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Effect of tyrosine and phenylalanine supplementation on the colour and behaviour of chinchillas

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ABSTRACT. The purpose of the study was to verify whether increasing the content of tyrosine and phenylalanine in the feed would result in more intense colouration of chinchilla fur and affect the animals' reactions to behavioural tests. Chinchillas were divided into 3 groups: G-1 fed complete commercial fodder (Tyr 6.91 mg/g, Phe 7.45 mg/g), G-2 fed commercial and experimental fodder (1:1 ratio), and G-3 fed with experimental fodder (Tyr 14.31 mg/g, Phe 21.65 mg/g). During the experiment, the fur colouration type was assessed using two methods: objective, with a colorimeter (CIE L*a*b* colour space: L* – light-dark axis, a* – red-green axis, b* – yellow-blue axis, C* – colour saturation), and subjective, by a qualified chinchilla judge. A behavioural hand test was used to categorise the chinchillas' responses to human intrusion into their cage. The results from the colorimeter measurements did not show any significant differences in the L* ($P > 0.05$) component; however, significant differences were observed for the a* ($P = 0.0029$), b* ($P = 0.0218$), and C* ($P = 0.0342$) components in relation to the fur colouration of individual groups. The assessment of the colour type by a qualified specialist found statistically significant differences ($P < 0.05$) between the initial and final measurements. Spearman's correlation coefficients between colour type assessment and colour components were mostly low or medium, but with stronger values observed for L* compared to a*, b* or C*. There were no significant effects of the feeding group on the results of the and test. However, a decreasing trend was observed in the hand test results for G-3 from week 5 of the measurements.

Introduction

The long-tailed chinchilla (*Chinchilla lanigera* Bennett, 1829) is native to northern Chile and can be found along the foothills of the Andes and the coastal mountains south to Talca, at an elevation of 400–1650 m. Human activities, such as poaching, hunting, grazing of cattle and goats, mining, firewood extraction, and the pet trade, have posed a significant threat to this species for years. Although legislation to protect the species has been in place since 1929, it was not efficiently enforced until the establishment of the Reserva Nacional Las Chinchillas in Auco, Chile in 1983. This species has been included in the CITES Appendix I since 1977. The current wild population is estimated at around 5350 mature individuals, and despite current protection measures, populations continue to decline, and the species is classified as endangered according to the Red List Category & Criteria (Jimenez, 1996; Roach and Kennerley, 2016). Currently, chinchillas are a popular pet and farm animal, but due to

their very valuable fur, their domestication began in the 1920s, with twelve wild individuals captured in the Andes by Matthew Chapman (Spotorno et al., 2004). In contrast to the low number of individuals in the natural environment, population of chinchillas kept in captivity can be estimated in the hundreds of thousands (Łapiński, 2018). Most breeding chinchillas represent a standard colour variant, similar to wild chinchillas with beautiful blue-grey fur, shaded darkest on the back, lighter grey on the sides, and white on the abdomen. Over time, selective breeding has produced several other colour mutations known as: Black Velvet, Beige, White, Ebony, Violet, Sapphire, etc. (Barabasz, 2008). The assessment of chinchillas often prioritizes animals with darker fur, as they tend to receive higher scores due to the currently applied evaluation methods (KCHZ, 2012; EPVC, 2019; ČSCH, 2021). Mammalian skin and hair colour is primarily determined by the type and amount of melanin present. Melanin is synthesised in in specialized pigment-producing cells called melanocytes, where it is generated as pigment granules known as lysosome-related organelles called melanosomes (Wasmeier et al., 2008; Videira et al., 2013). Melanin is produced from the amino acid tyrosine through a synthesis regulated by the enzyme tyrosinase. Melanin biosynthesis can be initiated either by the hydroxylation of phenylalanine to tyrosine or directly from tyrosine, which is subsequently hydroxylated to dihydroxyphenylalanine (DOPA). The oxidation of DOPA to dopaquinone is a crucial next step in both eu- and pheomelanogenic pathways. Eumelanogenesis involves the further transformation of dopaquinone to leukodopachrome, followed by a series of oxidoreduction reactions with the formation of dihydroxyindole (DHI) and DHI carboxylic acid (DHICA) intermediates that undergo polymerisation to form eumelanin (Prota, 1995; Ito, 2003; Simon et al., 2009). Pheomelanogenesis also starts with dopaquinone, which is then conjugated to cysteine or glutathione, yielding cysteinyldopa and glutathionyldopa transformed further into pheomelanin (Ito, 2003; Simon et al., 2009). Melanin in hair consists of a mixture of two pigments: eumelanin (brown and black pigments) and pheomelanin (yellow to reddish-brown pigment) (Ozeki et al., 1995). The precursor of both pigments is derived from tyrosine, which can be sourced directly from the diet (foods high in dietary tyrosine include cheese, soybeans, beef, lamb, pork, fish, chicken, nuts, eggs, dairy, beans, and whole grain), or generated by hydroxylation of the essential amino acid phenylalanine (Schallreuter, 2008; Watson et al., 2017; Kühn et al., 2019). Although hair colour is essentially determined by genetics, nutrition can play a significant role in its expression. A number of studies have been conducted that illustrate the significant effect of nutrition on pigmentation in dogs and cats; however, the relationship between nutrition and hair pigmentation is still not well understood (Morris et al., 2002; Watson et al., 2015).

Mutations in genes influencing melanocytes not only affect the animal's colouration but are also believed to impact physiological and behavioural functions (Finn et al., 2016). Quesada and Senar (2007) conducted an experiment on the great tit (*Parus major*) to analyse the relationship between nest defence and plumage colouration. Males with a large black tie defended their nests more vigorously, while no such effect was found for yellow-breasted males. This suggests that melanin-based colouration in the great tit is associated with aggression, including both dominance-aggression and nest defence, whereas carotenoid-based colouration is not. Mafli et al. (2011) proved that eastern Hermann's tortoises (*Eurotestudo boettgeri*) with eumelanic shell colouration were more aggressive in male-male confrontations and bolder towards humans. These relationships were independent of body size and ambient temperature. In the American red fox (*Vulpes vulpes fulva*), wild red and black animals were demonstrated to be the least trusting and the fear response (the average startle distance) was modified by the presence of mutant coat colour genes (Keeler et al., 1970). During a 40-year-long study on fox domestication, it was observed that mating individuals showing interest/less fear towards humans produced more friendly and cooperative offspring, with mutated coat colours, resulting in the characteristic "white star" – hair without pigmentation, which occurs in domesticated animals like horses, cattle, dogs, rabbits, etc. This was due to an increase in serotonin levels and a decrease in corticosteroids, which affected the production of melanocytes that make up the animal's hair pigment (Trut, 1999). One of the most important aspects of farm animal welfare is the proper human-animal relationship. Behavioural tests (e.g. stick test, hand test) are used as a non-invasive method to quantify and evaluate animal personalities and categorise them as fearful or shy. These tests have been widely applied in domestic, farm, and even wild animals. Chinchillas are typically confident and trusting animals, although some individuals may show undesirable reactions to humans like fear or aggression. Fearlessness towards humans can reduce

stress, and confident individuals can better tolerate farm conditions (Browning, 2020; Łapiński et al., 2023). However, if dietary supplementation was to increase aggressive or fearful behaviour, it would be detrimental to animal welfare.

The aim of our study was to verify whether increasing the content of tyrosine and phenylalanine in granulated feed would result in more intense staining of the chinchilla's hair coat. Additionally we sought to determine whether adding tyrosine, a precursor of norepinephrine, would alter the chinchilla's reaction to humans.

Material and methods

Animals, housing, and colour type measurements

Since this was a feeding experiment with no invasive procedures performed on the animals, it did not require the approval of the relevant Ethics Committee for procedures used in animal experiments.

The experiment was carried out on a chinchilla breeding farm (Myślenice, Poland) for 4 months, from December to April. Fifty-four four-month-old standard chinchillas were selected from the herd, with no indication of siblings or half-siblings based on pedigree control. The animals were then divided into the 3 following groups of 18 individuals each (9 males and 9 females): G-1 – control, received full dose of commercial fodder (K); G-2 – received commercial granulate mixed with the experimental fodder in a 1:1 ratio; G-3 – fed with the experimental fodder (E) with tyrosine (Tyr) and phenylalanine (Phe) addition. The chinchillas were given *ad libitum* access to the fodder and were housed individually in stainless-steel cages (0.40 m width × 0.50 m length × 0.35 m height) with a wire floor.

During the experiment, fur colour type was assessed using two techniques: objectively with a colorimeter CR-410 (Konica Minolta Co. Ltd., Osaka, Japan) and subjectively by a specialist from the National Animal Breeding Centre (KCHZ, 2012). Colorimeter measurements were performed monthly according to the manufacturer's instruction; the device was calibrated before each measurement session. The colorimeter was applied to the animal's back between the shoulder blades, allowing for the determination of colour system coordinates according to the CIE (Commission Internationale de l'Eclairage) system. The L*, a*, and b* characteristics, i.e. colour brightness and chromaticity coordinates (L* for light-dark axis, a* for red-green axis, and b* for yellow-blue axis) were determined. For L*, a value of 0 indicates pure black and a value of 100 indicates pure white. Higher values of the a* discriminant indicate a tendency towards red, and lower values indicate a tendency towards green. Positive values of the discriminant b* mean yellow, and negative values indicate blue. The a* and b* values were used to calculate colour saturation (chroma, C*) from the following equation:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Phenotypic assessment was performed at the beginning and at the end of the experiment, according to the chinchilla phenotype assessment template (Table 1) (KCHZ, 2012).

Table 1. Phenotypic assessment of the colour type of standard chinchillas (KCHZ, 2012)

Assessment	Requirements description	Points
Exemplary	Very dark type, graphite-black, veil evenly distributed on the back, sides, hips, contrastingly cut off from the belly, minimal lightening on the nape is allowed (up to 2 cm^2).	5
Minor flaws	Very dark type, graphite veil, evenly distributed on the back, with a slight lightening on the neck, sides or hips, contrastingly cut off from the belly.	4
	Dark type, graphite veil, thickened on the back, evenly passing to the sides, lightened on the neck and hips, contrastingly cut off from the belly.	3
	Medium type, veil thickened on the back, slightly extending to the sides, lightened on the nape and hips, separating from the belly.	2
Major flaws	Light type, veil brightened along the entire length of the back, neck, sides, and hips, indistinctly separating from the abdominal belt.	1
Disqualifying-defects	No veil or patchy distribution of the veil.	0

Behavioural study

The hand test following the methods of Łapiński et al. (2023) was used to categorise the responses of chinchillas to the intrusion of a researcher's hand into their cages. The tester, a stranger to the animal, placed one hand on the open front of the cage and moved it slowly inside. The chinchilla's reaction to the manipulation attempts was recorded using a five-point scale (Table 2). The duration of the test was approximately 15–30 s, depending on how much the animal interacted with the researcher. The test was carried out in each cage for each animal every two weeks during the four months of the experiment (9 replicates per animal). The mean scores obtained from this test were used to classify the animals' behaviour: 1.00–1.80 = confident (CON); 1.81–2.60 = cautious (CAU); 2.61–3.40 = timid (TIM); 3.41–4.20 = nervous (NER); 4.21–5.00 = aggressive (AGG).

Table 2. Hand test scores categorising chinchillas' responses to human intrusion into their cages

Score	Description
1	Chinchilla is not afraid, sniffs the hand, approaches with interest, allows its head and back to be stroked.
2	Chinchilla explores the hand at a distance (no physical contact), approaches but withdraws, does not allow itself to be touched.
3	Chinchilla makes warning calls (barks), moves away from the hand, runs around the cage.
4	Chinchilla runs around the cage and screams, often stands on two paws and attempts to spray the person with urine.
5	Before opening the cage, chinchilla makes warning calls, takes flight around the cage, tries to bite.

Fodder composition

At the feed mixing plant of the National Research Institute of Animal Production (IZ-PIB), 2 kg of L-Phe FCC (cat. no.: W358509; Sigma-Aldrich, Saint Louis, MI, USA) and 1 kg of L-tyrosine FG (cat. no.: W373605; Sigma-Aldrich, Saint Louis, MI, USA) were added to 150 kg of comminuted commercial fodder. Amino acids were added to the fodder based on literature data (Yu et al., 2001; Morris et al., 2002). The granulate was prepared again after mixing the feed. The composition of commercial and experimental fodder was tested in the laboratory of the Department of Animal Nutrition and Biotechnology, and Fisheries at the University of Agriculture in Krakow (Tables 3, 4).

Table 3. Composition of commercial (K) and experimental (E) fodder

	DM %	CA %	TP %	CF %	CF %
Fodder K	90.34	9.86	19.81	3.06	15.32
Fodder E	89.98	9.70	20.30	3.73	14.56

DM – dry matter, CA – crude ash, TP – total protein, CF – crude fat, CF – crude fibre

Statistical analysis

All analyses were performed using SAS software (SAS, 2014). Data were tested for normality before analysis using the Shapiro-Wilk test (Table 5). Fur colour components, i.e. L*, a*, b*, and C* had a normal distribution. The assumptions of normality were met for two categorised data types, i.e. hand test and colour type assessment.

Fur colour components were analysed with repeated measures data using the MIXED procedure of SAS (2014). The following linear model was applied:

$$Y_{ijkl} = \mu + FE_i + ME_j + (FE \times ME)_{ij} + AN_k + E_{ijkl},$$

where: Y_{ijkl} – fur colour components, i.e. L*, a*, b* and C*; μ – overall mean; FE_i – fixed effect of *i*-th feeding (i = 1, 2, 3); ME_j – fixed effect of *j*-th measurements (i = 1, 2, 3, 4); $(FE \times ME)_{ij}$ – interaction between feeding and measurements; AN_k – random animal effect (k from 1 to 54); E_{ijkl} – residual effect.

Table 4. Amino acid composition of the commercial (K) and experimental (E) fodder (mg/g dry matter)

Amino acid	Fodder K	Fodder E
Alanine	8.16	7.43
Arginine	12.61	11.14
Aspartic acid	19.21	16.58
Cysteine	3.43	2.86
Glutamic acid	30.56	29.17
Glycine	9.00	7.89
Histidine	5.52	5.36
Isoleucine	5.97	6.37
Leucine	12.25	11.92
Lysine	9.14	8.09
Methionine	3.94	3.47
Phenylalanine	7.45	21.65
Proline	9.93	9.46
Serine	8.85	6.31
Threonine	7.27	6.07
Tyrosine	6.91	14.31
Valine	8.43	9.15

Table 5. Statistical value and *P*-value for the Shapiro-Wilk test

Trait	Statistic value (W_0)	*P*-value
L*	0.992915	0.3958
a*	0.993432	0.4644
b*	0.989861	0.1375
C*	0.990491	0.1727
Hand test	0.789026	<0.0001
Colour type assessment	0.765120	<0.0001

L* – lightness of chinchilla fur, a* – redness of chinchilla fur, b* – yellowness of chinchilla fur, C* – chroma of chinchilla fur

The significance of differences was determined by the Tukey-Kramer test. The non-significant fixed effect of sex was removed from the model.

The effects of animal, feeding, and measurements on the hand test and colour type assessment were analysed using the NPAR1WAY procedure of SAS (2014) and the Kruskal-Wallis test. The significance of differences was determined using the Dwass-Steel-Critchlow-Fligner method. Additionally, the effect of feeding during the measurement week (hand test and colour type assessment) or month (colour components, i.e. L*, a*, b*, and C*) were analysed. All *P*-values less than 0.05 were considered statistically significant. Additionally, the Spearman's correlation coefficients between colour type assessment and colour components were calculated (PROC CORR).

Results

Colorimeter analyses did not show any significant differences in the L* ($P = 0.5353$) component, while the a* ($P = 0.0029$), b* ($P = 0.0218$), and C* ($P = 0.0342$) components differed significantly with respect to fur colour of chinchillas from individual feeding groups (Table 6). The results of subsequent L*, b*, and C* measurements carried out at monthly intervals differed significantly ($P < 0.05$) from each other (Figure 1). A significant interaction was observed between feeding and monthly measurements for a* ($P = 0.0338$), but not for L* ($P = 0.5623$), b* ($P = 0.1397$) or C* ($P = 0.1401$).

Table 6. Least square means (LSM) with standard errors (SE) of lightness (L*), redness (a*), yellowness (b*), and chroma (C*) of chinchilla fur (by feeding group)

Feeding group	L*		a*		b*		C*	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
G-1	25.99	0.7091	0.24^{a}	0.0152	2.27^{a}	0.0601	2.28^{a}	0.0609
G-2	26.91	0.7157	0.21^{a}	0.0154	2.46^{b}	0.0606	2.47^{b}	0.0614
G-3	26.31	0.7091	0.16^{b}	0.0152	2.41^{b}	0.0601	2.42^{b}	0.0609

G-1 – control, received full-dose commercial fodder (K); G-2 – received feed consisting of commercial granulate mixed with experimental fodder at a 1:1 ratio; G-3 – fed experimental fodder (E), with higher amounts of tyrosine (Tyr) and phenylalanine (Phe); ^{ab} – values in the same column marked with different letters differ significantly ($P < 0.05$)

Analysis of the hand test results showed significant differences ($P < 0.0001$) between individual animals, but the effect of feeding group was not significant (Table 7). However, the hand test results obtained at successive weekly measurements were statistically different ($P < 0.05$), showing a tendency for behaviour to change from timid (2.61–3.40, TIM) in weeks 1–7 to cautious (1.81–2.60, CAU) in the last two weeks, i.e. 8–9 (Figure 2).

The differences between animals in colour type assessment and the effect of feeding group on the colour type assessment were insignificant ($P = 0.9494$ and $P = 0.4984$, respectively) (Table 8).

Table 7. Characteristics of hand test results (by feeding group)

Feeding group	N	Mean	SD	Min	Max	Median
G-1	162	2.75	0.75	1	4	3
G-2	157	2.76	0.80	1	4	3
G-3	162	2.62	0.64	1	4	3
P-value	0.0731					

G-1 – control, received full-dose commercial fodder (K); G-2 – received feed consisting of commercial granulate mixed with experimental fodder at a 1:1 ratio; G-3 – fed experimental fodder (E), with higher amounts of tyrosine (Tyr) and phenylalanine (Phe); N – number of observations (number of animals × number of hand test per animal), SD – standard deviation; colour type evaluation score: 0 – 1 – 2 – 3 – 4 – 5

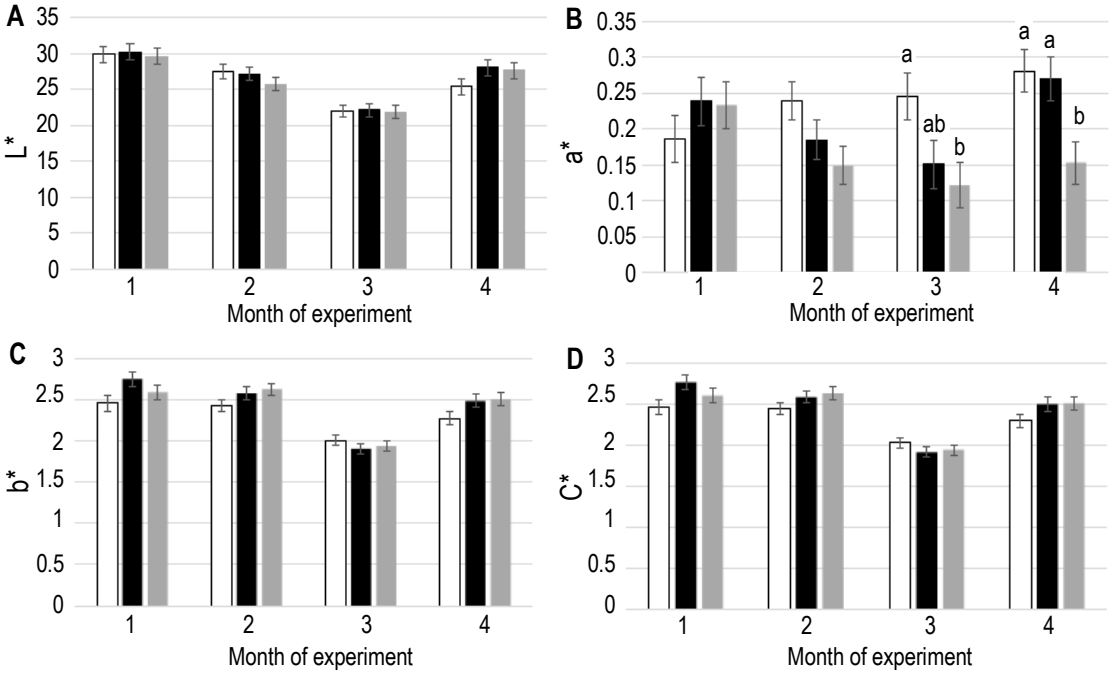

Figure 1. Least square means with standard errors of A) lightness (L*), B) redness (a*), C) yellowness (b*), and D) chroma (C*) of chinchilla fur, by feeding group and monthly measurements. Values with different letters in third and fourth month of experiment differ significantly ($P < 0.05$)

☐ G-1 – control, received full-dose commercial fodder (K); ■ G-2 – received feed consisting of commercial granulate mixed with the experimental fodder at a 1:1 ratio; ■ G-3 – fed experimental fodder (E), with higher amounts of tyrosine (Tyr) and phenylalanine (Phe)

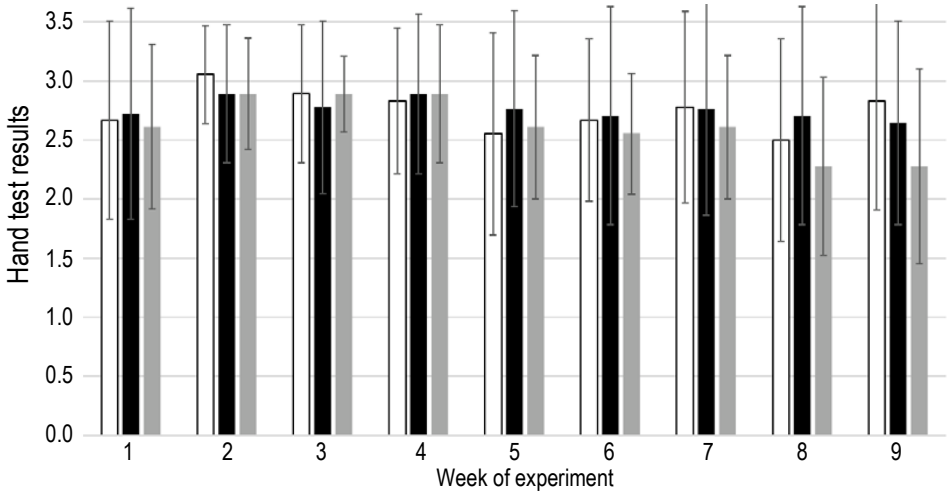

Figure 2. Mean values with standard deviations of hand test results, by feeding group and weekly measurements

☐ G-1 – control, received full-dose commercial fodder (K); ■ G-2 – received feed consisting of commercial granulate mixed with experimental fodder at a 1:1 ratio; ■ G-3 – fed experimental fodder (E), with higher amounts of tyrosine (Tyr) and phenylalanine (Phe); $P > 0.05$

Table 8. Characteristics of colour type assessment (by feeding group)

Feeding group	N	Mean	SD	Min	Max	Median
G-1	36	3.64	0.68	2	5	4
G-2	35	3.46	0.66	2	4	4
G-3	36	3.44	0.73	2	4	4
P-value	0.4984					

G-1 – control, received full-dose commercial fodder (K); G-2 – received feed consisting of commercial granulate mixed with experimental fodder at a 1:1 ratio; G-3 – fed experimental fodder (E), with higher amounts of tyrosine (Tyr) and phenylalanine (Phe); N – number of observations (number of animals × number of measurements per animal), SD – standard deviation; colour type evaluation score: 0 – 1 – 2 – 3 – 4 – 5

However, there were statistically significant differences ($P < 0.05$) between the initial and final measurements of the experiment when assessed by a qualified specialist (Figure 3).

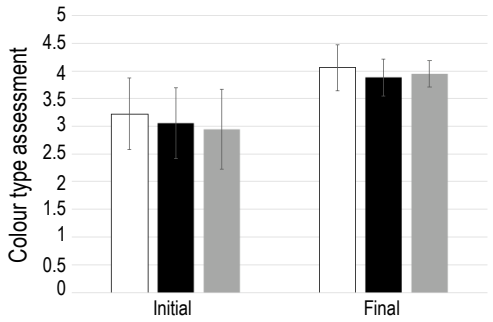

Figure 3. Mean values with standard deviations of colour type assessment, by feeding group and initial or final measurements

☐ G-1 – control, received full-dose commercial fodder (K); ■ G-2 – received feed consisting of commercial granulate mixed with experimental fodder at a 1:1 ratio; ■ G-3 – fed experimental fodder (E), with higher amounts of tyrosine (Tyr) and phenylalanine (Phe); $P > 0.05$

Spearman's correlation coefficients (Table 9) between colour type assessment and colour components had low to medium values (absolute value ranging from 0 to 0.51).

Table 9. Spearman's correlation coefficients between colour type assessment and colour components

Colour components	Measurement, month	Colour type assessment	
		initial	final
L*	1	−0.51	−0.23
a*		−0.07	−0.03
b*		−0.08	−0.22
C*		−0.08	−0.22
L*	2	−0.25	−0.33
a*		0.00	0.12
b*		−0.04	0.03
C*		−0.04	0.03
L*	3	−0.50	−0.42
a*		−0.30	−0.26
b*		−0.17	−0.18
C*		−0.18	−0.19
L*	4	−0.39	−0.42
a*		0.13	−0.11
b*		−0.07	−0.26
C*		−0.06	−0.26

L* – lightness of chinchilla fur, a* – redness of chinchilla fur, b* – yellowness of chinchilla fur, C* – chroma of chinchilla fur

Discussion

The colour of animals' coat is primarily genetically determined, but it is known that certain exogenous or environmental factors may also play an important role. For instance, exposure to ultraviolet (UV) light, particularly in combination with

humid conditions and high temperatures, can cause photobleaching of dark hair or photoyellowing of gray hair. The chemistry of such changes is not fully understood, but amino acids, particularly aromatic amino acids, are susceptible to damage or polymerisation when exposed to UV light and oxygen (Busch-Kschiewan et al., 2004; Watson et al., 2017).

To ensure the well-being of chinchillas, it is recommended to position their cages away from direct sunlight. Overexposure to sunlight can lead to an undesirable brown hue in their coats, and in summer, they may suffer from heat strokes due to overheating. Proper ventilation is also important to reduce excess water vapour, dust, and gases (ammonia, carbon dioxide, hydrogen sulphide). High concentrations of ammonia can cause irritation of the conjunctiva and mucous membranes and contribute to the development of many diseases. Additionally, ammonia can cause yellowing of the white belly and discoloration of black hair, negatively affecting coat quality (Barabasz, 2008).

Nutrition is another factor that influences hair colour, particularly through the synthesis and deposition of eumelanin and pheomelanin in the hair shaft. The optimal pigmentation of young animals is obtained by increasing dietary Tyr/Phe levels above the basal growth requirements, as evidenced by a reduction in the a* value determined by spectrophotometry (Watson et al., 2017). The present study revealed statistically significant differences in the a*, b*, and C* colour components between the feeding groups. Significant differences were also observed in monthly colourimetric measurements, as the group fed fodder with the highest Tyr and Phe content had the lowest a* component values after three and four months of the experiment. This tendency was consistent with the results of other studies, such as an experiment conducted on kittens, where animals fed a diet with a low level of phenylalanine and tyrosine developed reddish-black hair, and the supplementation with these amino acids prevented any change in hair colour from the pre-treatment black hair (Morris et al., 2002). The latter authors argued that the development of red-haired cats' fur was due to a lack of Phe and/or Tyr, which are involved in the formation of melanin pigment. In another study by Yu et al. (2001), it was found that feed with a low content of the amino acid tyrosine resulted in a change of cats' fur colour from black into red, as black female cats fed this feed gave birth to red-haired kittens. These authors concluded that the requirement to maintain black colour was more than 4.5 g Tyr plus 12 g Phe, but less than 24 g Phe/kg feed.

Shekar et al. (2008) have suggested that the a* dimension of the CIE Lab spectrocolourimetic index is a good approximation of hair pheomelanin concentration as it measures the continuum of the red-green spectrum. Therefore, a lower value of a* reflects a lower concentration of pheomelanin in the hair, manifested by a less reddened hair shaft. The results obtained in the present study seemed to confirm this view, as the group fed fodder with the highest Tyr and Phe content had lower a* values (Table 6, Figure 1). These findings are the first of their kind for chinchillas, and they highlight the importance of balancing complete feed mixtures to avoid the appearance of a brown shade of hair, especially in animals with darker coats (e.g. dark standard, ebony, black velvet).

The differences in colourimetric measurements recorded in the present study between subsequent months could also be related to hair maturation process in chinchillas (Barabasz, 2008). The results of the fur assessment conducted by the assessor also corresponded with colourimetric test results and indicated hair maturation. However, no differences between the feeding groups were observed with this method of measurement.

Correlations between colour type assessment and colour components were mostly low or moderate, but higher values were recorded for the L* component (light-dark axis) than for a*, b* or C*. This relationship was consistent with the chinchilla phenotypic assessment model, promoting darker animals (KCHZ, 2012). It is worth considering whether objective measurement methods, e.g. the use of a colorimeter, would be helpful in assessing colour type to the assessor. Such methods are currently practiced when sorting skins in auction houses, although the control and final decision are made by humans (Saga, 2019).

In a study on mink, Clausen and Sandbøl (2009) showed that increasing Phe + Tyr levels in two groups of mink resulted in a tendency ($P = 0.09$) for darker fur colour when compared to a control group. Additionally, the results showed a trend towards lower weight in mink kits with lower levels of Phe + Tyr, and a trend towards darker fur coats in both black and wildtype mink kits with higher levels of Phe + Tyr.

Increased Tyr content in the fodder did not result in significant differences in the chinchillas' reaction to humans. However, a decreasing trend in the hand test results was observed in G-3 from the 5th week of measurements (Figure 2). Behaviour is regulated by neurotransmitters and hormones, and changes in the availability of their precursors can affect it. Tryptophan, a serotonin precursor, may promote the occurrence of aggression, mutilation, and

resistance to stress. The latter may also be affected by dietary tyrosine levels, which is a precursor to catecholamines. Since diet composition influences nutrient availability and interactions, the presence of these precursors in the brain may influence behaviour or stress resistance (Bosch et al., 2007). In rats, a tyrosine-rich diet can prevent adverse behavioural and neurochemical effects caused by acute stressors, including hypothermia, restraint, and tail-shock. In stressed rats (tail-shock), ingestion of a high-tyrosine diet reversed the post-stress decline in brain noradrenaline and attenuated behaviour changes, i.e. decreased locomotion, standing on hind legs, and hole-poking in a novel open field (Lehnert et al., 1984; Rauch and Lieberman, 1990). This suggests that a tyrosine-rich diet may be beneficial during periods of severe stress as it prevents depletion of the substrate required for catecholamine synthesis during high catecholaminergic activity and demand (Bosch et al., 2007).

Fur chewing is one of the most common and difficult problems on chinchilla farms, affecting around 3.5% of animals on 85% of farms. The aetiology of fur chewing has not been conclusively established, but it may be related to long-term stress, which can lead to the development of repetitive behaviours induced by frustration, repeated attempts to cope, and/or a central nervous system dysfunction known as impulsive-compulsive disorder (Łapiński et al., 2014). The present study, as well as previous research concerning the effects of dietary tyrosine on the neurochemical and behavioural consequences of stress (Lehnert et al., 1984; Rauch and Lieberman, 1990; Bosch et al., 2007) suggest that feeding chinchillas with impulsive-compulsive disorders with increased Tyr and Phe doses may be justified.

The hand test results from consecutive weekly measurements for all animals showed statistically significant differences with a downward trend, which could be related to the age of the animals. Studies on raccoon dogs, minks, foxes, and chinchillas have shown that older animals tend to behave more calmly than younger ones (Łapiński et al., 2013; 2014; 2019; 2023), and this tendency was also observed in this study. This could be due to the animals' adaptation to the farm conditions and procedures, and repeated treatments may progressively evoke less aggression in the animals. Similar conclusions were drawn by Korhonen (1988), who observed that younger raccoon dogs were more excitable and active than older raccoon dogs.

Conclusions

In summary, the addition of Tyr and Phe to chinchilla diets with optimal nutrition did not result in visible effects on the colour of the hair coat, although this effect was recorded in the colourimetric measurements (CIE L*a*b* colour space). The lower a* value, reflected in a reduced pheomelanin concentration in the hair, and thus less reddening of the hair shaft, was obtained in the group fed with the highest Tyr and Phe proportion in the diet. Age-related changes in fur colour were noted in relation to coat maturation. The addition of Tyr and Phe did not statistically affect the human-animal relationship (hand test results), but it could be observed that the animals administered the feed with the highest Tyr and Phe content, reacted more calmly to humans. The results of the experiment seem very promising and suggest that further research is needed to gain a better understanding of the observed trends.

Conflict of interest

The Authors declare that there is no conflict of interest.

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