne and Radin, 1991). Drained blood content seemed most suited to detect long (>36 h) water deprivation. There was no distinct drop in drained blood content before 24 h of water withdrawal, in line with previous results in layers (Koike et al., 1983), but blood content more or less stabilized after 36 and 48 h of water withdrawal. However, considerable within-treatment spread was observed, which resulted in low sensitivity. When drained blood content was expressed relative to BW, no treatment effects were found. This may be because broiler chickens decrease their feed intake when water is absent (Viola et al., 2009), and will thus be lighter and will also have less blood in their bodies.

The last category of indicators consisted of those that could be used on-farm to detect birds that will only drink occasionally. This can occur when severely lame birds stand up to drink only when very thirsty, or when small birds can only access drinkers at specific times (e.g., when other birds lay down near the drinker and can be used as a stepladder, or when water is spilled). This situation was simulated by the long-term restricted access treatment, for which only drained blood content, capillary refill, skin turgor, and BW were measured. Neither indicator was significantly affected by long-term restricted access (compared with controls), although drained blood content and capillary refill were numerically decreased after applying such a treatment.

Feasibility of application under commercial circumstances is of great importance for welfare assessment in nonexperimental settings. Blood sampling could be carried out rapidly in the slaughter plant, but physiological blood indicators that require chemical analysis in the laboratory may be too time-consuming, impractical, or costly for large-scale monitoring applications. However, these physiological indicators show good potential and therefore it would be valuable to develop easy, not-too-costly methods for measuring them at the slaughter line.

In conclusion, these findings illustrate the potential of animal-based measures to assess different stages of dehydration in broilers. Such tests may form the basis of an on-farm or at-slaughter test that could be included in integrated animal welfare assessment schemes. Of all tested indicators, plasma chloride concentration may be most suitable to detect the effects of transport. The best indicators of medium-term water deprivation were creatinine and sodium. Measuring protocols for more easily applied indicators (blood content, capillary refill) should be optimized if they are to be used to evaluate dehydration because their sensitivity is currently poor.

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