TITLE: Diet modulates components of animal personality in house sparrows: insights into a possible hormone-mediated mechanism

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ABSTRACT

Animals do not eat whatever food item they find. They usually balance the intake of key nutrients, for example, essential amino acids that cannot be synthesized by animals and must be provided in the diet. However, ability to gain optimal ratios, proportions, and amounts of nutrients may be hampered by a changing environment, competitive conspecifics or species, and predators. Here, we used an experimental system in which house sparrows (*Passer domesticus*) were fed diet with different amino-acids composition (the experimental diet had phenylalanine and tyrosine (PT) content reduced to 42% of the control diet). PT are precursors of coping hormones: dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline) which are involved mainly in the expression of stress and fear, but also learning and long-term memory formation. In line with this, birds fed PT-deficient diet learned to avoid unpalatable food markedly slower, coped worse with stress in the presence of a novel object and were much more aggressive towards other sparrows than control birds. Surprisingly, circulating amounts of the catecholamines in blood plasma were higher in PT-limited birds than in sparrows fed the control diet. This study provides the first evidence that different amino acids composition in the diet is associated with variation in behaviours and hormone levels in birds. We conclude that food, besides its nutritional function, seems to represent one of the main modulators of behavioural expression making a balanced diet crucial for survival. Moreover, the dependence of behaviour expression on diet poses interesting questions about the sources of animal personality.

KEYWORDS: amino acids, animal personality, birds, catecholamines, deficient diet, hormones, house sparrow, nutritional ecology

1. INTRODUCTION

Free-living animals typically rely on diverse food sources, supplying individuals with a mixture of nutrients to meet energetic and structural needs, which is referred to as their intake target (Simpson et al. 2017). An animal that achieves its intake target will enjoy maximal Darwinian fitness (Simpson and Raubenheimer 2011). However, food choice options may be facilitated, complicated, or impeded by environmental and organismal determinants (Murphy 1994), modifying access to specific food compounds and in consequence causing nutrition imbalance. For instance, the array of foods available in the environment changes continuously. Thus, not all required nutrients may be accessible in a given space and time. For example, episodic thiamine (vitamin B_1) deficiency in Northern Hemisphere fauna, such as bivalves, ray-finned fishes, and birds was shown to cause population declines (Balk et al. 2016). Moreover, individual nutrient requirements may vary significantly through the stages of the annual or life cycle. For instance, calcium is probably the most limiting macronutrient required by the egg-laying bird (Reynolds and Perrins 2010). Poor reproduction in forest passerines in The Netherlands was a result of a decline in snail shells abundance causing calcium deficient diet and increased egg-shell defects (Graveland et al. 1994). Beyond vitamins and minerals, some amino acids can also often occur in limiting amounts modifying immunological (Chandra and Chandra 1986; Konashi et al. 2000), endocrine (Narita et al. 2011) and neuronal responses (Gietzen et al. 1998) leading to changes in specific behaviours or even in animal personality (Han and Dingemanse 2015).

Amino acids are among some of the most essential food components. Although animals can synthesise some of them, vertebrates require a core of nine exogenous amino acids for maintenance and growth purposes (D’Mello 2003). The need for these amino acids arises from the inability of all animals to synthesize their carbon skeletons. These amino acids are classified as ‘indispensable’ or ‘essential’ and they must be provided in the diet to meet requirements for maintenance, growth, development, and health (Hou et al. 2015). The remaining amino acids, which animals are able to synthesize are termed ‘dispensable’ or ‘non-essential’. Of the three aromatic amino acids (tryptophan, tyrosine and phenylalanine) required for protein synthesis and other functions, only tryptophan and phenylalanine are considered to be essential. Tyrosine is regarded as dispensable as it can be synthesized from phenylalanine (D’Mello 2003). Aromatic amino acids are central to a number of biochemical pathways as they not only play a role in protein synthesis, but also are precursors of melanin pigments (Hill and McGraw 2006). They also give rise to a number of endogenous signalling and regulatory compounds. Tyrosine is key to the synthesis of thyroid hormones (Hsu et al. 2019), whereas both tyrosine and phenylalanine are precursors of several endogenous amine compounds, e.g. the three catecholamines (CAs) — dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline, Purves et al. 2004).

Catecholamines, known as coping hormones, are involved mainly in the expression of stress and fear (Goldstein 2003). A quick release of catecholamines during acute stress leads to changes in the carbohydrate metabolism to provide glucose for important bodily functions which are key in overcoming the challenge of an adverse situation (Barth et al. 2007). By activating the autonomic nervous system, catecholamines cause tachycardia, increased respiration rate, elevated body temperature and redistribution of visceral blood volume towards skeletal muscles and the brain (Tilders and Berkenbosch 1986; O’Neill 2019). If activated for too long, CAs can create negative psychological and physical outcomes, augmenting both the risk of a mood disorders and the risk of worsening of a coronary disease (Goldstein 2003). Moreover, CAs are neurotransmitters in the central and peripheral nervous system. They affect brain function in a number of ways. Adrenaline plays a role in long-term stress adaptation and emotional memory. Noradrenaline enhances attention, is required for latent learning and long-term memory formation in conditioned learning (Kobayashi 2001). Level of brain noradrenaline affects attention in rats (Sirviö et al. 1993), and plays a role in vocal learning in birds (Poirier et al. 2011). Dopamine is usually seen in popular culture and media as the main chemical mediator of pleasure, but current opinion in that dopamine instead confers motivational salience. When catecholamine synthesis is compromised through restricted feeding or an imbalanced diet, animals become less resistant to stress, less motivated, and exhibit symptoms of attention deficit (Gibson et al. 1982; Avraham et al. 1996; Clark and Noudoost 2014). Despite the growing existence of the impact of nutritional condition on behaviour, only a few studies used experiments to investigate the effects of diet composition on behaviour, with most research focusing on invertebrates (Han and Dingemanse 2015).

Using an experimental system in which house sparrows (*Passer domesticus*) were fed a diet with different amino-acids composition, we tested the hypothesis that that shortage of specific components which have to be delivered to the organism from the diet causes consistent differences in components of animal personality. The foraging behaviour of the house sparrow is opportunistic. In cities, they eat discarded food which can make dietary complementation difficult to realize, especially during reproduction or moulting, both of which require certain nutrients in proper amounts (Carey 1996). Moreover, in a complex and fast-changing city's environment birds have to deal with neophobia, the fear of new things. At the same time, birds that forage in flocks increase competition among individuals for potentially limiting food resources (Coogan et al. 2018).

Here, we investigated whether diet composition, namely phenylalanine and tyrosine (PT) reduced diet decreased learning abilities, stress resistance in the presence of a novel object (neophobia) and affected aggressiveness in comparison to birds fed a control diet. We expected that the aforementioned changes in behaviour would be caused by decreased biogenic amine synthesis, namely catecholamines concentration.

2. MATERIAL AND METHODS

2.1 Birds capture and care

62 males and 8 females of house sparrows (unbalanced sex ratio was caused by a previous experiment focused on males, females were added to alleviate interspecific conflicts occurring in all-male flocks of sparrow) were caught with mist nets in autumn 2019 on several sites in Kraków, Poland. Birds were weighed and banded with metal bands and released to an outdoor aviary located on the campus of the Jagiellonian University, Kraków, Poland inside a closed patio of the Institute of Environmental Sciences. The aviary measured 3.5 m x 10 m (2.5 m high) and was outfitted with trees, bushes, perches, wooden shelters, water and food dishes. Initially, birds were maintained with a mixture of seeds: wheat, barley, millet and sunflower seeds and water, provided *ad libitum*. Additionally, they had access to sand with shells and sepia.

After two weeks of acclimation to captivity, the birds were randomly divided into two groups. In the first group, there were 32 males and 4 females and birds were fed experimental diet, whereas in the second there were 30 males and 4 females which had access to control diet (see Diet manipulation). The groups were assigned to each of the halves of the original aviary, divided into two smaller aviaries (A1 and A2, 3.5 x 5 m). After three weeks and six weeks of the experiment sparrows were swapped between aviaries. Both aviaries were visited at the same time. Moreover, the location of both small aviaries was symmetrical in terms of distances from building walls and other features.

2.2 Diet manipulation

For four months of the experiment, birds received synthetic diets *ad libitum*. The synthetic diet was a mixture of protein (WPC80, free amino acids and whey protein isolate BiPRO GMP 9000 (Agropur Inc., Appleton, USA)), fats, carbohydrates and fibre (Table 1). The ingredients were thoroughly mixed to produce small pellets (6 mm diameter) that the sparrows consumed readily. The experimental diet had phenylalanine and tyrosine at 42% (PT-reduced, n=36 individuals) of the control diet (n=34 individuals), (Table 2). Because diets differed only in the amino-acid composition, they were isocaloric. The diet was prepared by ZooLab (http://zoolab.pl/en/home/, Sędziszów, Poland). During the experiment, both treatment groups had unlimited access to food (except during the food deprivation periods that occurred two hours before feeding-dependent tests).

2.3 Experiment 1: avoidance of unpalatable food

2.3.1 Pre-treatment period - adapting to the new feeders

Prior to the experiment, A1 was separated visually from A2. During four consecutive days, birds were adapting to new coloured feeders. After two hours of starvation (first group from 1000 h to 1200 h and the second group from 1100 h to 1300 h, the order was changed daily), blue and green plastic containers (19 x 16 x 7 cm) with 350 g of food were placed in the middle of each aviary spaced apart by approx. 1 m. To reduce the loss of spilled food, the coloured feeders were placed on larger transparent dishes (34 x 39 cm). As soon as foods were placed in the aviary, birds were monitored and video recorded on camera (Logitech, C920) from an observation blind. After 1 h of continuous observation and recording, food was removed from the cages and weighed to the nearest 1 g. After exposition to the new feeders, the regular feeder used daily was put back into each aviary. The locations of the feeders in aviaries were changed every day.

2.3.2 Aggressive behaviour

During the period of adapting to the new feeders, number of aggressive behaviours during feeding was noted based on video recordings. Chasing, biting, or lunging at another sparrow was considered as aggression.

2.3.3 Unpalatable food preparation

Three grams of quinine were dissolved in 50 ml of warm water and sprayed over 300 g of food. In the process, the food pellets were constantly turned and mixed to promote even coverage. Untreated food

(300 g) was sprayed with 50 ml of water. The food was then dried in an oven at 60°C for 2 hours and left uncovered to absorb atmospheric moisture.

2.3.4 Learning food aversion

The experiment was carried out in a separate common garden aviary (A3) designed similarly to A1 and A2, and visually isolated from them. Each day, house sparrows in groups of 10 individuals (two sparrows from A1 did not take part in this experiment) were randomly caught from the aviary (A1 or A2) and moved to a dark box for a period of food deprivation. After 2 hours, birds were released in A3 in which all birds had a choice between the green feeder with 200 g of bitter-tasting quinine-treated food pellets and the blue feeder filled with 200 g of food without the distasteful chemical. As soon as foods were placed in the aviary, birds were monitored and video recorded on camera (Logitech, C920) from an observation blind. Half an hour later birds were re-caught from A3 and moved back to their original housing aviary. The procedure was repeated for the other experimental aviary. Learning trials were performed through 5 consecutive days from 1100 h to 1530 h. The trials were arranged to balance the use of birds in A1 relative to A2. The sequence of aviaries and the locations of feeders were changed daily. Thirty males from each aviary, randomly chosen in each 10 individuals' batch, were used for the experiment ($n_{total} = 60$).

2.4 Experiment 2: Phobia of novel object

To compare object neophobia between experimental groups, a second experiment using a similar protocol and the same individuals as in the learning food aversion was conducted. After 2 hours without access to food, randomly chosen sparrows in groups of 10 individuals were released in an additional aviary A3. Latency to approach and feed and number of individuals approaching and feeding in the presence of a novel object were recorded for one hour, again from behind a blind. The novel object was a bright yellow sponge attached to the feeder which birds used every day.

2.5 Video analysis of feeding behaviour

For each group, the following data were collected from video recordings: (a) feeder colour of the first feeding, (b) latency (in seconds) to approach (landing on) the green and the blue feeder, (c) latency (in seconds) to consume food from the feeders, (d) number of birds landing on the feeders, (e) number of birds feeding from the green and blue feeder. If birds had not approached / consumed food by the end of the recording, they were given a maximal score of 3600 s (1 hour) in the case of pre-treatment trials and neophobia test, and 1800 s (0.5 hour) for food aversion trials.

2.6 Blood collection and quantitative determination of catecholamines

After two days from finishing all behavioural tests, a small puncture was made with a sterile needle on a left brachial vein and blood was collected using a heparinized capillary tube (Microvette CB 300 LH, Sarstedt). For each bird, around 300 µL of blood was collected. The blood samples were kept on ice and centrifuged (13 000 rpm, 7 min) within half an hour from the time of collection. Then, the plasma was separated from the cells and frozen immediately in -20°C.

The quantitative determination of adrenaline (epinephrine, ADR), noradrenaline (norepinephrine, NADR), and dopamine (DOP) in plasma was performed using a competitive ELISA kit (3-CAT ELISA, Demeditec Diagnostics GmbH, Germany) according to manual instruction. Whenever necessary, plasma samples were diluted using saline to the required volume of 300 µL. Catecholamine concentrations were calculated from a reference curve constructed using the provided standards, and were then recalculated taken into account any dilution of the original sample. Final concentrations were expressed as picograms per mL.

After the experiments were completed, the house sparrows were kept in the aviary for additional four weeks. During that time, birds were fed both pellet food and mixture of seeds in order to supplement the shortage of experimentally limited amino acids. After ensuring the birds were healthy and behaved normally in the aviary, they were released in the places where they were initially caught.

This research was done under permit no. 25/2019 (with a supplementary permit no. 78/2020) from the 2nd Local Institutional Animal Care and Use Committee in Kraków.

2.7 Statistical analyses

In the pre-treatment period of the food aversion experiment, we tested whether eaten food mass differed between dietary treatments, feeder colours and days. These data met the assumptions of parametric statistic and we used a linear model (ANOVA) with eaten food mass as the dependent variable and treatment, day and feeder colour as fixed factors. Because of different numbers of individuals in two experimental groups, eaten food mass was calculated per individual.

General linear models (GLMs) with Poisson distribution were used for count data. To determine whether the number of aggressive behaviours differed between sparrows fed control and PT-reduced diet, we used a GLM with the diet type and the number of birds approaching each feeder as independent variables. Data was standardized due to non-equal groups by proportionally scaling the number of aggressive behaviours in the larger group to the number of individuals in the smaller group.

In the learning food aversion experiment, we collected data concerning feeder of the first choice. It allowed us to compare behaviour irrespective of activity level or motivation between treatments. We performed analysis using the Fisher's exact test to compare the frequency of choosing the green vs blue feeder as the first feeding choice in both experimental groups. In addition, we used a Generalized Additive Model (GAM) with Poisson data distribution to analyse the relationship between learning and treatment. The choice of GAM was dictated by non-linear characteristics of raw data and thus inappropriateness of a typical ANCOVA. The GAM model included number of feeding birds as the dependent variable and treatment, day of the experiment, feeder colour and recording time (noon versus afternoon) as fixed factors. We also modelled a three-way interaction effect between treatment × day × feeder colour.

An unpaired t-test was used to compare treatment effect on latency to approach the feeder in the neophobia experiment. Number of sparrows approaching the feeder was analyzed using a GLM with Poisson error structure.

Hormones' concentrations were log-transformed prior to the analysis to remove distribution skew. After checking the assumptions of a parametric test, data on ADR and NADR were analysed using a linear model with diet type and sampling group as fixed factors, and data on DOP were analyzed using the Kruskal-Wallis test. All analyses and graphs were performed in R (version 4.0.2, R Core Team).

3. RESULTS

3.1 Pre-treatment period

Neither the amount of food consumed per individual (mean ± SE: control = 0.58 ± 0.11, PT-reduced = 0.43 ± 0.16; $F_{1,12}$ = 2.16, p=0.17), nor the amount of food consumed during each day of the trial ($F_{1,12}$ = 0.03, p=0.86) varied significantly between dietary treatments. However, sparrows in both experimental groups consumed more food from the green feeder ($F_{1,12}$ = 40.05, p<0.001). Thus, based on this result the green feeder was chosen in the next step of the experiment (learning food aversion) as a feeder filled with unpalatable food.

3.2 Learning food aversion

Irrespective of activity level and motivation to eat, birds from PT-reduced diet tended to choose green feeder as a feeder of the first choice, whereas control sparrows preferred the blue feeder. The result appeared to be marginally significant (Fisher's exact test: $p = 0.065$).

GAM model indicated the existence of a significant interaction of the treatment group, feeder colour and day in the food aversion experiment. We have thus split our data according to feeder colour (linked to food palatability) to explore this pattern further. The effect of diet type on the number of sparrows feeding from the green feeder changed significantly between days (Figure 1A, Table 3) and this pattern was different in the two experimental groups (Figure 1A). This indicates that the ongoing learning process in which birds should stop eating from the feeder with unpalatable food (green) proceeded at a different rate depending on the dietary manipulation type. PT-deficient sparrows learned much slower than control birds. The learning process appeared similar in case of the blue feeder (Figure 1B). Similarly to the green feeder, there were significant changes in numbers of birds feeding from this feeder (Figure 1B, Table 4). However, again, this effect depended on the diet type. PT-deficient birds were learning to use the proper feeder markedly slower than control sparrows. Recording time did not affect the number of feeding sparrows in all of the analyses ($p>0.05$).

3.3 Aggression

Sparrows fed the two diets showed differences in numbers of aggressive behaviours such as chasing, biting or lunging at another sparrow. Specifically, among the PT-reduced birds we observed three times more offensive and attacking acts in comparison to the control birds (mean ± SE: control = 16 ± 2 , PT-reduced = 48 ± 12; ChiSq = 75.39, $p<0.001$). Naturally, such behaviours also depended on number of birds staying around the feeder (mean ± SD: control = 272 ± 16, PT-reduced = 217 ± 50; ChiSq = 19.24, $p<0.001$).

3.4 Neophobia

Latency to approach the feeder to which the novel object was attached differed significantly between diet type groups (mean ± SE (sec): control = 1377 ± 129, PT-reduced = 3300 ± 300; t = -5.89, p = 0.01). Sparrows fed control diet landed on the feeder faster than sparrows fed experimental diet. Similarly, significantly more sparrows fed control diet landed on the feeder than PT-reduced birds (mean ± SE: control = 18 ± 13, PT-reduced = 6 ± 6; ChiSq = 16.64, $p<0.001$).

3.5 Plasma catecholamines level

The concentrations of plasma catecholamines differed between treatment groups (Figure 2). Surprisingly, sparrows from the PT-reduced diet had higher concentration of all analysed hormones (adrenaline: mean ± SE (ng/mL): control = 1.13 ± 0.12, PT-reduced = 1.79 ± 0.16 ; $F_{1,53} = 12.84$ $p<0.01$; noradrenaline: mean ± SE (ng/mL): control = 2.99 ± 0.30, PT-reduced = 6.23 ± 0.44 ; $F_{1,53} = 35.76$, $p<0.001$; dopamine: mean ± SE (ng/mL): control = 0.09 ± 0.02, PT-reduced = 0.42 ± 0.10; ChiSq = 10.63, $p<0.01$). Manufacturer-supplied control samples assayed together with sparrow samples fell within the concentration limits specified in the kit manual. However, control samples for dopamine were slightly above those limits (manufacturer control 1: 80 ng/mL (± 40%), control 2: 300 ng/mL (± 40%); assayed concentrations 128 ng/mL and 482 ng/mL, respectively). Thus, in spite of systematic deviations from manufacturer values, control samples still reproduced the expected magnitude of concentration differences. Although absolute concentrations of DOP should be treated with caution, the difference in concentrations seems to be robust.

4. DISCUSSION

This study, to our knowledge, provides the first evidence that variation in amino acids composition of diet is associated with variation in behaviours and hormone levels in birds. We found that when house sparrows ate food with decreased phenylalanine and tyrosine content, they learned much slower, coped worse with stress in the presence of novel object and were much more aggressive towards other sparrows than control birds. Surprisingly, circulating amounts of the catecholamines in blood plasma were higher in PT-limited birds than in sparrows fed control diet. In the latter, ADR, NADR and DOP were at 63%, 48% and 22% of concentrations detected in PT-deficient birds, respectively. Although brain concentration of amino acids was not measured in this study, modified behaviour suggested their deficit.

There is little doubt that diet can affect brain functions (Fernstrom 1990). Indispensable amino acids, precursors of neurotransmitters which are central components of interneural communications, and critical to brain function, are neither synthesized nor stored, and thus must be obtained in the diet (Fernstrom and Fernstrom 2007). The PT-reduced sparrows displayed a deficit in learning in comparison to control birds. The behavioural changes in conditioned food aversion suggest the impairment of the consolidation process for long-term memory in PT-limited birds. The long term memory formation is highly susceptible to a reduction in catecholamine biosynthesis and the noradrenergic activity (Kobayashi 2001; Tully and Bolshakov 2010). Studies that experimentally increased brain noradrenaline noted improved attention in rats (Sirviö et al. 1993) and maze solving performance in mice (Avraham et al. 1996). Moreover, study on aged mice showed that oral administration of seven essential amino acids (precursors of neurotransmitters) to low-protein diet mice reversed behaviour abnormalities related to learning and memory impairment (Sato et al. 2020). Similar results have been demonstrated by Le Douce et al., (2020) where dietary supplementation of L-serine restored synaptic plasticity and memory in mice with previously reduced both L- and D-serine synthesis.

However, based on the observed decreased learning abilities and at the same time increased aggressive behaviours in our study, we may speculate that the concentration of the limiting amino acids was not uniformly depressed throughout the brain in birds fed the PT-limited diet. Regional differences in brain neurochemistry were previously reported (Gibson et al. 1982; Tilders and Berkenbosch 1986; Gietzen et al. 1989). This phenomenon may play a role in integrating signals underlaying the initial response to dietary amino acid imbalance. The imbalanced composition of diet could also make individuals increase the expression of behaviours that enable them to escape nutritional deficiency (Han and Dingemanse 2015). Most of the research to date reporting this phenomenon focused on invertebrates. An interesting example has been observed in Mormon crickets, where protein-deficient diet increased the expression of aggressive behaviour and cannibalism as the nearest possible source of high-quality protein turned out to be another cricket (Simpson et al. 2006).

Catecholamine-level manipulations have been also associated with a sense of fear and anxiety. The PT-reduced birds in our study exhibited the increased latency to approach the feeder in the presence of a novel object. This result seems to be in line with many studies supporting the role of forebrain catecholamines depletion (specifically noradrenaline) in neophobia (Mason et al. 1978; Tombaugh et al. 1983; Britton et al. 1984). However, the action of catecholamines in fear behaviour does not seem to be unequivocal, as many authors come to opposite conclusions (Steketee et al. 1989). Conflicting results may stem from differences in novelty perception between various species used in such studies and/or various testing protocols (Greggor et al. 2015).

Our finding that the amino acids-limited diet modified behaviour in a way suggesting catecholamine deficit and, at the same time, caused no reduction in circulating plasma levels of CAs, may demonstrate specificity in amino acid distributions throughout an organism’s body. It is well-recognized that catecholamines are generally unable to penetrate the developed blood-brain barrier (Kostrzewa 2007). Thus, amino acid concentration in the brain is not a simple reflection of the plasma amino acid profile (Gietzen et al. 1989). Peng, Tews, & Harper (1972) observed that alteration in the plasma amino acid pattern owning to ingestion of unbalanced diet is commonly associated with a low concentration of certain brain amino acids. In rats fed a high-protein diet, plasma amino acids were elevated whereas in brain they tended to be depressed.

To emphasize the above, it is also important to note that phenylalanine and tyrosine are key not only to protein synthesis and catecholamine signalling, but also play central roles in many CA-independent biochemical pathways, such as melanin pigments synthesis (Hill and McGraw 2006) and thyroid hormones production (Hsu et al. 2019). Trade-offs involving these interconnected processes could also contribute to reductions in CA availability in the brain, depending in physiological needs imposed by each individual pathway exploiting deficient amino acids. Finally, transporters responsible for the intake of phenylalanine and tyrosine into the brain are also responsible for the intake of other large neutral amino acids, including tryptophan (Pietz et al. 1999). Low concentrations of PT may lead to increased intake of the competing tryptophan molecules into the brain and consequently – imbalances in levels of serotonin (major neurotransmitter and derivative of tryptophan). Future studies looking at diet-behavior links should therefore broadly consider all interconnected signaling pathways and possible trade-offs to better understand observed behavioral changes.

Our results rely heavily on a uniform protocol applied when handling all animals since many factors can influence sympathetic activity and consequently – various hormones' levels. Collection of the blood samples in our study was not without stress which could itself greatly modify the circulating amounts of the catecholamines, even in spite of a rapid clearance and catabolism of adrenaline and noradrenaline (Dillon et al. 1992). This applies particularly to small animals, where the difficulty lies in providing stress-free conditions, contrasted with the need to collect amounts of blood that are large in proportion to the size of the animal (Buhler et al. 1978). Moreover, substantial between individual variation and different sampling techniques crucially affect the reliable measurement of the plasma catecholamine concentrations. For example, in rats differences in ADR, NADR and DOP between freely moving, undisturbed rats and handled animals were severalfold (Buhler et al. 1978). In addition, differential rates of metabolism of catecholamines due to interindividual variation might also produce differences in circulating levels unrelated to rates of release of these compounds. In our study, great care was given to treating all animals in the same way, maintaining repeatable sequences of procedures and randomizing handling order with respect to e.g. times of day whenever individuals could not be handled at the same time. Thus, we avoided overlaying any systematic handling differences with our experimental groups. It makes treatment-related conclusions valid even if observed levels of hormones were affected by specific procedures in the experimental protocol.

To conclude, our results demonstrated that food, besides its nutritional function, seems to represent one of main modulators of behavioural expression. Modified learning abilities, reaction to stress and levels of aggression inflicted by a nutrient-restricted diet may be crucial for survival. Our study emphasizes that after meeting the short-term energetic nutritional goals, it is the amino acid food composition (especially with respect to indispensable amino acids) that becomes a priority (Gietzen and Aja 2012). Behavioural modulation via diet observed here is also in line with evidence that many species (Roth et al. 2006; Fortes-Silva et al. 2012), including birds (Murphy and Pearcy 1993), have a demonstrated ability to perceive dietary limitations. Behavioural changes in response to the levels of indispensable amino acids may be extremely useful in helping animals to optimize foraging efficiency to ensure the adequacy of protein intake over time (Gietzen and Aja 2012). The dependence of behaviour expression on diet poses interesting questions about the among-individual differentiation in personality (Han and Dingemanse 2015) and may soon emerge as one of the major topics in personality-related study.

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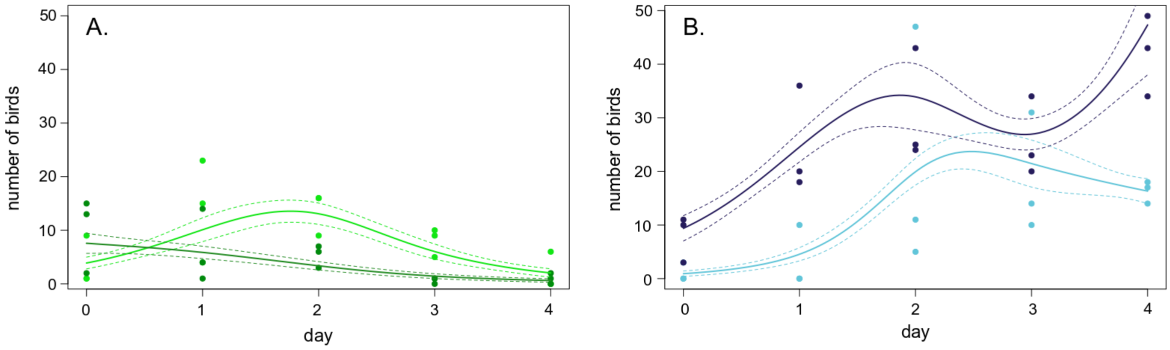
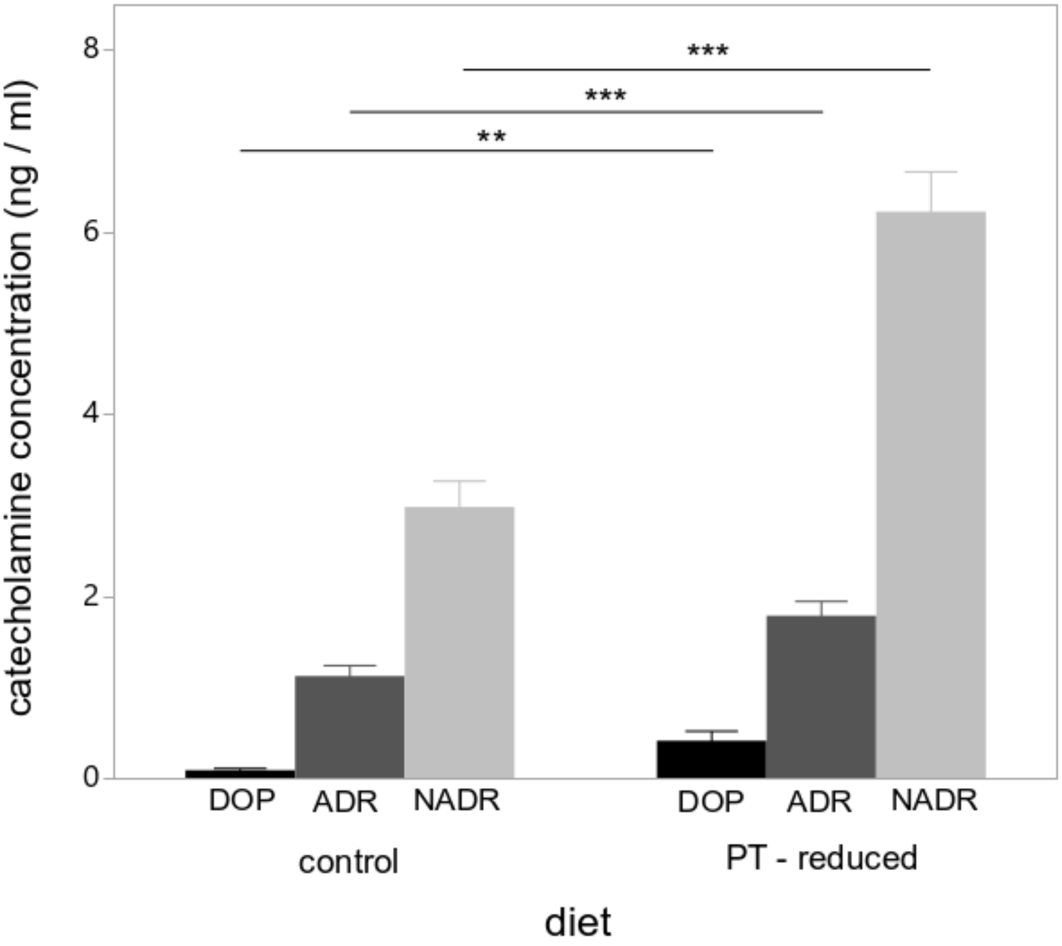
FIGURE LEGENDS

Figure 1. Number of house sparrows feeding on the green feeder filled with unpalatable food (A) and the blue feeder with control food (B) during five days of the experiment. Dark colours represent control birds, whereas bright colours refer to sparrows fed phenylalanine and tyrosine reduced diet. Points represent data for groups of 10 individuals, lines depict predictions from the GAM model with their standard errors.

Figure 2. Catecholamine concentrations (DOP – dopamine, ADR – adrenaline, NADR – noradrenaline) in plasma of house sparrows fed control (n=29) and phenylalanine and tyrosine (PT) diet (n=30). The asterisks indicate significant differences between the groups, ** $p < 0.01$, *** $p < 0.001$. Error bars represent standard errors.


A.
B.
number of birds
day
0
10
20
30
40
50
0
1
2
3
4


catecholamine concentration (ng / ml)
0
2
4
6
8
**
***
***
DOP
ADR
NADR
control
DOP
ADR
NADR
PT - reduced
diet

TABLES AND TABLE LEGENDS

Table 1. Formulation for synthetic diet used in experiment on house sparrows

Component	**g/kg**
Cornstarch	471.9
Protein and free amino acids	100.0
Maltodextrin	132.0
Saccharose	100.0
Vegetable Oil	70.0
Cellulose	50.0
Salt mix[1]	63.5
Vitamin mix[2]	10.0
choline bicarbonate	2.5
tert-Butylhydroquinone	0.014

[1] according to the composition contained in Fox Briggs N Mineral Mix (Dyets, Inc., Bethlehem, Pennsylvania, USA)

[2] according to the composition contained in NRC Chick Vitamin Mix (Dyets, Inc.)

Table 2. Percentages of amino acids present in diet treatments, including amino acids present in protein concentrate WPC80, glycomacropeptide BiPRO GMP 9000 and free amino acids

Amino acid	**Percentage**
Alanine	4.3
Arginine	7.1
Aspartic acid	8.1
Cysteine	0.9
Glutamic acid	19.3
Glycine	6.4
Histidine	2.8
Isoleucine	5.9
Leucine	7.9
Lysine	6.4
Methionine	1.5
Phenylalanine	1.8 *
Proline	6.7
Serine	4.6
Threonine	8.4
Tryptophan	0.8
Tyrosine	1.6 *
Valine	5.6

* birds on control diet received a mixture of amino acids that included 4.25% phenylalanine and 3.79% tyrosine.

Table 3. Results of the Generalized Additive Model showing effects of diet type on the number of sparrows feeding from the green feeder. The model included treatment, day of the experiment and recording time as fixed factors and two-way interaction between day x treatment. Reference levels for fixed effects: treatment – control; recording time: afternoon.

	Estimate	**SE**	***z***	***p***
Intercept	1.31	0.19	7.02	<0.001
treatment (PT-reduced)	0.77	0.21	3.68	<0.001
recording time (morning)	-0.32	0.20	-1.61	0.11

Approximate significance of smooth terms				
	edf	**ref.df**	**Chi.sq**	***p***
day : treatment (control)	1.95	2.34	26.30	<0.001
day : treatment (PT-reduced)	2.91	3.31	32.27	<0.001

Table 4. Results of the Generalized Additive Model showing effects of diet type on the number of sparrows feeding from the blue feeder. The model included treatment, day of the experiment and recording time as fixed factors and two-way interaction between day x treatment. Reference levels for fixed effects: treatment – control; recording time: afternoon.

	Estimate	**SE**	***z***	***p***
Intercept	3.09	0.09	32.93	<0.001
treatment (PT-reduced)	-1.17	0.15	-7.66	<0.001
recording time (morning)	0.13	0.18	0.73	0.47

Approximate significance of smooth terms				
	edf	**ref.df**	**Chi.sq**	***p***
day : treatment (control)	3.14	3.33	55.54	<0.001
day : treatment (PT-reduced)	3.09	3.42	47.36	<0.001