House sparrows (*Passer domesticus*) increase protein catabolism in response to water restriction

Alexander R. Gerson and Christopher G. Guglielmo

Department of Biology, Advanced Facility for Avian Research, University of Western Ontario, London, Ontario, Canada

Running head: Accelerated protein catabolism during acute dehydration

Corresponding Author:
Alexander R. Gerson
Department of Biology
University of Western Ontario,
1151 Richmond Rd.
London, ON, Canada
N6A 5B7
Email: agerson2@uwo.ca
Phone: 519-661-2111 ext 84646
Fax: 519-661-3935
Abstract:

Birds primarily rely on fat for energy during fasting and to fuel energetically demanding activities. Proteins are catabolized supplemental to fat, the function of which in birds remains poorly understood. It has been proposed that birds may increase the catabolism of body protein under dehydrating conditions as a means to maintain water balance because catabolism of wet protein yields more total metabolic and bound water (0.155 g H2O kJ⁻¹) than wet lipids (0.029 g H2O kJ⁻¹). On the other hand, protein sparing should be important to maintain function of muscles and organs. We used quantitative magnetic resonance body composition analysis and hygrometry to investigate the effect of water restriction on fat and lean mass catabolism during short-term fasting at rest and in response to a metabolic challenge (4 h shivering) in house sparrows (*Passer domesticus*). Water loss at rest and during shivering was compared to water gains from the catabolism of tissue. At rest, water restricted birds had significantly greater lean mass loss, higher plasma uric acid concentration and plasma osmolality than control birds. Endogenous water gains from lean mass catabolism offset losses over the resting period. Water restriction had no effect on lean mass catabolism during shivering, as water gains from fat oxidation appeared sufficient to maintain water balance. These data provide direct evidence supporting the hypothesis that water stress can increase protein catabolism at rest, possibly as a metabolic strategy to offset high rates of evaporative water loss.

Keywords:
Protein catabolism, dehydration, metabolic water, total evaporative water loss, magnetic resonance body composition analysis.
Introduction

Birds have an exceptional ability to rapidly mobilize and catabolize fat to fuel metabolically demanding activities such as flight or thermogenesis (21, 25, 36, 46). Supplemental to fat catabolism, it has become apparent that protein in lean tissue is also catabolized during flight, thermogenesis, and at rest (3, 7, 25, 27, 28, 31, 42, 45). Protein is primarily catabolized for energy during phase III of fasting when fat and glycogen stores have been depleted (13), but protein catabolism during phase I of fasting, while an animal still has sufficient energy stores remaining, may be in response to other physiological factors.

Catabolism of protein during flight in birds has been documented through gravimetric changes in muscles and organs (3, 5, 32, 42), and through changes in plasma metabolites such as uric acid (16, 22, 26, 27, 43, 49). Since there is no storage tissue for protein as there is for fat (adipocytes) or carbohydrates (liver and muscle glycogen), protein is used directly from muscles and organs with possible negative consequences to flight performance in the case of muscle catabolism, or nutrient absorption and processing in the case of organ catabolism.

There has been much discussion about the possible role for this seemingly maladaptive phenomenon (3, 7, 11, 12, 14, 25, 29, 31-33, 37, 42). Protein catabolism may be necessary for gluconeogenesis or for the anaplerosis of tricarboxylic acid (TCA) cycle intermediates, both of which may be necessary during sustained fat catabolism (14, 25). The breakdown of protein could also aid in
the maintenance of water balance under dehydrating conditions especially in
uricotelic animals, where the excretion of nitrogenous wastes requires less water
than in ureotelic animals (25, 29, 50). For the same amount of energy released,
catabolism of wet protein results in the release and production of 0.155 g H₂O kJ⁻¹,
approximately five-times more bound and metabolic water than the catabolism of
fat (0.029 g H₂O kJ⁻¹) (25). Thus, protein may serve as a source of endogenous water
to offset water losses, while additionally providing the metabolites necessary for
gluconeogenesis and anapleurosis of TCA cycle intermediates (29).

Many of the studies documenting substantial lean mass losses of birds were
performed on trans-Saharan migrants, or after multi-day non-stop flights in
shorebirds, where water stress is possible (3, 4, 7, 12, 28, 29, 33). Although there is
evidence that long-term dehydration can increase protein catabolism in humans and
Richardson’s ground squirrels (*Spermophilus richardsonii*) (6, 8, 23), to date there
are no studies showing a direct reduction in the total lean mass of an animal due to
acute dehydration. Thus, we postulate that the amount of lean mass catabolized
may not necessarily depend on energetic demands, and may instead be a response
to water deficit or other stressors. If this is the case, it is expected that water-
restriction will increase lean mass catabolism, ultimately resulting in maintenance
of water balance due to endogenous water gains.

Until recently, it has proven difficult to accurately measure the effects of
water restriction on body composition over time within an individual (35, 38).
Techniques such as heavy water dilution for estimating lean mass rely on total body
water and can be confounded by manipulation of water balance (44). Catabolism of
protein in a uricotelic animal results in respiratory quotients (CO$_2$ produced ÷ O$_2$ consumed) very similar to those of fat catabolism (31), precluding the use of respirometry for the determination of fuel mixture including a protein component. Destructive body composition analysis requires a large number of individuals, and would lack the power of a repeated measures design. Therefore, in this study we used a quantitative magnetic resonance body composition analyzer (QMR), which accurately and non-invasively measures lean mass, fat mass, and total body water in un-anaesthetized animals (47, 48). Changes in body composition were monitored longitudinally in individual birds throughout the course of an 18 h water restriction at rest, followed by a 4 h period of elevated metabolic rate (shivering). Our goal was to investigate whether water balance status affects the rate of lean mass catabolism during rest and simulated endurance exercise in house sparrows (Passer domesticus). In order for birds to maintain water balance during the resting phase of this experiment, additional endogenous water production should be necessary from lean mass catabolism. However, during the shivering phase of the experiment high metabolic rates will result in elevated metabolic water production primarily from fat. Depending of the rate of ventilatory water loss during shivering net metabolic water gains could reduce or preclude water required from lean mass catabolism.
Materials and Methods

Animal Care

Male house sparrows (*Passer domesticus*) were caught using mist nets during March of 2008 near the University of Western Ontario campus (London, Ontario, Canada). Birds were moved to the animal care facility within 1 h of capture where they were weighed, individually color banded, and placed in 40 cm X 45 cm X 45 cm cages individually with water and a diet consisting of a mixture of millet seed and Mazuri® Small Bird diet. Birds were maintained on a 12L:12D (lights on at 06:00) cycle at 24°C for the duration of the experiment. Birds were kept for a minimum of two weeks before the experiment began. All animal care protocols followed the Canadian Council on Animal Care guidelines and were approved by the University of Western Ontario Council on Animal Care and the Animal Use Subcommittee (Protocol # 2006-011-04).

Experimental protocol

Sparrows were randomly assigned to either a water-restricted (WR; n = 8) or control (CT; n = 7) group, which were housed individually in adjacent cages, thus controlling for minor fluctuations in light and disturbance in the housing facility.
These pairs of birds followed identical routines throughout the experimental period. Each experimental day between 15:30 and 17:30, one CT bird and one WR bird were weighed, scanned in duplicate using QMR (Initial scan) and returned to their cages. Each replicate QMR scan lasted approximately 90 s. After the initial scan, water was removed from the WR bird. At 07:00 the following morning, food was removed from the WR and CT birds. At 10:00 birds were assumed to be post-absorptive, and were placed in identical adjacent 1 L respirometry chambers maintained at 24°C for a period of two hours to determine resting metabolic rate (RMR) and resting rates of total evaporative water loss (see below), at this time the WR birds had experienced 16-18 h without access to free water. After two hours at 24°C, the birds were scanned in duplicate using QMR (before shivering), blood sampled from the right brachial vein (rest), and then placed in identical individual 1 L respirometry chambers that had been pre cooled to initiate a four-hour shivering trial at 5°C. Shivering metabolic rate (SHMR) and rates of total evaporative water loss were measured (see below). At the conclusion of the shivering trial, birds were scanned in duplicate a third time using QMR (post shivering) and a final blood sample was taken from the left brachial vein (shivering). The bottom of each metabolic chamber was lined with aluminum foil and all droppings were collected and immediately frozen at -30°C after the resting period and the shivering trial. Each individual bird was only used once during the course of the experiment.

Quantitative Magnetic Resonance
Quantitative magnetic resonance body composition analysis has been shown to be extremely accurate and precise, and the principle of the methods has been described elsewhere (36, 45, 49, 50). The instrument we used was specifically designed for use with small birds (model Echo-MRI-B, Echo Medical Systems, Houston, TX, USA). Our validation studies with house sparrows indicate that fat, wet lean, and total body water are measured with precisions (CV) of 3 %, 0.5 %, and 3 %, respectively, and relative accuracies of ± 11 %, ± 1 % and ± 2 %, respectively (Guglielmo, Gerson, McGuire and Seewagen unpublished data). Overall changes in body mass, lean mass, and fat mass were compared between WR and CT groups using repeated measures general linear model (GLM) with initial mass as a covariate. Between treatment effects for each experimental interval (Initial to before shivering and before shivering to post shivering) were assessed by comparing the differential in body mass, lean mass and fat mass for each interval using GLM (SPSS v. 17.0).

Respirometry, hygrometry, and estimated water budgets

Flow through respirometry was used to measure resting (RMR) and shivering metabolic rate (SHMR) simultaneously with rates of evaporative water loss. Incurrent air was scrubbed of CO₂ and water vapor using soda lime and Drierite®, respectively. All four respirometry chambers were well sealed and received constant flow of approximately 600 ml min⁻¹ (measured after the chambers with a Sierra Instruments 840-L mass flow meter, Monterey, CA, USA). Excurrent air was sub-sampled at a rate of 150 ml min⁻¹ through a H₂O analyzer (Licor LI-7000) after
which air passed through a Drierite column to the CO₂ (Sable Systems CA-2A, Las Vegas, NV, USA) and oxygen gas analyzers (Sable Systems FC-1B) with CO₂ and H₂O scrubbing between the two gas analyzers. Gas analyzers were calibrated with a certified standard (20.9% O₂, 2.0% CO₂ balanced with N₂; Praxair, London, Ontario, Canada). Multiplexing allowed measurement of each chamber in 30-minute intervals, with a 10-minute baseline measurement every hour. Two respirometry chambers were placed in a temperature controlled cabinet maintained at 24°C (PTC-1, Sable Systems), while another two chambers were maintained at 2-5°C in a Styrofoam cooler lined with copper tubing that was connected to a water bath (Lauda E100) circulating -8.0°C propylene glycol. This circulating temperature was most effective at maintaining chamber temperature between 2°C and 5°C during the shivering trial. All instruments were connected to an analog to digital converter (UI-2, Sable Systems), which was connected to a laptop computer. Data collection and analysis was done using Expedata software (Sable Systems). Fractional concentrations of O₂ and CO₂ were lag corrected and \( \dot{V}O_2 \) (ml/min), \( \dot{V}CO_2 \) (ml/min) and \( \dot{V}H_2O \) (mg H₂O/h) were calculated from the mean for the final 20 minutes of each 30-minute sampling period for each channel using equations 11.1, 11.6, and 11.9 respectively from (34), assuming that 1 mL of water vapor is equivalent to 0.803 mg H₂O (34). \( \dot{V}H_2O \) at rest and during shivering was extrapolated over time to estimate total water lost during those periods. Endogenous water production (sum of metabolic water and water liberated from catabolism of lean mass) was calculated from the values in (25), and the change in lean and fat mass measured by QMR. Estimated total evaporative water loss and endogenous water production
were compared between treatments using Student's T-test. Upon analysis of the respirometry data, the RQ values were unrealistically low, and it was determined that the fuel cell in the oxygen analyzer had expired, thus $\dot{V}O_2$ data was discarded. $\dot{V}CO_2$ and $\dot{V}H_2O$ during resting and shivering trials were compared between treatment groups using student's t-test.

Uric acid and osmolality determination

Uric acid was determined by endpoint assay (Wako Uric Acid 20R/30R kit) as in (49) for both plasma and droppings. Droppings were weighed and dried to constant mass at 45 °C. Dried excreta was then ground using a small glass mortar and Teflon pestle and dissolved 120-fold (w/v) in 0.1 M glycine buffer, pH 9.3 for analysis; plasma was analyzed undiluted. Excreted uric acid was only compared between treatments during the resting period due to a low number of dropping samples during the shivering trial. Plasma osmolality was measured in 10 μL of plasma using a Wescor Vapro 5520 vapor pressure osmometer calibrated as per the manufacturers instructions. Plasma uric acid and osmolality from rest and shivering blood samples were compared between water restricted and control birds using repeated measures GLM. Excreted uric acid was compared between treatments using t-test (SPSS v.17).
Results

Body Composition

All birds lost mass throughout the experiment (Figure 1a F_{2,20} = 124.769, P=0.001), but WR birds lost on average 1.01 g (4.3 %) more than CT birds (Figure 1a F_{1,10} = 6.785, P=0.026). There was no significant difference in initial mass between treatments (t-test: t = 1.150, DF = 13, P = 0.271) and most mass loss occurred during the resting period for both WR and CT birds. Although mass loss during this time was 0.73 g (3.0 %) greater in water-restricted animals, mass loss at rest was not significantly different between treatments (F_{1,13} = 2.811, P=0.117). The greater mass loss in water-restricted animals was a result of an additional 0.85 g (4.3 %) of lean mass loss overall (F_{1,12} = 21.372, P=0.001). The majority (74.02 %) of the lean mass
loss occurred between the initial and before shivering time points, and during this
time WR birds lost significantly more lean mass than CT birds (Figure 1b $F_{1,13} =$
5.435, $P = 0.036$); there was no significant difference between treatments in lean
mass loss during shivering ($F_{1,13} = 1.410, P=0.256$). No significant differences in fat
mass losses were evident between water restricted and control birds for either the
overnight interval ($F_{1,13} = 0.230, P=0.639$) or during the shivering trial ($F_{1,12} = 2.98,$
$P = 0.108$) (Figure 1c).

Respirometry

No significant differences were found between water restricted and control groups
in $\dot{V}_{CO_2}$ ($t = 0.320, DF = 13, P = 0.754$) or $\dot{V}_{H_2O}$ ($t = 0.675, DF = 13, P = 0.512$) at rest
or during shivering ($\dot{V}_{CO_2} t = -0.495, DF = 14, P = 0.628$; $\dot{V}_{H_2O} t = -1.421, DF = 14, P =$
0.177). Shivering resulted in a significant increase in $\dot{V}_{CO_2}$ over resting in both
treatments (WR: $t = -7.169, DF = 14, P<0.001$; CT: $t = -2.259, DF = 14, P<0.001$; Table
1).

Plasma osmolality and Uric Acid

Plasma osmolality at rest was 7.3% higher in water-restricted birds (Figure
2: $F_{1,11} = 20.080, P = 0.001$), and was 4.97 % higher in WR birds after the shivering
trial (Figure 2: $F_{1,11} = 9.639, P = 0.010$). Plasma concentrations of uric acid were also
elevated in the water-restricted group at rest (Figure 3: $F_{1,11} = 15.384, P = 0.002$).
However, post-shivering, there was no significant difference in plasma uric acid
between the water restricted and control samples (Figure 3: $F_{1,11} = 1.963, P = 0.192$;
Figure 3b). Both water-restricted and control animals experienced an increase in plasma uric acid with shivering (Figure 3: WR: $t = -3.984$, $DF = 7$, $P = 0.005$, CT: $t = -3.736$, $DF = 5$, $P = 0.013$). Treatment did not affect excreta uric acid concentration ($t = -1.60$, $DF = 6$, $P = 0.080$), water content ($t = 0.832$, $DF = 7$, $P = 0.432$), or the total amount of uric acid lost in excreta during the resting period ($t = -1.379$, $DF = 6$, $P = 0.108$), although the uric acid concentration of the excreta as well as the total amount of uric acid lost tended to be higher in WR birds (Table 2).

Water budgets

Water restricted sparrows produced significantly more endogenous water from the catabolism of lean mass at rest ($t = -2.489$, $DF = 14$, $P = 0.026$), but not during the shivering trial ($t = 0.93$, $DF = 14$, $P = 0.927$) and they tended to produce more water during the entire experiment from lean mass catabolism ($t = -2.089$, $DF = 13$, $P = 0.057$). As a result WR birds produced more total endogenous water (water from lean and fat) at rest ($t = -1.94$, $DF = 14$, $P = 0.036$) than control birds. During shivering, CT birds had greater total endogenous water production ($t = 2.27$, $DF = 12$, $P = 0.020$). There were no significant differences in estimated total evaporative water loss between treatments for either the resting ($t = 1.033$, $DF = 13$, $P = 0.320$) or shivering ($t = -0.69$, $DF = 13$, $P = 0.946$) periods. At rest, WR birds maintained positive net water balance, which was significantly higher than CT birds, where net water balance was negative ($t = -2.129$, $DF = 9$, $P = 0.033$). During shivering, metabolic water production exceeded water losses regardless of treatment, but CT birds had greater gains than WR birds ($t = 2.20$, $DF = 13$, $P = 0.023$). Total body
water as a percent of body mass did not change significantly between resting and shivering periods (Rest: $t = -0.045$, $DF = 11$, $P = 0.482$; Shivering: $t = -0.876$, $DF = 7$, $P = 0.204$) (Table 3).

Discussion

This is the first study, to our knowledge, to directly test the hypothesis that birds preferentially catabolize protein as a means to liberate endogenous water under conditions of water stress. The elevated lean mass loss as a result of acute water-restriction as shown by the direct measurement of body composition over time within individuals represents clear support for this hypothesis. Although this hypothesis has been proposed mainly as a strategy for long distance migratory flight
(25, 29), this experiment serves as a proof of concept that a physiological mechanism exists whereby water balance status can influence fuel mixture. Whereas a greater reduction in lean mass occurred only at rest in the water restricted group, it is important to note that during shivering, metabolic water production far exceeded water losses, consequently additional water production from lean mass was unnecessary.

The strongest evidence for accelerated lean mass catabolism as a result of water restriction comes from the QMR data, which was corroborated by the elevated plasma uric acid levels. Changes in body composition coupled with $\dot{V}H_2O$ allowed the estimation of water budgets for each phase of this experiment, which indicate that higher rates of lean mass catabolism in the WR birds resulted in endogenous free water gains sufficient enough to offset evaporative losses at rest. During shivering, metabolic rate was elevated approximately 2.5-fold regardless of treatment. This elevation in metabolic rate was primarily fuelled by fat catabolism, and the relative contribution from lean was similar for each treatment. Since water balance was maintained during the resting period, at the expense of lean mass in the WR birds, elevated lean mass catabolism during shivering was unnecessary for the WR birds.

Birds typically maintain plasma volume during water restriction (10), discounting the possibility that elevated osmolality and uric acid were simply a product of reduced plasma volume, rather than an increase in the actual metabolites responsible. In fact, elevated plasma osmolality may be a response to dehydration that facilitates the maintenance of plasma volume by favoring the movement of
intracellular water to blood vessels, down the osmotic gradient thus resulting in
cellular dehydration (1). There is evidence indicating hyperosmolality alone may
influence cellular metabolism in mammals, ultimately resulting in elevated protein
catabolism (6, 23), whether this mechanism exists in birds has yet to be explored.
The resulting endogenous water production may alleviate cellular dehydration
while amino acids and peptides may bring intracellular osmolality towards
equilibrium with the interstitial fluid and plasma. This mechanism would not only
liberate water, but would reduce the osmotic gradient between the intra- and inter-
cellular compartments. Taking this into account, the decrease in plasma osmolality
during the shivering trial in the WR birds could be a product of water gains due to
higher metabolism overall, and thus greater metabolic water production, resulting
in the expansion of plasma volume during the shivering period.

Responses of birds to extended dehydration typically include reduced
glomerular filtration rate and greater tubular water re-absorption, resulting in
production of more concentrated urine (19, 20). Although no significant differences
were found between treatments, the trends in uric acid excretion are consistent
with this response. However, the degree of the response is subtle, perhaps because
the period of water restriction in the current experiment was relatively short. Birds
are uricotelic and utilize extrarenal water reabsorption in the colon to minimize
excretory water losses (20). For this reason, birds may be distinct from mammals
in their ability to benefit from a protein-for-water strategy so long as urine does not
reach a concentration where extrarenal absorption is repressed (19). Although,
highly concentrated urine is unlikely in the current study due to the relatively short
water restriction and the maintenance of water balance from elevated protein catabolism. Body water as a percent of body mass did not change throughout the experiment, but it has been noted that the amount of water relative to body mass is not a good indicator of water stress or dehydration (29).

Due to the limited amount of plasma available, other analysis were not possible in this study. The response of arginine vasotocin (AVT), prolactin, aldosterone, or corticosterone to water restriction could have provided insight into possible control of fuel selection during water restriction. Although, this study did not differ substantially in terms of magnitude or duration of water restriction from many other studies that have thoroughly investigated the hormonal response to acute water restriction in birds at rest and during exercise (2, 17, 19, 39). Corticosterone does affect fuel use in birds, and would be the likely hormone responsible for elevated lean mass catabolism. However, experimental design must incorporate careful control of handling and other stressors during the experiment for measurements of corticosterone to be meaningful.

The protein for water phenomenon could have broad implications during many life history stages of birds including breeding and migration, especially in light of projected changes in climate (24). The use of protein for water may help explain recent evidence that birds actually fly under conditions unfavorable to water balance (41), taking advantage of favorable winds, and possibly maintaining water balance at the expense of protein. If water stress during migratory flight or stopover refueling results in additional lean mass catabolism, refueling rates may be
reduced (40) leading to increased stopover duration, delaying arrival on the
breeding grounds (9).

Perspectives and Significance:

Since much of the discussion surrounding the hypothesis that protein can be stored
and used for water production has focused on migratory flight, it would seem logical
to design experiments in order to test the possibility that water balance may
influence the proportions of fat and lean mass utilized during flight. To this end, we
feel that the present study utilizes a simple yet informative suite of minimally
invasive techniques that could be implemented to study the possible effects of
environmental conditions on fuel mixture utilization in avian flight. It would then be
interesting to modify existing fuel use models for bird flight to examine the possible
consequences of dehydration to flight range in terms of changing fuel mixture (11, 12, 29, 30). It should be noted that due to the very high metabolic rates experienced
during flight, a vast amount of metabolic water is produced from the catabolism of
fat alone. Whether this water production is balanced by water loss depends entirely
on the ambient conditions experienced during flight. Rates of water loss are
constant across temperatures during flight, until a threshold temperature is reached
above which evaporative water loss increases with temperature (15, 18). This
threshold temperature has been identified to be around 20°C for both rose-colored
starlings (Sturnus roseus) and pigeons (Columbia livia) (15, 18). Flying at
temperatures above this threshold may result greater lean mass utilization for
water in flight. Future studies should investigate this phenomenon in resting and
exercising animals in order to more fully understand the mechanisms involved in
the control of lean mass catabolism as well as the osmoregulatory consequences of
lean mass catabolism during water restriction at both the cellular and whole animal
levels.

Acknowledgements and Funding
We would like to thank Alice Boyle, Tara Crewe, Liam McGuire, Silke Nebel and
Bethany Thurber for helpful advice on statistical analysis and with the preparation
of this manuscript. Edwin Price for advice on the manuscript and for assistance with
captive birds. Dr. Brent Sinclair and Caroline Williams for access to and assistance
with the Licor LI-7000 and Dr. Donglin Bai and Dr. Xiang-Qun Gong for access to the
vapor pressure osmometer. ARG was supported by an NSERC Alexander Graham
Bell Canada Graduate Scholarship. This study was funded by an NSERC Discovery
Grant and a Leaders Opportunity Grant from the Canada Foundation for Innovation
and Ontario Research Fund.
References


Figures:

Figure 1. a) Water restriction resulted in greater mass loss overall. b) Lean mass loss in WR birds was greater during the resting period and overall. c) No significant differences in fat mass losses were evident between water restricted and control birds. WR: n = 8, CT: n = 7. Values are means ± SD. * Indicates significant difference between WR and CT at P < 0.05.

Figure 2. Plasma osmolality was significantly elevated by WR both at rest, and post-shivering (WR: n = 8, CT: n = 7). Values are means ± SD. * Indicates significant differences between treatments at P< 0.05.

Figure 3. Plasma concentrations of uric acid were elevated in the water-restricted group at rest, but not after shivering. Both treatments had increased plasma uric acid post-shivering (WR: P = 0.005, CT: P = 0.013). Values are means ± SD. * Indicates significant differences between treatments at P< 0.05.
Table 1. $\dot{V}CO_2$ and $\dot{V}H_2O$ at rest and during shivering in WR and CT birds. No significant differences were found between WR and CT. Shivering resulted in significantly elevated $\dot{V}CO_2$ over resting values within each treatment group. * Indicates $P < 0.05$. Values are means (SD).

Table 2. Uric acid concentration, moisture and total uric acid lost in excreta at rest during respirometry from WR and CT birds. No significant differences were evident between treatments. Values are means (SD).

Table 3. Estimated water budgets for WR and CT treatment groups throughout an 18 – 20 h dehydration period, followed by a 4 hour shivering trial. Total endogenous water production was calculated from changes in body composition, assuming 0.155 g H2O kJ^{-1} for lean mass and 0.029 g H2O kJ^{-1} for fat as in (25). Net water balance is the difference between total evaporative water loss and total endogenous water production, CT birds had access to water during the resting period. Body water % is the QMR value for total body water divided by total body mass for the before and after shivering time points. * indicates significant differences ($P < 0.05$) between treatment groups, but within metabolic states. Values are means (SD).
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>WR</strong></td>
<td></td>
<td><strong>CT</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>Shivering</td>
<td>Rest</td>
<td>Shivering</td>
</tr>
<tr>
<td>$\dot{V}_{CO_2}$</td>
<td>2.00</td>
<td>5.02 (1.68)*</td>
<td>1.88 (1.35)</td>
<td>4.54 (2.21)*</td>
</tr>
<tr>
<td>(ml CO$_2$ min$^{-1}$ g$^{-1}$)</td>
<td>(0.28)  &amp; 5.40 (1.22)</td>
<td>4.33 (1.64)</td>
<td>4.85 (1.01)</td>
<td></td>
</tr>
<tr>
<td>$\dot{V}_{H_2O}$</td>
<td>3.89</td>
<td>5.40 (1.22)</td>
<td>4.33 (1.64)</td>
<td>4.85 (1.01)</td>
</tr>
<tr>
<td>(ml H$_2$O min$^{-1}$ g$^{-1}$)</td>
<td>(0.77)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REST</td>
<td>Uric Acid (mg/g)</td>
<td>% Moisture</td>
<td>Uric Acid lost (mg)</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Water Restricted</td>
<td>418.01 (66.61)</td>
<td>5.97 (6.09)</td>
<td>344.01 (65.78)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>309.91 (135.20)</td>
<td>9.74 (8.06)</td>
<td>261.62 (116.28)</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.080</td>
<td>0.147</td>
<td>0.108</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------</td>
<td>---------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>WR</td>
<td>CT</td>
<td>WR</td>
<td>CT</td>
</tr>
<tr>
<td>Total Evaporative water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>loss (mL)</td>
<td>1.85 (0.42)</td>
<td>2.20 (0.85)</td>
<td>0.554 (0.11)</td>
<td>0.550 (0.11)</td>
</tr>
<tr>
<td>Endogenous water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>production Lean (g)</td>
<td>1.40 (0.41)*</td>
<td>0.769 (0.59)</td>
<td>0.487 (0.11)</td>
<td>0.567 (0.15)</td>
</tr>
<tr>
<td>Total Endogenous water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>production (g)</td>
<td>2.19 (0.79)*</td>
<td>1.48 (0.68)</td>
<td>0.964 (0.14)*</td>
<td>1.14 (0.17)</td>
</tr>
<tr>
<td>Net water balance (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.348 (0.54)*</td>
<td>-0.443 (1.43)</td>
<td>0.409 (0.18)</td>
<td>0.598 (0.15)</td>
</tr>
<tr>
<td>Body water (% of Mb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>63.07 (2.32)</td>
<td>63.00 (3.91)</td>
<td>64.41 (3.12)</td>
<td>62.93 (2.52)</td>
</tr>
</tbody>
</table>