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Assessment of stable areas for young ostriches based on physiological stress parameters

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ABSTRACT

Commercial ostrich farming continues to develop globally, however, there is a lack of scientific research on ostrich welfare related to husbandry conditions. To assess needed stable size, young ostriches were kept in three groups of 8 animals on 20 m^2 (i.e. 2.5 m^2/animal), 40 m^2 (i.e. 5 m^2/animal) and 80 m^2 (i.e. 10 m^2/animal) of stable space with access to 2500 m^2 of pasture for each group. Feather corticosterone (fCort) as well as blood heterophil/lymphocyte (H/L) ratio, creatine kinase (CK), aspartate aminotransferase (AST) and uric acid (UA) were evaluated at 6, 9, 12 and 15 months of age as indicators of chronic stress. fCort dropped over time in groups in larger stables while fCort of ostriches on 2.5 m^2/animal remained near starting levels ($p = 0.0599$). H/L ratio, AST and CK yielded no differences between groups. UA plasma concentrations were significantly higher in the group on 2.5 m^2/animal ($p < 0,01$). During a period when birds had to be stabled because of avian influenza restrictions, fCort and H/L ratio showed an upward trend though this was overshadowed by individual differences. Our findings suggest a negative effect on ostriches kept on 2.5 m^2/animal but no additional benefits for ostriches kept on stables of 10 m^2/animal over those on 5 m^2/animal. For further assessment of stable sizes ethological studies are necessary.

1. Introduction

Ostrich farming started in the 1860s in South Afrika, when ostrich feathers were in high demand. When the feather market crashed with the first world war, the number of farms declined drastically. With the increasing demand for ostrich leather and meat, ostrich farming was revived and in the 1980s farms were established in America, Asia and Europe (Shanawany, 1995). In Germany ostrich farming has since created a stable niche in the market for itself (Kistner, 2019). While intensive farming systems are common in ostrich production all over the world, German farms exclusively use extensive, pasture-based systems, in compliance with national legislation. Since the very beginning animal welfare concerns have been raised regarding the suitability of European climate conditions for ostriches. However, by now it has been shown that access to open, adequately constructed stables, providing protection against adverse weather conditions, allows ostriches to thrive under European conditions (Wöhr and Erhard, 2005). The need for stables is depicted in the various husbandry guidelines published by both legislators (BMEL, 2019; Europarat, 1997) and institutions (Kistner, 2003; Korbel et al., 2015; TVT, 2011). While it is generally agreed that an adequate stable must accommodate all ostriches at the same time, these guidelines are based on empirical values and experiences resulting in different opinions on required minimal stable size as reviewed by Rückschloss et al. (2025). Especially for the age group over 6 months until breeding age requirements differ widely spanning from 2 to 10 m^2 per animal. To our knowledge only one study has been conducted to assess the influence of stable space on ostriches, evaluating planimetric values, growth, performance and feather quality (Rückschloss et al., 2025). They found that young ostriches kept with access to 2.5 m^2 stable space/animal showed poorer condition of plumage and integument, lower weight gains and subsequently lower slaughter weights than ostriches kept on larger stable spaces. These effects were detected although all groups of ostriches had equal access to 2500 m^2 of pasture

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List of abbreviations

fCort	feather corticosterone
H/L ratio	heterophil/lymphocyte ratio
CK	creatine kinase
AST	aspartate aminotransferase
UA	uric acid
AI	avian influenza

(Rückschloss et al., 2025).

Due to its role in the response to stimuli generally regarded as stressful, corticosterone is often, if overly simplified, referred to as "stress hormone" (MacDougall-Shackleton et al., 2019). It is released from the hypothalamic-pituitary-adrenal axis, raising blood corticosterone concentrations within minutes, and can induce changes in metabolism, behaviour and the immune system (Eugen et al., 2019; Ralph and Tilbrook, 2016). Measuring blood corticosterone provides insight into a bird's physiological state. However, it only represents a single point in time and is susceptible to falsification due to the acute stress of sampling (Monclús et al., 2017).

During feather growth corticosterone is deposited from the blood stream via the blood quill (Jenni-Eiermann et al., 2015). Thus, feather corticosterone (fCort) provides a long-term measure, integrating corticosterone baseline values with elevations caused by singular events relative to their frequency and magnitude. Depending on the speed of feather growth, a timeframe of days to months is represented (Bortolotti et al., 2008; Romero and Fairhurst, 2016). The use of fCort is popular amongst researchers for assessing the physiological state of wild birds as well as for the evaluation of poultry welfare. The extraction of fCort has not been established for ostriches so far, but has been validated for many species, e.g. chickens, ducks and various non-domesticated birds (Bortolotti et al., 2008; Freeman and Newman, 2018; Häffelin et al., 2020; Johns et al., 2018; Monclús et al., 2017). Nevertheless, due to its complex and not yet fully understood role, corticosterone levels should not be interpreted in isolation.

The heterophil-to-lymphocyte ratio (H/L ratio) obtained from a differential leucocyte count can be used to measure the immune response to stressors. During a stressful event, lymphocytes migrate out of the bloodstream into tissues while more heterophils are released from the bone marrow into the circulation, resulting in an increased H/L ratio. This can be caused by both physiologic and pathologic stressors, including infectious and non-infectious causes, but has been shown to be clearly linked to elevated corticosterone concentrations. In some cases H/L ratio has been shown to be more sensitive to subtle stressors than blood corticosterone levels (Davis et al., 2008; Müller et al., 2011).

Making use of the widespread effects of stress on the metabolism, a variety of blood chemical factors like creatine kinase (CK), aspartate aminotransferase (AST), uric acid (UA), lactate, calcium, sodium and phosphorus have also been used to evaluate stress and welfare in birds (Krivankova et al., 2020; Xie et al., 2023).

Studies on the effects of stable size on physiological parameters and stress indicators commonly used to evaluate poultry husbandry are not available for ostriches so far. Therefore, in this study we examined concentrations of fCort, AST, CK, UA and the H/L ratio to compare chronical stress experienced by young ostriches kept in differently sized stables. Our research hypotheses were that ostriches in smaller stables would have higher fCort concentrations, increased H/L ratios and increased blood concentrations of AST, CK and UA.

2. Material and methods

Ethical statement

This experiment was conducted in accordance with the German animal welfare regulations and with the approval of the responsible German authority (reference number ROB-55.2-2532.Vet_02-20-91)

2.1. Birds and experimental design

For this study crossbreeds of blue-necked ostriches (*Struthio camelus australis*) and black-necked ostriches (*Struthio camelus domesticus*), commonly used in commercial farming, were used. The ostriches were bred, eggs artificially incubated, hatched and raised at a commercial farm in southern Germany. At the age of 6 months, 24 birds were randomly selected and assigned to one of three experimental groups, forming three groups of 8 birds each. Individuals showing heightened fear or aggression towards humans were not included.

All groups were housed in the same stable building, ensuring identical exposure to environmental influences and identical treatment during daily management. The ostriches did not get any vaccination as is the norm in Germany. Clinical health was visually inspected daily. The building was open to outdoor climate and natural lighting, following the natural circadian rhythm. Stable departments were filled with straw. Water and feed (a grass silage, maize silage and bruised grain mixture) were provided *ad libitum* inside the stable. In every group, all ostriches were able to feed simultaneously although the feeding fence was shorter the smaller the stable size. Each group had constant access to 2500 m^2 of pasture in accordance with German legislation (BMEL, 2019). However, due to a temporarily increased risk of avian influenza (AI) in the area around the farm, all ostriches were kept in the barns for a period of 48 days during the study. Experimental animals were 9 months old at the beginning of stabling. Nettings were used to prevent contact with wild birds.

Stable sizes differed between groups with **group s** (small) having access to 20 m^2 (i.e. 2.5 m^2/animal), **group m** (medium) having 40 m^2 (i.e. 5 m^2/animal) and **group l** (large) having 80 m^2 (i.e. 10 m^2/animal) of stable space available.

2.2. Sampling

Every three months (at the age of 6, 9, 12 and 15 months respectively) 3–5 feathers were plucked from the cranial contour of the left thigh. Additionally, blood samples were collected from the subcutaneous ulnar wing vein (*V. ulnaris subcutanea*). From each bird 10 ml of blood was taken using heparin as anticoagulant. Blood samples were immediately placed in a cooler; feathers were kept at room temperature in the dark. We first sampled group s, then group l and finally group m, with each group taking around 2 h. Before sampling began all ostriches were stabled to facilitate capture and individual ostriches were released onto the pasture after being sampled. The building layout allowed the birds to observe all previous captures, but not the sampling procedure. Birds were stabled for AI protection one week before the second sampling at 9-months and were kept stabled for another 5 weeks afterwards.

2.3. Corticosterone measurement

Extraction of corticosterone was performed as described by Monclús et al. (2017) with modifications. Three (first sampling) or two (subsequent samplings) feathers were chosen for extraction. The whole length of the feathers was used to represent the full time span of feather growth. After removal of calamus and rachis, the vanes were minced with scissors before being shredded in a ball mill (MM 400, Retsch, Haan, Germany) at 25 HZ for 1 min. The powdered samples were weighed in to 15 mg (± 0.5 mg; Explorer Pro EP 64 C, Ohaus, Nänikon, Switzerland), suspended in 10 ml of HPCL-grade methanol and placed in an ultrasonic

bath for 30 min. After shaking incubation at 37 °C for 18 h, feather material was filtered through a filter paper (Whatman™ grade 595 ½, Cytiva, Wilmington DE, USA). The original vial and the filter paper were then washed twice with an additional 1 ml of methanol. The wash was added to the original extract. Liquids were evaporated in a water bath at 40 °C under a fume hood and the resulting dried residues were stored at −20 °C until further analysis.

Corticosterone concentrations were determined using an ELISA (Corticosterone ELISA Kit ADI-900-097, Enzo Life Sciences Inc., New York, USA). Samples were reconstituted in 500 µl assay buffer by shaking for 0.5 h. Further procedures were carried out according to the instructions of the manufacturer. Each sample was analysed as a duplet. Photometric measurements and calculations were performed using ELISA reader RT – 6500 Microplate Reader (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, P. R. China). A coefficient of variation of less than 20 % within duplets was considered acceptable.

2.4. Blood analysis

The cooled blood samples were transported to the laboratory where three blood smears per animal were made. The remaining blood was centrifuged within approximately 12 h after sampling. Plasma was used to analyse CK, AST and UA using VetScan® Avian/Reptilian Profile Plus reagent rotors (Abaxis, Inc., California, USA). Blood smears were stained using a modified Wright-Giemsa stain (Haema-Schnellfärbung LT-Sys®, Eberhard Lehmann GmbH, Berlin, Germany) and evaluated under a light microscope. For each blood smear 200 white blood cells were differentiated to calculate H/L ratio by dividing the number of heterophile granulocytes by the number of lymphocytes. Microphotographs were taken with an Olympus DP 23 camera using the Olympus cellSens Entry software (version 4.1) mounted on an Olympus BX41 microscope.

2.5. Statistical analyses

A dependent sample *t*-test was conducted to determine whether the number of artifact leucocytes of unknown origin in the blood smears significantly affected the H/L ratio.

fCort values were adjusted for variance of extraction by the following method: two samples of 15 mg were taken after pulverisation of one to two feathers. The corresponding samples were extracted in different passages and analysed on the same ELISA plate. The differences between the pairings were calculated and the standard deviation was subtracted from all fCort results. Further statistical analyses were performed using R (version 4.4.2). To account for the non-negativity of the continuous response variable, a gamma distribution was assumed for the response. To quantify covariate effect sizes, generalized linear mixed models (GLMMs) were fitted, using R-packages lme4.

To examine if the development of any of the parameters measured differed between ostriches over time depending on the treatment, the two groups m and l were combined. This reduced the number of parameters estimated by the model, a necessary adjustment due to the limited sample size. In the following this model will be referred to as interaction model. Statistical significance was determined at a threshold of $p < 0.05$.

3. Results

3.1. Feather corticosterone

There were no significant differences between the three groups (Table 1). On average, fCort values decreased over time. This temporal effect was significant ($p < 0.01$). However, Fig. 1 suggests that the average of fCort over time is mainly due to groups m and l, where this

Table 1
Effects of stable size on stress parameters (mean ± standard deviation). The combined values of all groups show the effects of age.

age (month)	6	9	12	15
fCort (pg/mg)				
group s	4.2 ± 0.6	4.0 ± 1.4	4.7 ± 0.7	3.8 ± 1.0
group m	5.2 ± 1.3	3.8 ± 0.6	4.3 ± 1.0	3.3 ± 1.0
group l	4.2 ± 1.0	4.2 ± 1.1	3.9 ± 0.6	3.4 ± 1.4
overall	4.5 ± 1.1	4.0 ± 1.1	4.3 ± 0.9	3.5 ± 1.2
H/L ratio, Method 1				
group s	5.2 ± 2.5	5.9 ± 2.0	3.5 ± 0.9	4.5 ± 1.6
group m	5.3 ± 2.3	5.0 ± 1.4	3.4 ± 0.8	3.1 ± 0.6
group l	6.5 ± 2.9	5.9 ± 2.0	3.4 ± 0.8	4.2 ± 1.8
overall	5.7 ± 2.5	5.6 ± 1.9	3.4 ± 0.8	3.9 ± 1.6
H/L ratio, Method 2				
group s	4.6 ± 2.1	5.3 ± 2.0	3.3 ± 1.0	3.8 ± 0.9
group m	4.9 ± 2.4	4.4 ± 1.1	3.1 ± 0.7	2.9 ± 0.6
group l	5.7 ± 2.2	5.4 ± 1.7	3.2 ± 0.7	3.9 ± 1.6
overall	5.1 ± 2.0	5.0 ± 1.8	3.2 ± 0.8	3.5 ± 1.2
AST (IU/l)				
group s	461.9 ± 48.0	492.4 ± 56.6	384.1 ± 64.2	370.0 ± 66.0
group m	530.9 ± 121.6	539.4 ± 109.0	449.5 ± 130.7	538.8 ± 273.1
group l	463.0 ± 26.2	508.9 ± 50.1	430.1 ± 58.6	397.5 ± 46.6
overall	485.3 ± 85.3	513.5 ± 80.6	421.3 ± 96.6	435.4 ± 184.0
CK (IU/l)				
group s	3526.0 ± 571.3	4025.0 ± 749.5	3871.8 ± 1757	3260.8 ± 1140.1
group m	2600.9 ± 360.2	3381.9 ± 696.5	3070.0 ± 398.8	2706.4 ± 255.9
group l	2615.9 ± 292.9	3974.6 ± 1088.4	3619.5 ± 1319.8	3240.0 ± 1161.4
overall	2914.3 ± 600.7	3793.8 ± 894.4	3520.4 ± 1339.3	3084.8 ± 1014.1
UA (mg/dL)				
group s	10.4 ± 2.1	7.5 ± 1.5	9.7 ± 3.8	7.5 ± 1.9
group m	9.2 ± 1.2	6.3 ± 1.0	5.2 ± 0.7	5.0 ± 1.1
group l	8.1 ± 1.6	6.3 ± 1.1	4.4 ± 1.0	4.8 ± 1.1
overall	9.2 ± 1.9	6.7 ± 1.4	6.4 ± 3.4	5.8 ± 1.1

fCort = feather corticosterone; H/L ratio = heterophil/lymphocyte ratio; CK = creatine kinase; AST = aspartate aminotransferase; UA = uric acid.

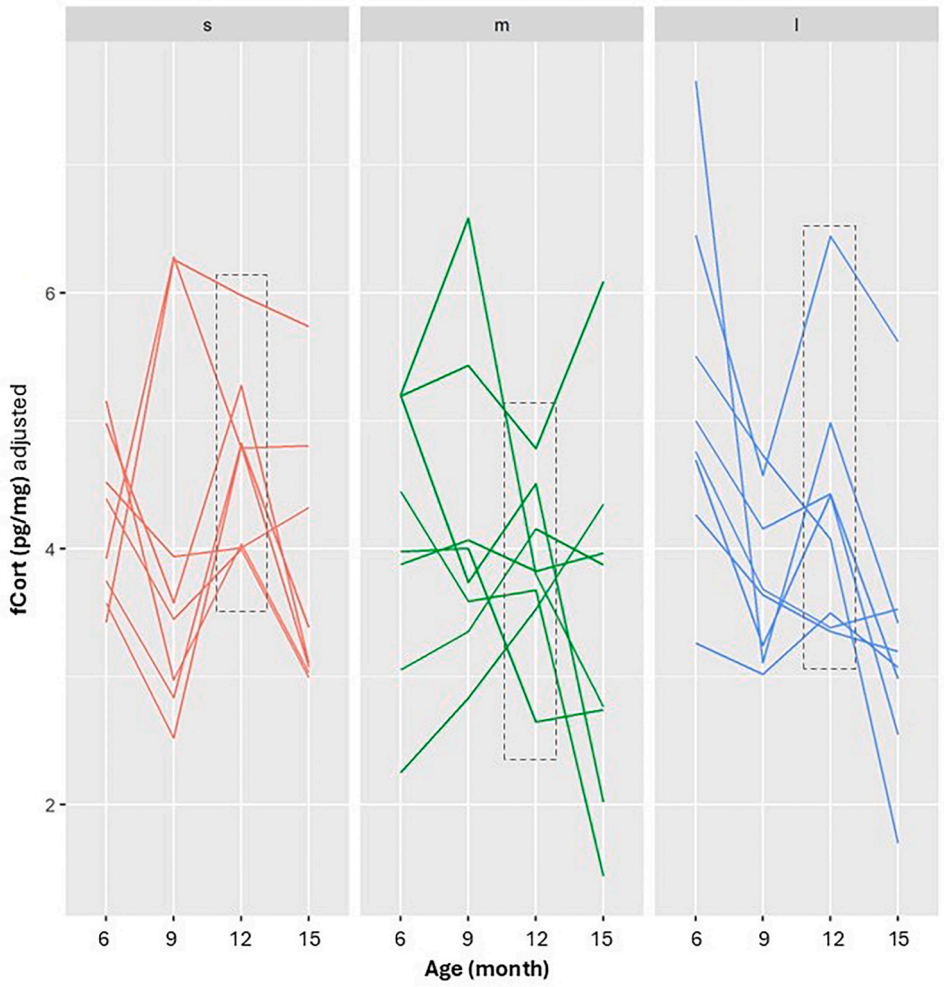

Fig. 1. Individual curves of feather corticosterone (fCort). Adjusted fCort concentrations (pg/mg) of ostriches of group s (red), group m (green) and group l (blue). The dotted rectangles indicate values obtained at the time ostriches were stabled.

effect is visible. The interaction model supports this suggestion as the average fCort values of ostriches in group s decrease over time, but this effect is not significant. For the m/l group however, fCort values decreased on average by a factor of exp(-0.033) = 0.97 per month of age. With a p-value of 0.0245 this effect is significant to the chosen 5 % level (Fig. 2).

At the third sampling (corresponding with feathers grown during stabling because of AI regulations) the individual fCort values of many birds showed a peak. This could be most clearly seen as a trend in group s. The individual curves in group l were more diverse and in group m only three birds showed a peak at all.

3.2. H/L ratio

All birds appeared clinically healthy during the time of the experiment and there were no obvious aberrances visible in the blood smears. During differentiation of leucocytes, mononuclear cells with altered cell morphology were found in blood smears of all the three groups (Fig. 3). Comparison with other cell populations within the slides, alongside information from the literature, indicated a preparation artifact is the most probable cause of the unusual morphology. We could not determine whether the altered cells originated from monocytes or lymphocytes. These cells were present in 85 out of 96 blood smears and, although they were not numerous, depending on their classification the resulting H/L ratios differed significantly from each other as shown by the *t*-test. Therefore, we analysed both possibilities: For H/L ratio 1 we treated the altered cells as monocytes, for H/L ratio 2 we treated them as lymphocytes. Both analyses yielded very similar results (Fig. 4). No statistically significant differences between groups or timepoints were found using either H/L ratio 1 or 2. Group l showed the lowest mean value throughout the experiment. In all groups mean values dropped over time, and variation within groups decreased (Table 1). However, this was less pronounced in group s. At 9-months-old, during AI stabling, groups s and l showed a slight peak, in group m this peak could only be seen in individuals. The mean remained around the same level as three months before.

3.3. Blood chemistry

For both AST and CK we found no significant differences between time or groups in any model. At 9-months-old a peak could be seen in both enzymes. AST values showed a downward trend over time ($p \ll 0.01$) (Table 1).

Group l contained a bird, which showed elevated AST values at every sampling, without displaying any noticeable abnormalities. At 15-months-old one bird yielded both an abnormally high AST value and a CK value of zero, which we suspect to be due to a faulty measurement.

For UA, we found significant differences between group s and group m as well as group s and group l (both $p < 0.01$) (Fig. 5). Overall UA decreased significantly over the experimental period ($p < 0.01$) (Table 1). The interaction model revealed that the decrease for group m/l was significantly steeper than for group s ($p < 0.01$) (Fig. 6).

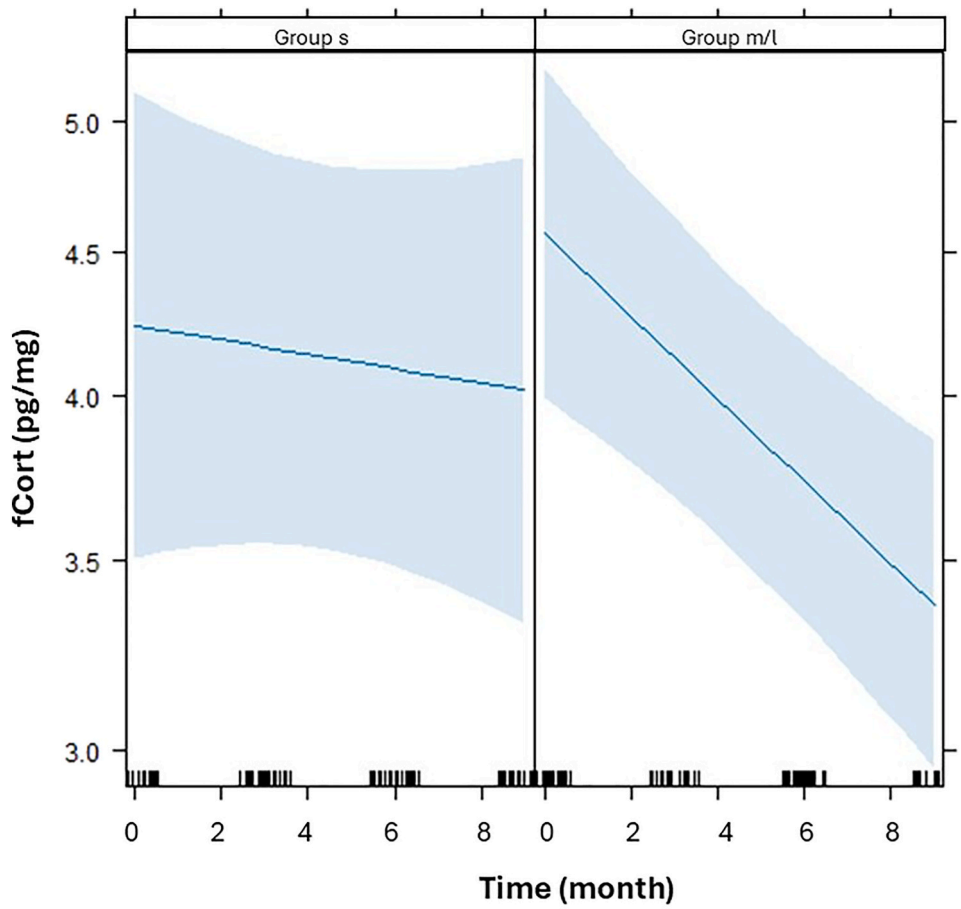

Fig. 2. Feather corticosterone (fCort) in the interaction model. Represented is the calculated monthly change of adjusted fCort concentrations for ostriches of group s (left side) and group m/l combined (right side) respectively. The black lines at the x-axes mark the sampling points.

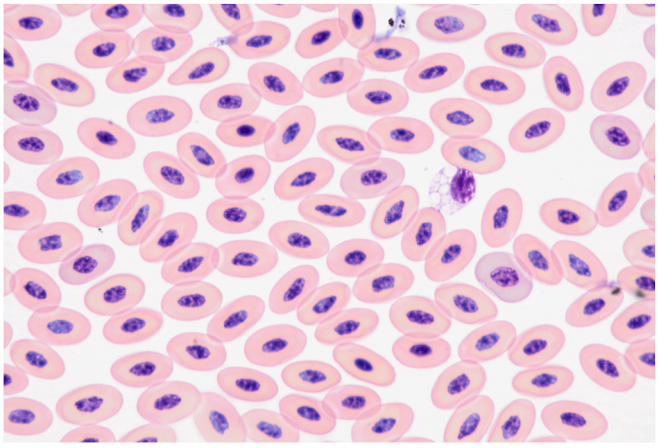

Fig. 3. Leucocyte with altered morphology. Leucocyte with altered morphology among erythrocytes in the blood smear of an ostrich. The basophilic cytoplasm is filled with vacuoles. The microphotograph was taken with an Olympus DP 23 camera using the Olympus cellSens Entry software (version 4.1) mounted on an Olympus BX41 microscope; settings at 1000 magnification (eyepiece WHN 10×/22, objective UPlanSApo 100×/1.40 oil/0.17/FN26.5, light set to level 6). The image was scaled using Adobe Photoshop Elements 2022 Editor (version 20.0, Adobe Systems Software Ireland Limited, Dublin, Republic of Ireland) according to the following scheme: 7.76 MB, 2008 × 1350 pixels, 17 × 11.43 cm, resolution 300 pixels per inch.

While the value curves of individual birds in groups m and l showed very similar courses with little scattering, the curves of group s were more diverse with a broader range (Fig. 7). For individual values of all investigated parameters see Table S1.

4. Discussion

This study aimed to provide scientifically backed data on the stable space needed for young ostriches. We expected that ostriches would show elevated stress indicators when kept in enclosures providing the legally required minimum stable size of 2.5 m^2/animal. While not all the data confirmed these hypotheses, the overall picture is indicating that birds on 2.5 m^2 stable area/animal were under strain, while there seemed to be no additional benefits of 10 m^2/animal over 5 m^2/animal. fCort and UA decreased over time in groups m and l but not in group s, a trend that is also implied for the H/L ratio. Furthermore, UA was significantly higher in group s. In an associated investigation Rückschloss et al. (2025) used the same ostriches to examine weight, growth and quality of plumage and integument as well as food intake. As described elsewhere, the ostriches of group s had lower weight gain and lower carcass weights and they consistently did worse in plumage scoring than the other groups (Rückschloss et al., 2025).

Differences between groups were less pronounced than expected. The free access to 2500 m^2 of pasture equally available for each group may have allowed the ostriches to compensate for smaller stable space. Therefore, results could be interpreted as effects of the total space available rather than the stable space alone. So, effects seen in this study might be not due to classical crowding but a result of changed behaviour around stable usage, i.e. feed intake and taking shelter, except for values

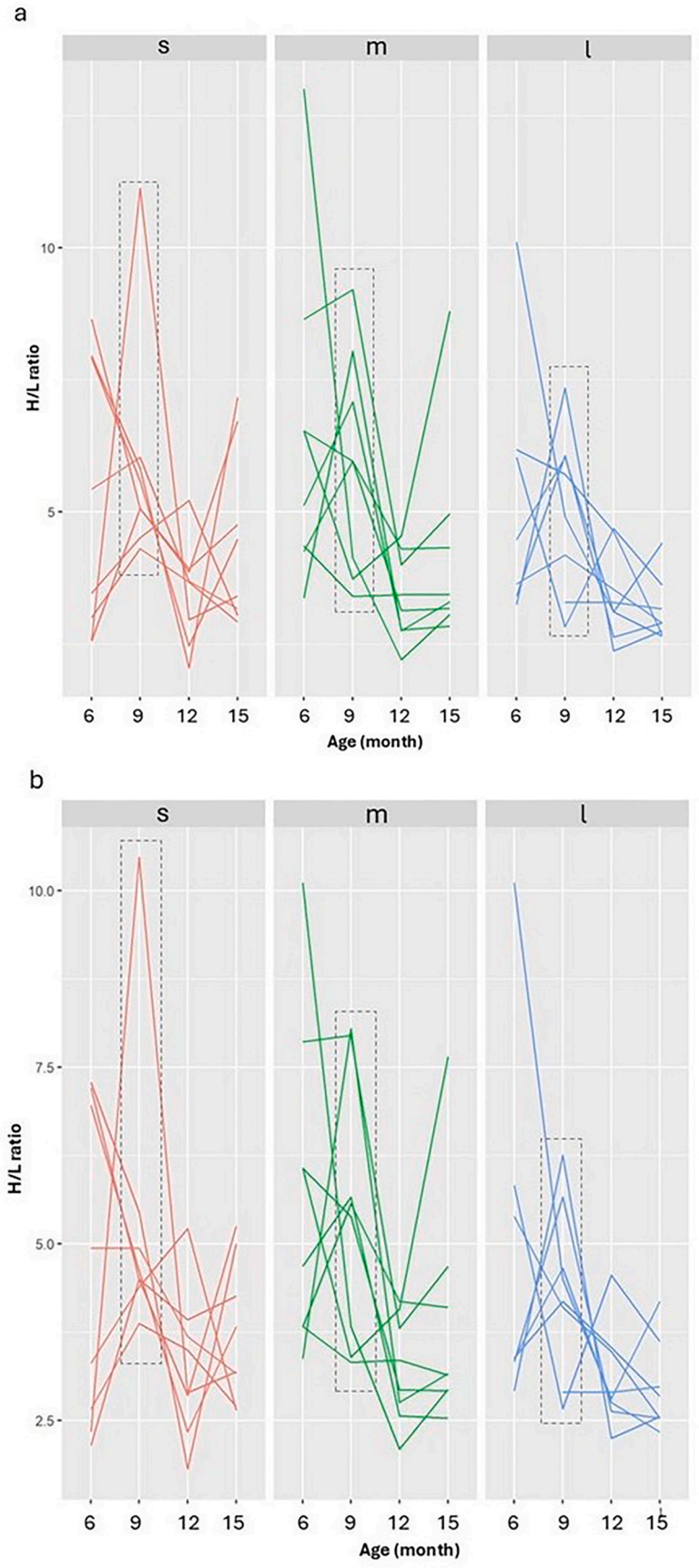

Fig. 4. Individual curves of Heterophil/Lymphocyte (H/L) ratio H/L ratios of ostriches of group s (red), group m (green) and group l (blue). H/L ratio was calculated by treating altered cells as monocytes (a) or lymphocytes (b). The dotted rectangles indicate values obtained at the time ostriches were stabled.

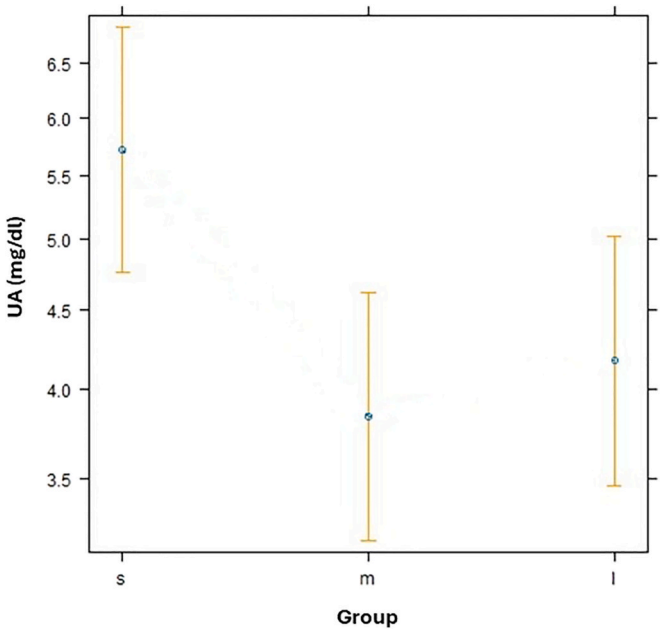

Fig. 5. Comparison of uric acid (UA) between groups. Mean UA values plasma concentrations (mg/dl) of ostrich groups s, m and l over the four samplings.

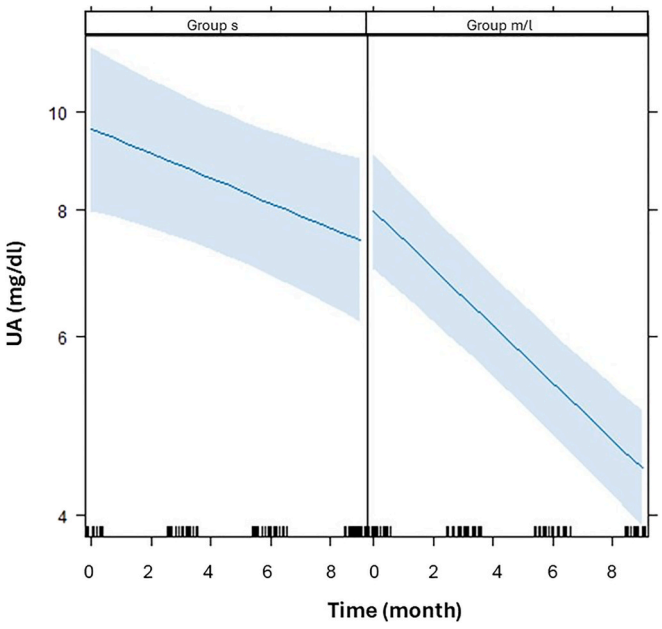

Fig. 6. Uric acid (UA) in the interaction model. Represented is the calculated monthly change of UA plasma concentrations for ostriches of group s (left side) and group m/l combined (right side) respectively. The black lines at the x-axes mark the sampling points.

taken during stabling due to AI regulations.

In this study ostriches were given access to pasture to reflect extensive farming conditions which are standard in Germany and to comply with legislation. However, given the growing threat of AI to poultry and ostrich husbandry the need to temporarily stable birds should be planned for. During the experiment exclusive stabling because of AI requirements occurred over a 48-day period starting one week before the second sampling at 9-months old and lasting further 5 weeks afterwards and we expected a simultaneous rise in stress indicators. While we did find a peak in all parameters but UA, the effect was far less than anticipated. It was overshadowed by individual differences between birds, leading to only a slight increase of group means. For both fCort and H/L ratio, the trend was most uniform in group s. Interestingly, in group m only three birds showed increased fCort, and the mean H/L ratio was only slightly higher than in the previous sampling. The latter may be simply due to the relatively high group mean at the initial sampling. In group l there is a visible, albeit small, upwards trend. The small effect AI stabling had on fCort and H/L-ratio especially in group m, supports the view that the greater space available to group l did not grant benefits over group m.

Corticosterone has been used to investigate the influence of housing conditions on poultry welfare, with varying results (Ralph and Tilbrook, 2016). To our knowledge this study is the first to measure fCort in ostriches. Therefore, no physiological, standard fCort ranges of unstressed ostriches are available. For the aim of this study, we focused on the comparison of the three experimental groups. We found no significant differences in fCort levels between groups, but fCort of groups m/l dropped noticeably over time, while fCort of group s remained at the same level. Our results of no differences in fCort levels between groups are supported by similar findings of Campbell et al. (2022), who detected only subtle differences of fCort between groups of laying hens kept in different housing systems but found higher differences in fCort between different points in time, possibly facilitated by age or climatic stressors. Similarly, stocking density hardly affected blood corticosterone according to studies on stocking density within the same type of housing in broilers, goslings and pigeons during rearing (Liu et al., 2021; Thaxton et al., 2006; Xie et al., 2023). Notably the latter three studies were done on young birds. In contrast Kang et al. (2016) and Onbaşılar and Aksoy (2005) reported significantly higher serum corticosterone values in mature laying hens kept in higher density ground pens and cages respectively.

Considering the time course of fCort concentrations during the observation period, we expected the groups to start with the same initial fCort levels at first sampling due to the retrospective nature of fCort, which provided measures reflecting the time pre-experiment, when all birds were housed together in a single group. The observed gradual development of differences between the experimental instead of an abrupt change could have been caused by the growth of the birds. As the birds doubled their space coverage during the experiment, shown by planimetry (Rückschloss et al., 2025), it is possible that the relative increase in spatial limitation over time caused the growing divergence in fCort.

fCort only yields information on the time the tested feather part grew. Therefore, the maximum timeframe that can be examined depends on the speed and duration of feather growth (Romero and Fairhurst, 2016). To our knowledge there is no data on the growth speed of ostrich body feathers available but except for the first sampling, we always found some feathers that were still growing at the predefined sampling site (the cranial contour of the thigh on the left side). We made sure to use the whole length of a feather, to represent the longest possible time frame. At the final sampling, birds were moulting into their adult plumage, so the feather growth may not have been induced by the previous sampling. While the examined feathers may thus not always reflect the full three-months period between samplings, they should cover at least a majority of that period in a comparable way for all three study groups.

Considering leucocytes as another parameter investigated, neither variant of H/L ratio that we applied revealed any significant differences between groups or ages, although group l had the lowest mean values at every sampling. Previous studies conducted on chicken have shown that the H/L ratio reacts to stocking density. Laying hens had higher H/L ratios in addition to higher corticosterone values when kept at a higher stocking density (Kang et al., 2016; Onbaşılar and Aksoy, 2005). Broilers examined by Thaxton et al. (2006) had the highest H/L ratio when kept at the highest stocking density. While H/L ratio was found to react even more sensitive to stressors than corticosterone (Müller et al., 2011), increases were generally directly linked to elevated blood corticosterone

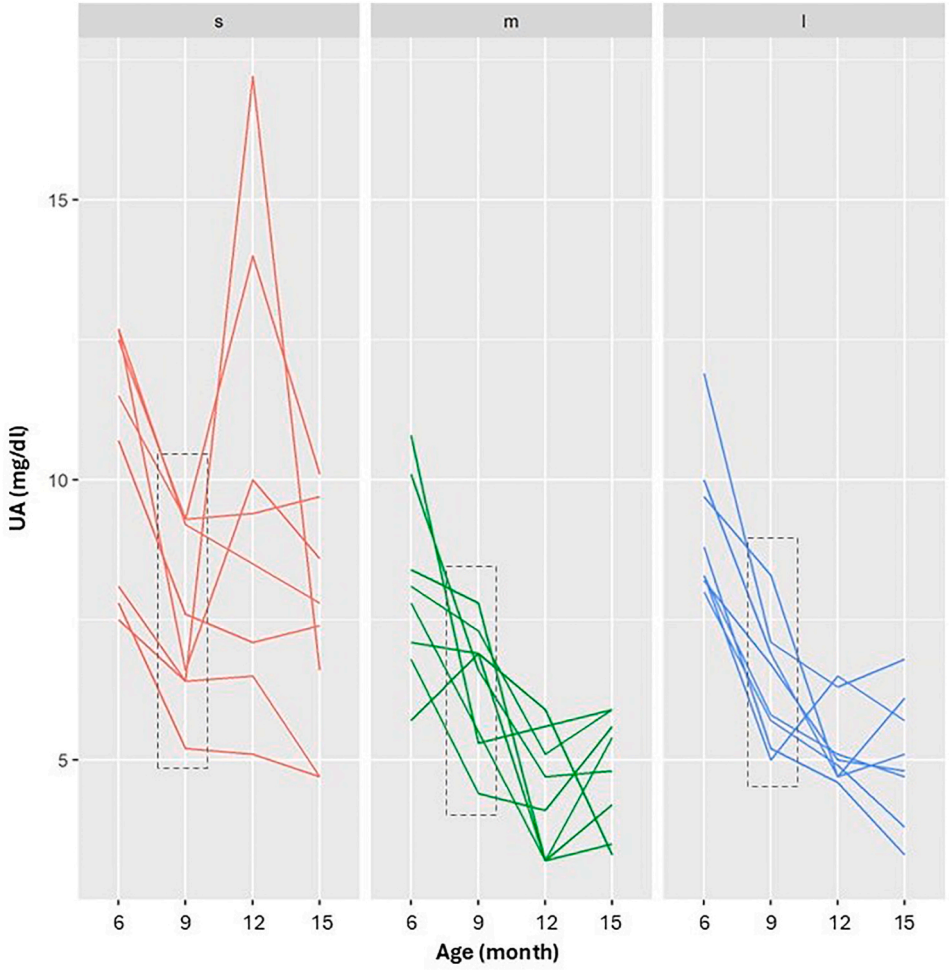

Fig. 7. Individual curves of uric acid (UA) UA plasma concentrations in mg/dL of ostriches of group s (red), group m (green) and group l (blue). The dotted rectangles indicate values obtained at the time ostriches were stabled.

levels, even so a direct correlation between corticosterone levels and H/L ratio has not been found (Jones et al., 1988; Lentfer et al., 2015). Furthermore, the immune response of individuals may appear within different ranges, so individual stress responses may get lost within evaluations of whole groups (Lentfer et al., 2015). In this study group sizes were small, and individuals were tracked for the duration of the experiment, so we do not think individual reactions were missed.

The H/L ratio relies on the proportions and does not include information about absolute leucocyte numbers, therefore, changes in H/L ratio may be caused by changes of either heterophils or lymphocytes but unilateral changes cannot be detected. Higher H/L ratio may represent immunocompetence in presence of pathogens instead of stress (Lentfer et al., 2015).

Although the H/L ratio does not rise as quickly as corticosterone, significant increases in the H/L-ratio within an hour of a stressor have been reported (McRee et al., 2018) making it suitable for indicating acute stress reactions as well. In ostriches elevated H/L ratios have been reported by Minka and Ayo (2008) when measuring stress imposed by transportation. After being loaded and transported by road for five to 7 h, ostriches showed significantly higher H/L ratios. Similarly, the H/L ratios in our study may have been affected by sampling procedure. As previously described, we sampled group s, group l and group m in sequence, taking around 2 h for each group, and ostriches were able to observe the captures. It is possible that birds reacted to the ongoing sampling procedure with acute stress resulting in short-term elevation of the H/L ratios and masking effects due to chronic stress. This could explain the high mean values observed in group m as the group last sampled.

Many factors influence the H/L ratio. Therefore, it is difficult to attribute changes or the lack thereof to one single factor. It is always possible that other stressors interfere with the parameter being investigated (Lentfer et al., 2015).

Elevated levels of the enzymes CK and AST are indicators of increased muscle metabolism, including muscle damage. In particular, elevated levels of CK have been linked to elevated blood corticosterone levels (Malheiros et al., 2003), suggesting that CK levels could be useful in assessing stress. In our study however, neither CK nor AST revealed any differences between groups or over time, nor did they indicate any pronounced damage of muscle tissue. The means of CK concentrations obtained in this study were within the upper range of reference values of 1648–4894 IU/l provided by Verstappen et al. (2002) for adult ostriches kept in the Netherlands, with individual values exceeding the reference range. The mean AST values were also above the reference values of 243–418 IU/l given by Verstappen et al. (2002) for adult ostriches. In our study, juvenile ostriches were included, and we cannot exclude an effect of age on CK or ALT standard values. Samour et al. (2011) examined changes in growing ostrich chicks kept in Abu Dhabi, that were sampled monthly until one year old and reported significant age-related increases in CK and decreases of AST values during the observation period. CK-values increased from 4919.9 IU/l at 6-months-old to 7504.9 IU/l at 12-months-old and AST dropped from 453.8 IU/l at 6-months-old to 177.1 IU/l at 12-months-old. In contrast, we found no significant differences in CK and AST values at 6- and 9-months-old, and only a slight increase in CK values and a small drop of AST at

12-months-old. Our findings are very similar to those reported by Thompson et al. (2008) in their study on 7- and 12-months-old ostriches in New Zealand, who give mean CK-values up to 4382 IU/l and mean AST-values up to 557 IU/l. Since they found the highest values in birds with the best performance, they proposed that the high CK and AST enzyme values were a consequence of rapid growth. AST was lower in older birds, supporting the trend seen in our results. Regarding these results neither CK nor AST seem to be suitable indicators of the well-being of growing ostriches under these husbandry conditions.

UA is one of the metabolic products of protein breakdown in birds, and thus a marker of protein metabolism. It is also a major molecular antioxidant and has been shown to react to corticosterone levels alongside other antioxidant components (Cohen et al., 2008; Lin et al., 2004; Padrones et al., 2023). In our study, blood levels of CK and AST gave no indication of muscle breakdown in any group, but UA levels were significantly higher in group s compared to the other groups. The mean values of all groups were within reference values given by Verstappen et al. (2002) of 351–649 µmol/l, but we found individual values both above and below the reference range in all groups. We can only speculate about the reasons for the observed differences in UA levels. The measured intake of feed given per group within the stables was very similar between the three groups (Rückschloss et al., 2025). Assuming similar grazing intake, all groups should therefore have ingested roughly the same amount of protein. However, group s still showed the smallest weight gain and importantly the highest variance in weight (Rückschloss et al., 2025), suggesting, as assumed before (Rückschloss et al., 2025), that weaker animals particularly of group s were supressed by stronger ones. Animals of lower rank may have experienced more social stress which could lead to higher UA as result of an altered metabolism. Alternatively, differences in UA may result from different proportions of feed and grazing intake among individuals, if ostriches of a higher rank restricted access to feed for those of a lower rank in the smaller stable space. Liu et al. (2012) found elevated UA in the blood of laying hens treated with subcutaneous injections of corticosterone over seven days. They also observed elevated insulin levels, leading them to assume that the corticosterone-treated birds became “metabolically inflexible”, meaning they had to rely on amino acids for gluconeogenesis. However, we did not find elevated fCort levels indicating elevated blood corticosterone in group s in accordance with this hypothesis. Nevertheless, it is notable that differences in UA levels became more apparent at later sampling points, when fCort levels diverged too. It is known that blood UA concentrations can increase because of dehydration reflecting reduced blood volume. Since haematocrit values or other possible indicators were not available to us, we cannot rule out dehydration in individual animals.

5. Conclusion

This study found no unequivocal signs of chronically elevated stress parameters related to stable size for young ostriches with simultaneous access to 2500 m^2 of pasture. However, our findings suggest, that birds in group s with access to only 2.5 m^2 of stable space/animal were negatively affected. Therefore, we recommend providing ostriches with more than 2.5 m^2 of stable space per animal, especially in view of the rising risk of diseases such as AI, which may require to keep ostriches stabled for extended periods. We did not find any additional benefits for ostriches with access to a size of 10 m^2 stable space/animal compared to 5 m^2/animal. Given the economical background, especially the cost of stable buildings, we caution against demanding overly high minimum stable spaces without a proven benefit to animal welfare. To improve our insight on the matter, further research using an ethological approach will be necessary. Likewise, the interaction between group size and available stable space should be investigated.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eups.2025.100013.

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