

Daily energy expenditure and water turnover in two breeds of laying hens kept in floor housing



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ARTICLE INFO

Article history:

Received 18 June 2020

Received in revised form 4 August 2020

Accepted 5 August 2020

Available online 15 December 2020

Keywords:

Chicken

Field metabolic rate

Isotopes

Poultry

Water metabolism

ABSTRACT

Laying hens are increasingly kept in barn or free-range systems, which not only allows birds to move freely but also potentially entails higher energy expenditures due to higher locomotor activity. Therefore, the aim of our study was to quantify the daily energy expenditure (DEE) and water turnover in freely moving laying hens. For that purpose, 10 Lohmann Selected Leghorn (LSL) and 10 Lohmann Brown (LB) hens were obtained from a conventional breeding company at 17 weeks of age. The trial started when birds reached an age of 34 weeks. All 20 birds were kept together in the same littered floor pen (12.1 m²). The pen was equipped with perches, a nest box, feeding and nipple drinkers. The DEE was determined individually for all experimental birds ($n = 20$) for a total of nine days using the doubly labelled water (DLW) method. Lohmann Brown hens were heavier than LSL hens, but laying rate did not differ between the two breeds, that is, one egg per hen and day during the study period. Average egg mass was 63.1 ± 0.20 g in LB and 61.7 ± 0.12 g in LSL hens, which converted to an egg energy content of 420 and 410 kJ/egg, respectively. Dilution spaces for oxygen and hydrogen differed between the breeds but not the respective turnover rates. Total body water as a percentage of body mass (LB: 54.4%, LSL: 53.8%; SEM = 0.7, $F_{1,18} = 0.41$, $P = 0.513$) and total water intake (TWI) per day (LB: 275 ml/day, LSL: 276 ml/day; SEM = 20, $F_{1,17} = 0$, $P = 0.994$) did not differ between LB and LSL hens. Individual DEE increased with body mass in LB but not in LSL hens. Average DEE did not differ between the two breeds (LB: 1501 kJ/day; LSL: 1520 kJ/day; SEM = 32.1, $F_{1,17} = 2.54$, $P = 0.131$). However, when comparing the DEE on a metabolic mass basis, LSL hens expended with $984 \text{ kJ/kg}^{0.75}$ on average significantly more energy per day than LB hens ($895 \text{ kJ/kg}^{0.75}$; SEM = 20.3, $F_{1,18} = 10.1$, $P = 0.005$). Our results suggest that the DLW technique is a viable method to measure the energy expenditure and water turnover over several days in laying hens. Furthermore, we show that laying hens kept in floor pens fit into the general pattern of DEE among wild birds.

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Implications

Our study provides the first quantitative data on the daily energy expenditure in two breeds of freely moving laying hens. We show that on a metabolic mass basis Lohmann Selected Leghorn hens expend more energy compared to Lohmann Brown hens and that established recommendations for the energy supply in laying hens seem to slightly underestimate the energy requirements in Lohmann Selected Leghorn hens. Furthermore, we show that laying hens kept in floor pens fit into the general pattern of daily energy expenditure among wild birds.

Introduction

From an animal welfare perspective, husbandry systems for laying hens in Europe have improved in recent years. In the European Union, battery cages for chickens were banned in 2012 (EU council directive 1999/74/ECC). Since then, laying hens are increasingly kept in barn or free-range systems, which not only allows birds to move more freely but also potentially entails higher energy expenditures for the chickens, for example, due to higher locomotor activity. While some studies have investigated the metabolic rate and thus the energy expenditure under laboratory conditions using open flow respirometry or indirect calorimetry in poultry (Van Kampen, 1976a, 1976b and 1976c; Fuller et al., 1983; MacLeod et al., 1988; Kim et al., 2014), so far there is no published study that measured the energy expenditure in unrestrained freely moving chickens. One of the most widely used methods to measure the metabolic rate of animals in the field, also known as field metabolic rate, and thus in their natural habitat is

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the doubly labelled water (**DLW**) method (Lifson and McClintock, 1966; Speakman, 1997; Butler et al., 2004). The method allows measurements of the total amount of energy expended by an animal over a certain time while carrying out all its normal activities such as thermoregulation, reproduction and locomotion. The method involves the enrichment of the body water of an animal with both oxygen and hydrogen isotopes. While the hydrogen isotope leaves the body as water only, the oxygen isotope is lost not only as water but also as respiratory CO₂. The difference between the elimination rates of hydrogen and oxygen isotopes is used to estimate the CO₂ production, which together with the respiration quotient yields the O₂ production and thus an estimate of the daily energy expenditure (**DEE**) (Nagy, 1987; Speakman, 1997; Butler et al., 2004). The DLW technique is currently the most reliable method for estimating energy expenditure in unrestrained free moving animals and has been used to measure the DEE in numerous mammalian and bird species (for review, see Speakman, 2000; Nagy, 2005; Capellini et al., 2010; Riek and Bruggeman, 2013; Hudson et al., 2013). Furthermore, the DLW method also allows the measurement of the total water turnover of an individual animal including water from drinking, feed and metabolism. Recommendations for water requirements in poultry however are usually reported on a flock basis rather than on a single animal basis, mainly due to difficulties of measuring individual water intake in poultry kept in flocks (National Research Council, 1994; Manning et al., 2007). The isotope dilution technique offers thus a viable and precise method to measure individual water turnover in poultry.

Therefore, the aim of our study was to quantify the DEE and water turnover in freely moving laying hens. We compared the DEE between two widely used laying hen breeds, that is, Lohmann Selected Leghorn (**LSL**) and Lohmann Brown (**LB**). Additionally, we compared the measured DEE with established recommendations on energy requirements for laying hens and with published DEE data of other bird species.

Material and methods

Animals and management

Ten LSL and ten LB hens were obtained from a conventional breeding company (Zahrte, Wrestedt, Germany) at 17 weeks of age. The trial started when birds reached an age of 34 weeks (240 days; i.e., approx. time of peak egg production) and lasted for nine days. All 20 birds were kept together in the same littered floor pen of 12.1 m² in size from an age of 17 weeks. The pen was equipped with a total of 7.8 m of perches at a height of 80 cm above the ground attached to the manure pit, a nest box (0.54 m²), two feeding troughs (40 cm diameter) and one watering trough with eight nipples. Animals had ad libitum access to food and drinking water throughout the trial. Feed offered was a commercial laying hen feed with the following composition: 88.7% DM, 15.5% CP, 5.2% crude fat, 3.4% crude fibre, 39.4% starch, 3.3% sugar, 12.8% ash, 3.5% calcium, 0.55% phosphorus and 11.2 MJ metabolizable energy. The light schedule was 14 h light to 10 h dark (light onset at 0400 h, light offset at 1800 h, with a 30 min dim phase at each transition), with 20 lx light intensity. The daily number and combined weight of eggs were recorded for LB and LSL separately (note: LB eggs are brown and LSL eggs are white). Three weeks before the start of the experiment, birds were equipped with numbers on their backs to distinguish birds individually for measurements of energy expenditure and water turnover. Ambient temperature (T_a ; resolution: 0.0625 °C) and relative humidity (RH; resolution: 0.04%) were not strictly controlled but recorded continuously throughout the trial with miniature data loggers at 10 min intervals at approx. 1.5 m above the ground (i-Button, DS1923#F5, Maxim Integrated Products, Sunnyvale, CA, USA).

Daily energy expenditure and water turnover

The DEE was determined individually for all experimental birds ($n = 20$) for a total of nine days using the DLW method (Lifson and McClintock, 1966; Speakman, 1997). On the first and last day of the DEE measurements, the body mass for each bird was recorded with a mobile scale (Weighing System IP 65, resolution: 0.001 kg, Sartorius GmbH, Göttingen, Germany). On day one of the DEE measurement, a blood sample of 1 ml was drawn from the wing vein of every bird using a indwelling cannula (Braun, Melsungen, Germany) to estimate the background isotopic enrichment of ²H and ¹⁸O in the body fluids (method D of Speakman and Racey, 1987). Subsequently, each bird was injected intramuscularly with 0.52 ± 0.06 g DLW/kg body mass (65% ¹⁸O and 35% ²H; 99.90% purity). The individual dose for each bird was determined prior to the injection according to its body mass. The actual dose given was gravimetrically measured by weighing the syringe before and after administration to the nearest 0.001 g (Sartorius model CW3P1-150IG-1, Sartorius AG, Göttingen, Germany). All birds were then held without access to food or water for a 2 h equilibration period, after which a further 1 ml blood sample was taken. Further blood samples were taken at five and nine days after dosing to estimate the isotope elimination rates. All blood samples were drawn into blood tubes containing EDTA. Whole-blood samples were pipetted into 2 ml glass vials and stored at -20 °C until determination of ¹⁸O and ²H enrichment. Blood samples were vacuum distilled (Nagy, 1983). Water from the resulting distillate was used to estimate the isotope enrichments of ¹⁸O and ²H by Off Axis Integrated Cavity Output Laser Spectroscopy using a liquid water analyser (Los Gatos Instruments Inc., San Jose, USA). This method has been validated for DLW measurements against indirect calorimetry (Berman et al., 2012; Melanson et al., 2018).

Samples were run alongside five lab standards for each isotope (calibrated to the IAEA International Standards: SMOW and SLAP) to correct delta values to ppm. Isotope enrichment was converted to values of CO₂ production using a single pool model as recommended by Speakman (1993). We assumed a fixed evaporation of 25% of the water flux, as this has been shown to minimize error in a range of applications (Visser and Schekkerman, 1999; Van Trigt et al., 2002). Specifically, carbon dioxide production rate per day in moles was calculated using eq. 7.17 from Speakman (1997). The DEE was calculated from carbon dioxide production by assuming a respiration quotient of 0.85. Isotope analyses and calculations were made blind of the status of the animals (i.e. breed and animal ID). Total body water (mols) was calculated using the intercept method (Speakman, 1997) from the dilution spaces of both oxygen (N_O) and hydrogen (N_H) under the assumption that the hydrogen space overestimates total body water by 4% and the oxygen-18 space overestimates it by 1% (Schoeller et al., 1986). The DEE measured by the DLW also includes the part of the energy required for egg production, but not the energy of the egg. Thus, the daily average egg weight was multiplied with the egg energy content (i.e. 670 kJ/100 egg mass, Jeroch et al., 1999) and added to the DEE to give total metabolizable energy demands. The TWI (ml/day) that consists of drinking water, preformed water ingested in food and metabolic water, was estimated as the product of the deuterium space and the deuterium turnover rate (Ofstedal et al., 1983).

To compare the measured DEE with energy recommendations for laying hens, apparent metabolizable energy (**AME**, kJ/day) was calculated using the following published formula:

$$AME = [480 + (15 - T_a) \times 7] \times BM^{0.75} + 23 \times dW + 9.6 \times dEM$$

where T_a = ambient temperature (correction for $T_a < 15$ °C, did not apply in our trial), dW = change in BW, BW = body weight, dEM = daily

egg mass. For birds kept in floor pens, as it was the case in our study, an additional 10% was added to maintenance requirements (Gesellschaft für Ernährungsphysiologie, 1999; Jeroch et al., 2011).

Statistical analysis

To compare the two breeds (LSL and LB), a mixed model was used with breed as a fixed factor and body mass a covariate for physiological measurements (TWI and DEE) using the MIXED procedure in SAS version 9.4 (SAS, Inst. Inc., Cary, NC, USA). Data are expressed as Least Square-means \pm SEM or means \pm SD where appropriate.

We also compared the relation between body mass and DEE in laying hens with published DEE values in wild bird species measured by the DLW method (Nagy et al., 1999). For that purpose, we assessed our results with published data on DEE and body mass in avian species using the phylogenetic general least square (PGLS) approach in order to account for the potential lack of independence between species, because of their shared evolutionary history. The statistical procedures have been described in detail elsewhere (Felsenstein, 1985; Garland et al., 1992; Garland and Ives, 2000; Rohlf, 2001; Freckleton et al., 2002). In brief, the phylogeny was derived from a published avian supertree (Jetz et al., 2012 and 2014) available at birdtree.org. The avian supertree was pruned to include only the species of concern (i.e. species for which DEE values were available, see Supplementary Material S1) using the treehouse application (Steenwyk and Rokas, 2019) in R version 3.6.0 (R Core Team, 2018). The method of PGLS was implemented for the log-transformed trait data using the 'Comparative analyses of phylogenetics and evolution' package (CAPER; Orme et al., 2012) in R using Pagel's branch length transformation (λ), determined by maximum likelihood (Pagel, 1992).

Results

For the duration of this study, T_a and RH were almost constant and were on average 20.8 ± 0.03 °C and $35.1 \pm 0.10\%$, respectively. The T_a did not fall below 18 °C at any time during the study. Lohmann Brown hens were heavier than LSL hens (Table 1) but egg laying rate did not differ between the two breeds, that is, one egg per hen and day during the study period of nine days. Average egg mass was 63.1 ± 0.20 g in LB and 61.7 ± 0.12 g in LSL hens, which converts to an egg energy content of 420 and 410 kJ/egg, respectively. Dilution spaces for oxygen and

Table 1
Body mass, dilution spaces for ^{18}O (N_{O}) and ^2H (N_{H}), respective turnover rates (k_{O} , k_{H}) and measured physiological variables in Lohmann Brown (LB) and Lohmann Selected Leghorn (LSL) hens over a measuring period of nine days (240–249 days of life).

Parameters	LB	LSL	Effect of breed		
	(N = 10)	(N = 10)	SEM	F-value	P-value
Body mass (g)	2073	1817	80	10.22	0.005
N_{O} (g)	1100	958	29	11.97	0.003
N_{H} (g)	1127	976	29	13.53	0.002
k_{O} (per day)	0.014	0.015	0	0.84	0.372
k_{H} (per day)	0.011	0.011	0	0.45	0.513
Total body water (% of BM)	54.4	53.8	1	0.41	0.531
Total water intake ¹					
(ml/day)	275	276	20	0	0.994
(ml · kg ^{-0.83} BM/day)	156	163	6	0.66	0.426
Energy expenditure ^{1,2}					
(kJ/day)	1511	1565	50	1.18	0.292
(kJ · kg ^{-0.75} BM/day)	895	984	20	10.12	0.005
Calculated energy requirements ³	1517	1419	19	13.55	0.002
(kJ/day)					
Energy expenditure/Calculated energy requirements (%)	101.6	108.4	2	6.53	0.020

Values are LS-means with respective model parameters.

¹ Body mass included as a covariate.

² Including daily egg production.

hydrogen differed between the breeds but not the respective turnover rates (Table 1). Total body water as a percentage of body mass was nearly identical for both breeds. Furthermore, the TWI whether expressed as a total amount or on a body mass basis did not differ between LB and LSL hens (Table 1). Individual DEE increased with body mass in LB but not in LSL hens (Fig. 1A). Average DEE did not differ between the two breeds (Table 1). However, when comparing the DEE on a metabolic mass basis, LSL hens expended on average significantly more energy per day than LB hens (Table 1, Fig. 1B). Calculated energy requirements were higher in LB than in LSL hens. Comparing measured DEE with calculated energy requirements revealed that LB hens closely followed calculated recommendations, whereas LSL hens had on average 8% higher energy expenditures than predicted from calculations (Table 1).

Published DEE values of 95 avian species, ranging in body mass from 3.7 g (*Archilochus alexandri*, Black-chinned hummingbird) to 88.3 kg (*Struthio camelus*, Ostrich) and our results on laying hens, were available

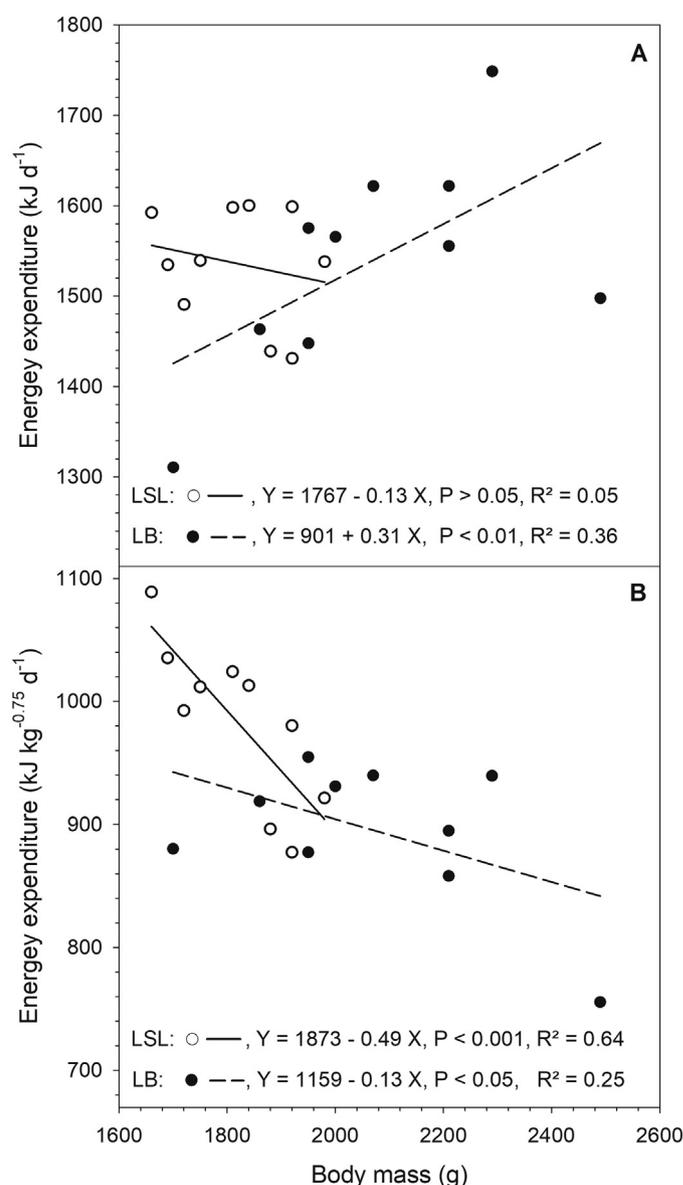


Fig. 1. Relationship between body mass and energy expenditure (expressed as kJ/day, A and on a metabolic mass basis, that is, kJ · kg^{-0.75} per day, B) in freely moving Lohmann Brown (●, LB; n = 10) and Lohmann Selected Leghorn (○, LSL; n = 10) hens. Energy expenditure was measured using the doubly labelled water method (see text for details).

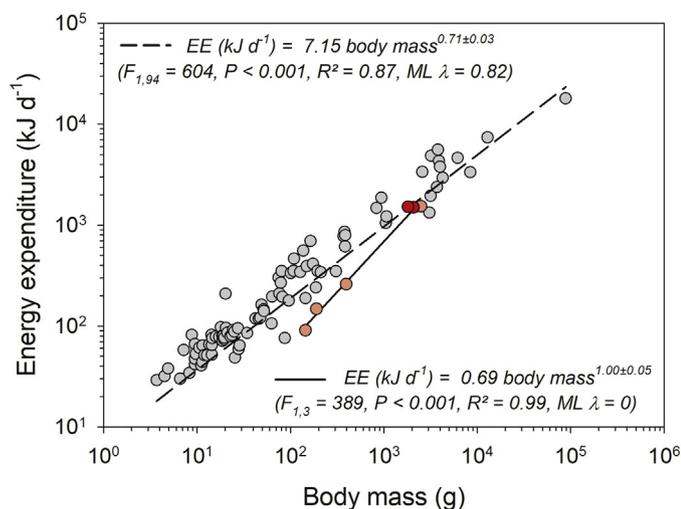


Fig. 2. Relationship between average energy expenditure (EE) and body mass in birds measured using the doubly labelled water method. Each grey and pink data point represents a different avian species ($n = 95$) published in Nagy et al. (1999) and the two red data points are results from the present study on laying hens (see Table 1). Red and pink data points represent species of the order Galliformes. The dashed line is the phylogenetic corrected relationship for all data points and the solid line for Galliformes species only.

for phylogenetic analysis. The resulting phylogenetically corrected allometric regression equation was DEE (kJ/day) = 7.15 body mass $^{0.71 \pm 0.03}$ with an estimated maximum likelihood λ of 0.82 (Fig. 2).

Discussion

No other study has been published measuring the DEE in laying hens using the DLW method. There are however studies that have investigated the energy expenditure in poultry under laboratory conditions using open flow respirometry or indirect calorimetry (e.g. Van Kampen, 1976b, 1976a and 1976c; Fuller et al., 1983; MacLeod et al., 1988; Kim et al., 2014). In these studies, birds were confined to metabolic chambers to measure the energy expenditure of specific behaviours or physiological processes. Thus, these studies are not directly comparable to our study since they did not measure energy expenditure over an extended timespan in unrestrained freely moving birds. Comparing our results on DEE with established energy recommendations for laying hens (Gesellschaft für Ernährungsphysiologie, 1999) suggests that current energy recommendations slightly underestimate actual energy expenditure for freely moving LSL hens kept indoors. There are anecdotal reports that LSL hens have a higher locomotor activity compared to LB hens. Thus, a possible explanation for the higher actual energy expenditure in LSL hens compared to energy recommendations could be that the extra 10% added for maintenance requirements in laying hens kept in floor pens (see energy recommendation equation above) might be too low for LSL hens but just right for LB hens. This would be in agreement with our result that LSL hens expended on a metabolic mass basis significantly more energy per day compared to LB hens. However, this cannot be verified as so far no published studies have quantified the differences in locomotor activity between LB and LSL hens. Therefore, future studies should look specifically at the differences in locomotor activity considering different husbandry systems (e.g. floor pens, free range, etc.) over an extended timespan between different genetic lines of the domestic chicken.

The differences in measured dilution spaces of ^{18}O and ^2H were in the range of reported values for birds (for review of different bird species measured using the DLW technique, see Nagy et al., 1999). Similarly, the mean ratio of $\text{N}_2/\text{H}_2\text{O}$ of all birds (1.022 ± 0.01) was close to reported values in other bird species (1.0 – 1.1). In our study, body

water expressed as a percentage of body mass was with approx. 54% the same for both breeds and in the range of reported values for layers (Johnson and Farrell, 1988), and thus slightly lower than values published for turkeys (Riek et al., 2008), emus (Dawson et al., 1983) and domesticated ostriches (Degen et al., 1991). Furthermore, the average TWI was nearly identical for both breeds. The TWI includes not only drinking water but also preformed water from ingested feed and metabolic water. Metabolic water, that is, water produced by the oxidation of nutrients, can be calculated from the feed composition where 1 g of metabolized protein, fat and carbohydrate yields 0.50, 1.07 and 0.56 g of H_2O , respectively (Schmidt-Nielsen, 1997). Thus, in our study, hens ingested from feed on average 113 ml of preformed water per kg of feed intake and produced 277 ml of metabolic water per kg of DM intake. We did not record individual feed intake, because an important part of our study was measuring the energy expenditure in freely moving chickens kept in a flock to let hens exhibit associated behaviours such as nesting, scratching and social interactions. However, data from published studies suggest that approx. 78% of the TWI in laying hens is ingested via drinking water (Vogt, 1987), which translates in our study to an average of 215 ml of drinking water per day. This is in close agreement with measured drinking water intakes in adult brown and white leghorn laying hens (214–228 ml/day; National Research Council, 1994). The remaining 65–66 ml per day of the TWI in our study can thus be attributed to preformed water from feed and metabolic water. Therefore, the present results of TWI in laying hens demonstrate that the isotope dilution method is a viable method for measuring individual water intakes in laying hens as it has been already shown for turkeys (Riek et al., 2008). Thus, this method could be of use, for example, for establishing reference values for water consumption in different poultry species kept in large flocks under various husbandry systems.

We compared our DEE results in laying hens to DEE measurements in wild bird species measured by the DLW method, published by Nagy et al. (1999). For that purpose, we calculated a phylogenetic corrected regression equation using the PGLS approach which includes the derivation of the parameter λ . Intermediate values of λ ($0 < \lambda < 1$) indicate that the trait evolution is phylogenetically correlated, but does not follow fully a Brownian motion model (Pagel, 1999; Freckleton et al., 2002). The phylogenetic signal λ thus describes a pattern in which close evolutionary relatives have more similar trait values than more distant relatives (White et al., 2009). In our case, λ was 0.82 which indicates that DEE in birds is strongly phylogenetically correlated. The phylogenetic corrected regression equation allows the comparison between predicted and actual DEE values (DEE , kJ/day = 7.15 body mass $^{0.71 \pm 0.03}$, Fig. 2). Predicted DEE values for laying hens from the equation for all birds were 1626 and 1480 kJ/day for LB and LSL hens, respectively. This is about 8% higher than the actual measured DEE for LB hens (1501 kJ/day) and only 3% lower than the actual measured DEE for LSL hens (1520 kJ/day). This seems to suggest that domestication and selection for a high egg laying frequency in laying hens did not substantially increase overall energy expenditure compared to wild birds of similar size. Interestingly, the exponent of the phylogenetic corrected regression equation for the relationship between DEE and body mass in all birds (0.71 ± 0.03 , 95% CI 0.56–0.96) is very close to the one found for mammals (0.70 , 95% CI 0.65–0.74; Capellini et al., 2010). We also compared our results from laying hens with other published DEE values of wild Galliforme species published in Nagy et al. (1999). The resulting regression equation was different in slope and intercept (DEE , kJ/day = 0.69 body mass $^{1.00 \pm 0.05}$, Fig. 2) compared to the equation for all bird species (see above). At the lower end of the body mass range of Galliforme species, the DEE seems to be lower compared to similar sized birds of other orders. Furthermore, our results on DEE in laying hens are on average nearly identical to the only other measured similar sized bird species of the order Galliformes so far, that is, the Sage grouse (*Centrocercus urophasianus*) with 1540 kJ/day (Vehrencamp et al., 1989). However, these results need to be treated with caution until further systematic studies on domesticated bird species are available. Furthermore, it needs to be emphasized that

predicting values for missing species that have not been measured from phylogenetic regression equations, as it is sometimes done in comparative analysis, are likely to be incorrect as the fit-lines are phylogenetically controlled and thus will not account for the phylogenetic history of the missing species.

Conclusion

Our results suggest that the DLW technique is a viable method to measure the energy expenditure and water turnover over several days in laying hens. Lohmann Brown and Lohmann Selected Leghorn hens have a similar total energy expenditure, but the DEE of those two breeds differs on a metabolic mass basis. Comparing our results with established energy recommendations for laying hens indicates that for LSL hens these recommendations might slightly underestimate the actual energy expenditure. However, as we did not measure the expense of energy for specific behaviours and physiological process separately, these results need to be treated with caution until more studies on DEE in poultry are available.

Supplementary materials

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.animal.2020.100047>.

Ethics approval

Procedures performed in this study were in accordance with the German animal ethics regulations and approved by the State Office of Lower Saxony for Consumer Protection and Food Safety, Germany (Ref. no.: 33.19-42502-04-18/2937).

Data and model availability statement

None of the data were deposited in an official repository. The data gathered during the current study and models used are deposited at the Institute of Animal Welfare and Animal Husbandry of the Friedrich-Loeffler-Institut, Germany. Anybody who is interested can apply for access rights at the Friedrich-Loeffler-Institut.

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Author contributions

Alexander Riek: conceptualization, methodology, formal analysis, investigation and writing – original draft. Stefanie Petow and John R. Speakman: methodology, writing – review and editing. Lars Schrader: funding acquisition, writing – review and editing.

Declaration of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Gabriele Kirchhof, Silke Werner, Klaus Gerling and Karsten Knop from the Institute of Animal Welfare and Animal Husbandry of the Friedrich-Loeffler-Institut for technical help and Catherine Hamby from the Institute of Biological and Environmental Sciences of the University of Aberdeen for the isotope analysis.

Financial support statement

This research received no specific grant from any funding agency, commercial or not-for-profit section

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