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Linköping University Post Print



N.B.: When citing this work, cite the original article.

Original Publication:

Beatrix Agnvall, Rebecca Katajamaa, Jordi Altimiras and Per Jensen, Is domestication driven by reduced fear of humans? Boldness, metabolism and serotonin levels in divergently selected red junglefowl (*Gallus gallus*), 2015, *Biology Letters*, (11), 9.

<http://dx.doi.org/10.1098/rsbl.2015.0509>

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Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-123162>

**Is domestication driven by reduced fear of humans? Boldness, metabolism and serotonin levels in divergently selected Red Junglefowl (*Gallus gallus*)**

Beatrix Agnvall, Rebecca Katajamaa, Jordi Altimiras, Per Jensen\*

IFM Biology, AVIAN Behaviour Genomics and Physiology Group

Linköping University

\* Corresponding author: [perje@ifm.liu.se](mailto:perje@ifm.liu.se)

## **Abstract**

Domesticated animals tend to develop a coherent set of phenotypic traits. Tamelessness could be a central underlying factor driving this, and we therefore selected Red Junglefowl, ancestors of all domestic chickens, for high or low fear of humans during six generations. We measured basal metabolic rate, feed efficiency, boldness in a novel object test, corticosterone reactivity, and basal serotonin levels (related to fearfulness) in birds from the fifth and sixth generation of the high and low fear lines (44-48 individuals). Corticosterone response to physical restraint did not differ between selection lines. However, basal metabolic rate was higher in low fear birds, as was feed efficiency. Low fear males had higher plasma levels of serotonin and both low fear males and females were bolder in a novel object test. The results show that many aspects of the domesticated phenotype may have developed as correlated responses to reduced fear of humans, an essential trait for successful domestication.

Keywords: genetics, domestication, stress

## 1. Background

Animal domestication changed human history, and was used by Darwin as a proof-of-principle for evolution [1]. During domestication, animals adapt through genetic and learning mechanisms to living with humans, and simultaneously develop a suite of traits, the “domesticated phenotype” [2]. This may include increased size and reproduction. Mechanisms underlying this phenotypic complex recurring in many species are largely unknown, but it has been suggested that they may partly be side effects of reduced fear of humans [3,4]. However, it is not clear if tameness is a unique trait or reflects generally increased boldness and reduced stress susceptibility.

Chickens were domesticated 8000 years ago, and are now the most abundant food-producing animals [5]. In a previous experiment, ancestral Red Junglefowl selected only for reduced fear of humans developed correlated traits, e.g., higher food-competitiveness, larger eggs and bigger offspring [6]. Hence, less fear of humans could be associated with higher basal metabolic rate (BMR) and more efficient food conversion [7], for example through linkage effects [8]. Changes in levels of serotonin (5-HT) may be involved, since high peripheral 5-HT-levels are associated with less fear and anxiety in chickens [9].

We studied BMR, feed conversion and 5-HT-levels, as well as boldness and stress responses in Red Junglefowl, selected during five or six generations for high or low fear of humans.

## 2. Material and methods

The experiments were approved by the Linköping Animal Ethics Committee, license no 122-10.

We studied Red Junglefowl from the 5<sup>th</sup> (S5) and 6<sup>th</sup> (S6) generation of populations, divergently selected for low (L-birds) vs high (H-birds) fear of humans. The breeding scheme and housing conditions have been described elsewhere [6,10]. Briefly, birds bred and maintained with identical experiences of humans were selected in each generation based on divergent scores in a fear-of-human test. Each generation was maintained at about 50 birds per selection line, from 5-10 families per generation and selection line. The birds were hatched and reared under standardized conditions in mixed groups (see below).

In S5, we measured basal metabolic rate (BMR) using open flow respirometry as previously described [11] in 48 birds (6 females and 9 males from the low fear line; 15 females and 18 males from the high fear line) at 5-6 weeks of age. Four birds were individually placed in 3.6 l opaque tight plastic containers (CurTec) in a thermostatically controlled chamber at 24 °C (Rubarth GmbH type 3001) for overnight measurements in darkness (1800 h to 0600 h, similar to their normal diurnal light pattern). Airflow through the chambers was set at 1000 ml min<sup>-1</sup>. Flow was measured continuously in all chambers using a multi-channel flow meter (FlowBar8, Sable Systems Inc.). A computer controlled multiplexer (Sable Systems Inc.) allowed the cycling of oxygen measurements between all chambers and a baseline channel. Oxygen concentration in the sample channel was

measured with a FoxBox oxygen meter (Sable Instruments Inc.). BMR was assessed from the lowest 90 min average oxygen consumption during the night, which corresponds to three consecutive measurements per chamber

When 19 weeks old (135 days), birds were placed in individual battery cages (60 x 40, height 40 cm) with water, perch, nest box and dust-bath, and we measured the feed intake during seven days in eight females and 10 males from the low fear line, and 14 females and 12 males from the high fear line. Feed was offered *ad lib* in a spill-safe food-container, and consumption was assessed by weighing feed remains daily. As a proxy for feed conversion (“feed efficiency”), we divided the average weekly growth of each bird between days 112 and 200 by its total feed intake during these seven days.

At 21 weeks of age, when the birds were still in the cages, we performed a Novel Object (NO) test in the same birds, as a measure of boldness. An egg was cracked in the feed trough (known to be attractive food), and when the bird started eating from it, a novel object (33 cl Coca Cola can) was placed 10 cm from the egg. During the test, the observer was hidden behind a screen. We recorded latency to start feeding after the NO had been placed in the trough, with a max time of 180 s.

In S6 at 28 weeks of age we assessed plasma corticosterone levels before and after a short period of physical restraint, using methods described in detail earlier [12]. Briefly, each bird (eight males and seven females from the low line; six males and eight females from the high line) was removed from its individual

cage, and a blood sample was obtained. It was then stressed by being placed in a hanging fish net for ten minutes, followed by a second blood-sample, after which it was returned to its home cage. A third blood-sample was collected 30 min after the first one. When the S6-birds were 47 weeks old, blood samples were obtained in undisturbed conditions during mid-day, from 19 males and 10 females from the low-fear line, and 6 males and 10 females from the high-fear line, for serotonin analysis. Blood was centrifuged at 900 x g at room temperature for 20 min and the plasma was stored at -18 °C until analysis.

Corticosterone was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, NY, USA) following the manufacturer's instructions. All samples were tested in duplicate and the analytic range of the assay was 32-20 000 pg/ml. The concentration of serotonin in the plasma samples was determined in duplicates using a commercial serotonin ELISA kit (analytic range 6.25-200 ng/ml, MyBiosource, San Diego, USA).

Data were analyzed with Generalized Linear Models (Linear Mixed Models for repeated measures on corticosterone), using SPSS v 22.0, with "selection" (high vs low) and sex (male vs female) as well as their interaction as predictors. We used the scale response "linear" and the link function "identity", except for the truncated variable "latency to eat" in the NO test, where we used gamma distribution with log link. The Omnibus test was significant in all cases ( $P < 0.05$ ). Significance was determined by the Wald  $\chi^2$ -statistics for Generalized Linear

Models, and F-statistics for Mixed Models, and estimated marginal means were calculated where applicable.

### **3. Results**

Basal metabolic rate (BMR) was significantly higher in L-birds (Fig 1A). There was also a significant sex effect, where females had lower BMR than males, but no interaction was found between sex and selection.

There was a tendency for higher feed efficiency in L-birds, but no sex effects (Fig 1B). However, the interaction was significant, showing that the females principally drove the effect. L-birds weighed significantly more than H-birds at 112 (825 ± 10.9 g vs 691 ± 12.7 g;  $\chi^2(1) = 64.2$ ,  $P < 0.001$ ) and 200 days (1054 ± 16.0 g vs 845 ± 13.2 g;  $\chi^2(1) = 101.4$ ,  $P < 0.001$ ).

In the NO test, L-birds (particularly females) were significantly bolder (Fig 1C). There was a significant effect of time after restraint on corticosterone levels ( $F_{1,65} = 94.7$ ,  $P < 0.001$ ) but no effects of selection or sex or its interactions (Fig 2A). Serotonin levels were significantly higher in L-males, as shown by a significant selection x sex interaction (Fig 2B).

### **4. Discussion**

Red Junglefowl, intentionally selected during few generations for reduced fear of humans only, had higher basal metabolic rate and tended to have higher feed

efficiency, traits normally associated with the domesticated phenotype. The effects may be mediated by increased levels of serotonin.

Tameness is a product of both genetics and experience [2]. We maintained all birds under identical rearing conditions and selected them only based on fear of humans in a standardized test, so the differences between the selection lines should be mainly a consequence of genetic differences [2].

It is debated whether domestication phenotypes evolve due to independent selection of separate traits, or because they are genetically linked to some selected key trait. For example, Trut and coworkers argued that morphological, physiological and behavioural traits evolved as a complex in artificially selected foxes because of linkage or pleiotropy in genes controlling tameness [3], whereas Rubin and coworkers believed that the most likely explanation for domesticated color phenotypes is independent selection specifically for each desired trait [13]. Our results suggest that tameness may be a driving factor for the domesticated phenotype. They also indicate that increased secretion of 5-HT may be a mediating factor, at least in males, supporting earlier research showing a relationship between high 5-HT in blood and less fear in chickens, perhaps also affecting gastro-intestinal function [9]. Serotonin in plasma is correlated with platelet levels, but results should be interpreted with care [14]. The profound differences between the selection responses of the two sexes corroborate previous results on sex-specific genetic and behavioral responses to domestication [15].

We expected that low fear of humans in the selected Red Junglefowl would cause a general reduced stress and fear response. However, even though the selected birds differed in boldness (approaching a novel object), they did not differ in corticosterone response to restraint.

In conclusion, our results support the hypothesis that reduced fear of humans during the early phases of chicken domestication may have caused birds to evolve a phenotypic complex involving increased boldness and a more growth-promoting metabolism. This could be driven by an enhanced serotonergic response.

## **5. Acknowledgements**

The project was financed by the research council Formas, and ERC (grant 322206 GENEWELL).

**Authors declare no competing interests.**

**Author contributions:** BA and RK collected and analysed data; JA supervised the metabolic measurements and analyses; PJ conceived and coordinated the study, and wrote the paper with input from all others. All authors gave final approval for publication.

Supplemental material: Table S1, Data set

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## Figure legends

### Fig 1.

Metabolic rates, feed efficiency and boldness in the two selection lines (High and Low fear of humans; Estimated marginal means with SEM).

A. Basal metabolic rate (ml oxygen per min and kg bodyweight) in males and females. Effects of Selection:  $\chi^2(1) = 5.4$ ,  $P = 0.02$ ; Sex:  $\chi^2(1) = 17.1$ ,  $P < 0.001$ ; Selection x sex:  $\chi^2(1) = 0.16$ ,  $P = 0.68$ .

B. Feed efficiency, in males and females. Effects of Selection:  $\chi^2(1) = 3.5$ ,  $P = 0.061$ ; Sex:  $\chi^2(1) = 1.52$ ,  $P = 0.21$ ; Selection x sex:  $\chi^2(1) = 10.5$ ,  $P = 0.001$ .

C. Latency to eat in the novel object test for males and females of both selection lines. Selection:  $\chi^2(1) = 4.1$ ,  $P = 0.044$ ; Sex:  $\chi^2(1) = 6.37$ ,  $P = 0.012$ ; Selection x sex:  $\chi^2(1) = 1.67$ ,  $P = 0.20$ .

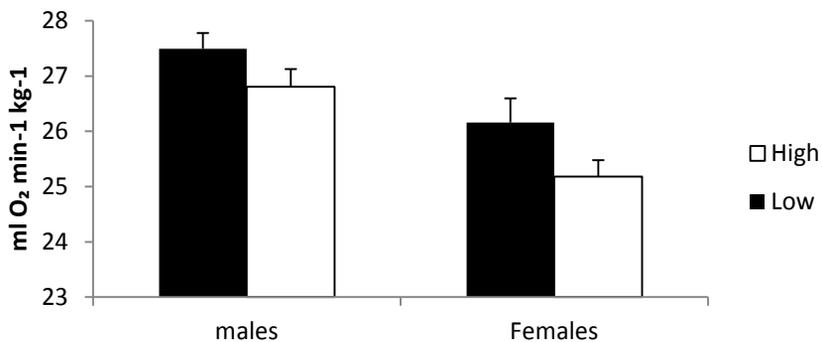
### Fig 2.

Physiological responses to selection in the two lines (High and Low fear of humans; Estimated marginal means with SEM).

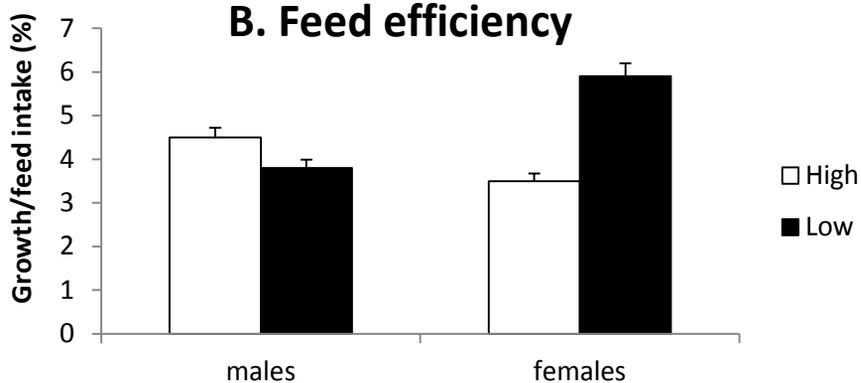
A. Plasma levels of corticosterone in males and females at baseline, after 10 min of physical restraint, and 30 min after first blood sample. Effects of Selection:  $F_{1,41} = 0.17$ ,  $P = 0.68$ ; Sex:  $F_{1,41} = 1.02$ ,  $P = 0.32$ ; Selection x sex:  $F_{1,41} = 0.27$ ,  $P = 0.61$ .

B. Plasma levels of serotonin (5-HT) in males and females. Effects of Selection:  $\chi^2(1) = 1.1$ ,  $P = 0.29$ ; Sex:  $\chi^2(1) = 6.0$ ,  $P = 0.014$ ; Selection x sex:  $\chi^2(1) = 5.7$ ,  $P = 0.017$ .

## A. Basal metabolic rate



## B. Feed efficiency



## C. Novel object test

