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HISTOMORPHOMETRIC CHARACTERISTICS OF THE INTEGUMENTARY SYSTEM OF THE POLISH POPULATION OF FARMED AND WILD FOXES*

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Abstract

The aim of the study was to determine the degree of differentiation between selected histomorphometric characteristics of common fox (*Vulpes vulpes*) skins with regard to the origin of animals (farmed vs wild population). Skin size parameters with evaluation of hair coat quality, trace element composition of hair, and histomorphometric characteristics of cutaneous tissue were studied. The domestic population of wild foxes was characterized by low body weight and poor hair coat quality, which showed considerable felting (10–35% of skin area), absence of down, and deficiency of elements needed for proper hair development. Histological analysis of cutaneous tissue in wild foxes showed a lower number of bundles per tuft ($P \leq 0.05$), a lower number of down hair per tuft and bundle ($P \leq 0.01$) and a greater number of sebaceous glands, which had greater length ($P \leq 0.01$) and area ($P \leq 0.05$). Analysis of the level of trace and major elements in the hair of wild and farmed foxes revealed highly significant differences in the amounts of iodine, lead, selenium and sulfur. All of these elements were more abundant in farmed foxes except for lead, which was higher in wild foxes.

CECHY HISTOMORFOMETRYCZNE UKŁADU POWŁOKOWEGO POPULACJI KRAJOWYCH LISÓW HODOWLANYCH I DZIKO ŻYJĄCYCH

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Abstrakt

Celem badań było określenie stopnia zróżnicowania wybranych cech histologiczno-morfometrycznych skór lisów pospolitych (*Vulpes vulpes*) z uwzględnieniem pochodzenia zwierząt (hodowla fermowa w stosunku do populacji dziko żyjącej). Badano parametry wielkości skór wraz z oceną jakości okrywy włosowej, składem mikropierwiastków we włosach i oceną histologiczno-morfometryczną tkanki skórnej.

Krajowa populacja lisów dziko żyjących odznaczała się niższą masą ciała i jakością okrywy włosowej, która charakteryzowała się znacznym sfilcowaniem (10–35% powierzchni skóry), niewykształceniem puchu i brakami pierwiastków sprzyjających jej właściwemu rozwojowi. W ocenie histologicznej ich tkanki skórnej wykazano niższą liczbę pęczków w kępce ($P \leq 0{,}05$), liczbę włosów puchowych w kępce i pęczku ($P \leq 0{,}01$) oraz większą liczbę gruczołów łojowych odznaczających się większą długością ($P \leq 0{,}01$) i powierzchnią ($P \leq 0{,}05$). W ocenie poziomu mikro- i makroelementów we włosach lisów dzikich i hodowlanych wykazano wysoko istotne zróżnicowanie w ilości jodu, ołowiu, selenu i siarki. Z wymienionych pierwiastków wyższe wartości stwierdzono u lisów hodowlanych z wyjątkiem poziomu ołowiu, który był wyższy u lisów dzikich.

Introduction

Out of the many species of foxes living in the world, only two are raised in cages: the arctic fox (*Vulpes lagopus*) and common fox (*Vulpes vulpes).* The common fox is a carnivorous mammal (*Carnivora*) of the family *Canidae*. In its natural state, it inhabits the Northern Hemisphere, from the Arctic Circle to North America, Europe, Asia and North Africa. It easily adapts to different environments. Foxes are on the IUCN's (International Union for Conservation of Nature) list of the world's 100 worst invasive species (STATHAM et al. 2011, *100 of the word's*... 2016).

The history of fur animal domestication is relatively short. Originally, common foxes were only farmed in North America, where semi-feral reproduction with partial human intervention was used in the 18th century. The first fox farm was established on Prince Edward Island (Canada) in 1894. Fur-bearing carnivores came to European farms as breeding animals in the 1920s. The interest in cage farming increased as a result of pelts from farmed foxes receiving four times the price of best pelts from caught foxes at a large fur auction held in London in the years 1904–1910. In Poland, the origins of fur farming date back to the interwar period (1918–1939) when the first farms of silver foxes were set up in Silesia and later near Gdańsk (PIÓRKOWSKA 2015). JEŻEWSKA-WITKOWSKA et al. (2012) demonstrated that Polish farmed foxes originate from North America (Canadian), *Vulpes vulpes* subspecies.

Human domestication of the wild fur animals was aimed to modify morphological, physiological, developmental and mental characteristics so as to obtain desirable traits (GUGOŁEK et al. 2013, GUGOŁEK et al. 2014). After many generations of breeding work on fox farms, the productive traits of farmed foxes came to differ considerably from those of wild animals. Clear differences

are observed in the productive traits being improved, notably hair coat quality, coat colour, body weight, measurable traits of the organs of the skeletal and digestive systems, and even animal temperament (KULAWIK et al. 2013, GUGOŁEK et al. 2014).

The aim of the study was to determine the degree of differentiation between selected histomorphometric characteristics of fox skins with regard to the origin of animals (farmed vs wild population).

Materials and Methods

The experiment used 40 raw skins of foxes. Twenty skins originated from a breeding farm belonging to the Experimental Station of the National Research Institute of Animal Production Chorzelów Ltd., and another 20 from wild animals harvested in north-eastern Poland during late autumn. An equal sex ratio was maintained in each group.

Pretreatment, fleshing, drying and preservation of the skins were performed in accordance with relevant standards for this animal species.

Physical parameters of the raw skins and quality traits of the hair coat were evaluated based on the methods described by KASZOWSKI and KAWIŃSKA (1960) and PIÓRKOWSKA (2001, 2002). The tests included body weight at slaughter, measurement of skin size parameters (weight, length of skin and tail, width of skin, planimetric area). Based on skin weight and area, the weight of 1 dm^2 skin was calculated to determine its lightness. All skins were graded for size according to auction sale requirements (SKINPOLEX 1994, *Sagafurs*® 2016). The following skin measurements and corresponding sizes were accounted for [cm]:

Auction size	Size [cm]
30 (000)	> 115.1
20 (00)	106.1–115.0
0	97.1–106.0
1	88.1–97.0
2	79.1–88.0

The experimental skins were evaluated organoleptically for defects and damage to the integumentary system (cutaneous tissue and hair coat). The defects and their extent were defined, and measurable traits were measured.

Histological and morphometric tests were performed on cutaneous tissue samples fixed in 6% buffered formalin. Samples were dehydrated in a graded ethanol series and cleared in xylene. Sections made from paraffin blocks were

cut on a microtome into 6–8 μm thick slices and stained differentially with Delafield's hematoxylin and eosin or with Mallory's stain based on three stain types: acid fuchsin, phosphomolybdic or phosphotungstic acid, and Orange G with aniline blue and oxalic acid (ZAWISTOWSKI 1970). The preparations were analysed and their microphotographs were taken using a Nikon Eclipse E-400 microscope and MultiScan program with the ScanBase image and text database (computer image analysis system MultiScanBase v.18.03).

Chemical analysis of hair was performed as a method of evaluating the body's mineral status. Concentration of bioelements (iron, zinc, copper, manganese, cobalt, selenium, sulfur, iodine, silicon, calcium, magnesium, lead, mercury) in hair was determined using an X-ray fluorescence (XRF) spectrometer. This method analyses the amount of X-ray radiation reaching the detector after being reflected from the sample.

The results were analysed by one-way ANOVA F-test in an orthogonal design. The calculations were made with Statistica 7.1 PL package using the following linear model:

$$y_{ij} = \mu + a_i + e_{ij}$$

where:
y_{ij} – observed value of a trait
μ – mean value of a trait in the population
a_i – effect of experimental group (1, 2)
e_{ij} – random error.

Results

The mean body weight of farmed foxes was highly significantly higher than that of wild foxes (6.74 and 5.42 kg, respectively; Table 1). The low body weight of wild foxes translated into the size and area of their skins, which differed considerably from those in the skins of farmed animals. Highly significant differences in favour of farmed foxes concerned all of the skin size parameters. In relation to the wild skins, skins from farmed foxes were about 34% heavier, 12% longer and 5% wider, with surface area greater by 25%. Comparison of the furs for lightness showed that the skins of wild foxes were lighter than those of farmed foxes by 1.24 g per dm^2 of skin.

The largest proportion of wild fox skins (85%) were auction size 2 and 1 (Figure 1). Two skins (10% of all wild fox skins) were too small for auction sale. The skins of farmed foxes were classified into four auction sizes, 55% of which were size 20 and 0, corresponding to skin sizes ranging between 97.1 and 115 cm.

Table 1

Measurements of raw skins from foxes

Item	Foxes			
	farmed	SD	wild	SD
Weight body [kg]	6.74^{A}	0.659	5.42^{B}	0.793
Weight of skin [g]	454.49^{A}	57.31	301.54^{B}	63.12
Total length of skin [cm]	134.82^{A}	6.249	118.70^{B}	9.608
Auction length of skin [cm]	99.37^{A}	4.874	86.32^{B}	6.736
Tail length [cm]	35.45^{a}	2.145	32.32^{b}	4.774
Width of skin [cm]	30.85^{A}	0.875	29.30^{B}	1.218
Area of skin [dm^2]	44.503^{A}	3.975	33.516^{B}	3.898
Weight of 1 dm^2 skin [g]	10.194^{A}	0.649	8.958^{B}	1.264

Means in rows with different letters differ significantly ($^{a, b}$ – $P \leq 0.05$; $^{A, B}$ – $P \leq 0.01$)

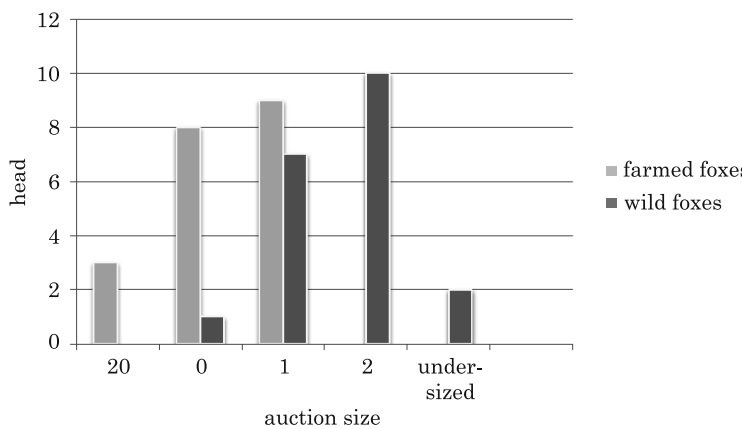

Fig. 1. Proportion of analysed skins according to auction size

Hair coat was felted in both farmed and wild foxes, but in the former this only concerned the pelvic girdle to a shallow/low extent (3 skins) and to a deep/high extent (1 skin), where hair tangling exceeded 1 cm in the cut sample. In wild foxes, felting spread from the sacrum to tail head and side, and the degree of felting was medium (2 skins) and deep/high (9 skins) – Table 2. Hair felting in wild foxes affected up to 35% of the skin area.

The histological examination of the wild foxes revealed a significantly lower number of bundles per tuft and a highly significantly lower number of down hair per tuft and bundle. The area, length and diameter of a hair bundle was highly significantly lower in these animals. No statistically significant differences were found in the number of down and awn hair. The skins of wild foxes had a greater number of sebaceous glands, which were highly significantly longer and had a significantly greater area (Table 3, Figure 2 and Figure 3).

Table 2

Degree of hair cover felting in foxes*

Foxes	Hair coat felting*						
	Site of occurrence	No. of skins acc. to degree of felting**:			No. of skins with defects	% of skin with defects	% of skin area felted, min – max
		shallow/ low	medium	deep/ high			
Farmed	pelvic girdle	3	–	1	4 (20)	20	1–5
Wild	from sacrum to tail head, side	–	2	7	9 (20)	45	10–35

* skins were grouped according to the highest degree of coat felting
** deep/high felting 1 cm and greater on a cut sample, counting from cutaneous tissue, medium felting from 0.5 to 1 cm, shallow/low felting up to 0.5 cm

Table 3

Results of histological examination of fox skins

Item	Foxes			
	farmed	SD	wild	SD
Thickness of skin layers [μm]:				
– epidermis	5.15	1.120	4.36	2.012
– dermis	219.30	46.25	222.75	45.11
No. of bundles per tuft	2.8^{a}	1.033	2.3^{b}	0.852
No. of down hair per tuft	41.3^{A}	14.46	30.2^{B}	17.41
No. of down hair per bundle	14.8^{A}	3.542	13.4^{B}	4.076
Hair bundle:				
– area [μm^2]	25064.9^{A}	5974	14695.5^{B}	4319
– length [μm]	276.63^{A}	47.15	216.67^{B}	32.16
– diameter [μm]	125.10^{A}	22.45	93.44^{B}	17.88
Guard hair:				
– area [μm^2]	5786.4	4558	7232.8	2549
– length [μm]	100.46	38.51	112.00	20.20
– diameter [μm]	73.39	24.48	82.89	14.93
Awn hair:				
– area [μm^2]	1355.9	290.5	1445.1	624.2
– length [μm]	50.37	8.256	48.97	11.04
– diameter [μm]	37.53	3.539	38.34	7.675
Sebaceous gland:				
– area [μm^2]	3823.4^{a}	1715	5633.1^{b}	3969
– length [μm]	121.07^{A}	29.22	174.51^{B}	84.19
– width [μm]	46.76	13.03	51.77	22.38

Explanations as in Table 1

Fig. 2. Microphotograph of a tuft with sebaceous gland from farmed fox (100x magnification, Mallory's staining): *1* – down hair; *2* – sebaceous gland; *3* – guard hair

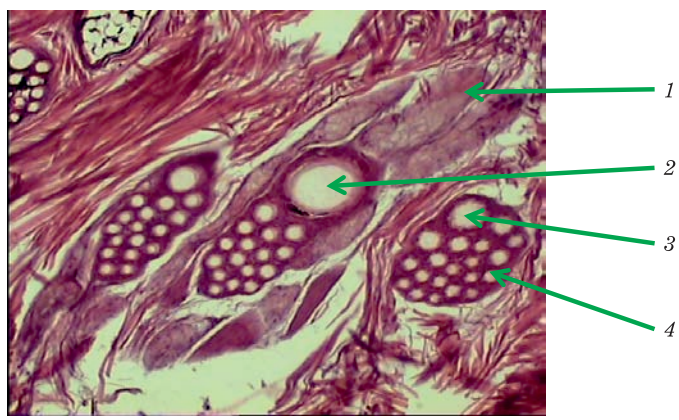

Fig. 3. Microphotograph of a tuft with sebaceous gland from wild fox (100x magnification, hematoxylin and eosin staining): *1* – sebaceous gland; *2* – guard hair; *3* – awn hair; *4* – down hair

Analysis of the level of trace and major elements in the hair of wild and farmed foxes revealed highly significant differences in the amount of iodine, lead, selenium and sulfur (Table 4). All of these elements were more abundant in farmed foxes except for lead, which was higher in wild foxes.

Table 4

Chemical analysis of hair [mg/kg]

Element	Foxes			
	farmed	SD	wild	SD
Ca (calcium)	348.25	154.1	301.94	137.8
Co (cobalt)	0.012	0.027	0.019	0.027
Cu (copper)	10.402	2.517	9.805	4.323
Fe (iron)	136.71	63.33	115.97	33.18
Hg (mercury)	0.241	0.113	0.284	0.107
I (iodine)	0.223^{A}	0.077	0.121^{B}	0.045
Mg (magnesium)	57.49	59.89	43.77	17.16
Mn (manganese)	0.782	0.446	0.958	0.363
Pb (lead)	0.996^{A}	0.452	1.528^{B}	0.334
Se (selenium)	0.429^{A}	0.109	0.304^{B}	0.124
S (sulfur)	245.30^{A}	54.85	132.48^{B}	65.42
Si (silicon)	372.93	173.6	289.81	67.78
Zn (zinc)	35.193	36.58	22.846	6.833

Explanations as in Table 1

Discussion

Fur-bearing carnivores have been farmed in Europe for about 95 years. According to authors (BRZOZOWSKI 2002, GUGOŁEK et al. 2008), differences between the skins of farmed and wild animals were small only during the initial period of farming. Today the differences in the size and quality of fur material are so large that over 86.5% of the skins offered by the auction houses originate from farmed animals (GUGOŁEK et al. 2008, GUGOŁEK 2015).

The considerably greater body weight and thus the skin size of the farmed foxes compared to the wild foxes, found in the present experiment, is the consequence of long-term breeding work and the use of properly balanced diets, which meet strict quality standards. Skin size may be indicated by its length, which reflects the animal's size. Similar results were obtained by JANISZEWSKI at al. (2010), LOREK et al. (2001), KULAWIK et al. (2013), PIÓRKOWSKA (2015), and PRZYSIECKI et al. (2006). Changes in the conformation of fur-bearing carnivores as a result of breeding work were investigated by BRZOZOWSKI (2002). The analysis covering the years 1975–2002 demonstrated that during this period the skin size of farmed foxes increased by two auction sizes, i.e. by around 18 cm. According to the information provided by the auction houses, after the year 2000 fox skin sizes 4, 3 and 2 were not included in the auctions (*Sagafurs* 2016).

The fur faults that considerably reduce the fur value of fox skins include felting, cutouts, greasiness, hair whorls, differences in the thickness and length of individual hairs and their ratio. In long-haired skins, the ratio of down hair to guard hair should be 70:30 to provide the coat with proper softness and thermal insulation. Adult foxes molt once a year – during the spring. During the summer hair growth, many hair roots remain dormant and winter hair begins to grow as late as the end of August (NATANEK et al. 2001). The lack of proper nutrients during this period may contribute to abnormal winter coat development, which we observed in foxes living in the wild. The number of down hair, in both tufts and bundles, was much smaller than in the farmed animals. The significantly larger area (thickness) of guard hair in the skins of wild foxes is evidence that their hair coat was rough and less noble (primitive).

Because hair felting, which is found in both farmed and wild animals, is a latent defect, it can be easily overlooked during preliminary visual assessment. This fault can be identified by the presence of soft, flabby and sticky hair that is devoid of elasticity. Organoleptic evaluation shows granularity at the base of hair and tufts of matted hair mass (BLOMSTEDT 2000). Many studies have shown that this defect is heritable (heritability of 0.26–0.38), similarly to hair coat quality, which means that it can be reduced through selection and deliberate selective breeding (BLOMSTEDT et al. 2001). The felting of skins from farmed foxes is probably a relic of the period when selection was for skin size as a factor having the largest effect on its price.

Histological examination is useful in evaluating the skins because it reveals changes occurring in different disease conditions or abnormalities in cutaneous tissue and hair structure. Histological analysis also allows for an accurate determination of hair coat density, making it useful for determining the hair-forming potential of skin.

Anatomically, the skin consists of three distinct layers – the epidermis, dermis and subcutaneous layer, which differ in structure, chemical composition and function – and hair coat, which is a product of the epidermis. Individual hairs penetrate deep into the dermis, to which they are attached through hair roots. Sebaceous gland secretion, which consists of fatty acids and cholesterol, protects the epidermis from drying out and the hair from being penetrated by water. Unlike the size of sebaceous glands, their number in animals does not change throughout their lifetime. In our study, the area, length and width of sebaceous glands (Figure 1 and Figure 2) were smaller in farmed foxes than in wild foxes, which can be seen as the result of domestication.

Farmed foxes, which are kept in covered cages, are not as exposed to the changing environmental conditions as animals living in the wild. Sebum

production is a natural process that creates a protective mantle for the skin and epidermis, and shields them from mechanical and chemical agents, microorganisms, changing environmental conditions, and UV radiation.

The hair coat of common foxes is classified as long due to the length of guard hair (45–110 mm). Hairs in the coat are arranged in tufts, and their number is about 10,000 per cm^2 (DUDA 1992). Hair coat characteristics have been analysed by many authors (SOCHA 1999, NOWICKI et al. 2010, 2012, PIÓRKOWSKA 2015), because these traits are continually changing under the influence of selection and environmental conditions.

Improper diet, ongoing pathological changes and the adverse environmental impact are reflected, among others, in the mineral composition of hair. The elemental concentration in hair largely depends on the diet currently in use (SKIBNIEWSKA et al., 2011). Organic trace elements are absorbed by the body and later deposited in the hair coat. Many studies suggest that there is a relationship between elemental levels in hair and internal organs (BIAŁKOWSKI and SABA 1987, ŁUCZAK-ZIELKIEWICZ and SZUTOWSKI 2013, SABA et al. 1982, *Life Line*... 2016). Thus, hair reflects the body's health status (KARCZEWSKI 1998, RADOMSKA et al. 1991). From blood tests it is impossible to conclude the accumulation of elements in different tissues in which they are stored, because their concentration varies with emotional changes or with the type of food consumed. No such variation is observed in hair. In a study on the mineral content of silver fox hair, SABA et al. (1982) determined that the mineral quantities were in the order of Na > Ca > K > Mg > P > Zn > Fe > Cu > Mn > Co, whereas the level of elements fell into the following ranges depending on the season: Na, 900–1150 ppm; Ca, 760–1170 ppm; K, 320–680 ppm; Mg, 200–300 ppm; P, 200–300 ppm; Zn, 49–64 ppm; Fe, 38–58 ppm; Cu, 10–14 ppm; Mn, 1–4 ppm; Co, 0.5–0.6 ppm.

The hair coat of wild foxes was found to contain a relatively high level of toxic element lead, which indicates that their ecological niche covers areas close to urban roads, where in fields and meadows adjacent to the roads the foxes forage for small rodents, which are the staple of their diet.

The element iodine, the content of which was almost twice as high in the hair of farmed foxes, plays a major role in hair growth and prevention of hair loss. It has an effect on the general appearance of the hair coat, and protects hair from becoming brittle and decoloured. Sulfur improves the appearance of hair and plays an essential role in its growth, whereas selenium, considered as one of the most important antioxidants, can alleviate the symptoms of hair loss. Low or zero levels of individual elements contributes to inhibited growth and loss of hair (the defect of absence of down).

The use of properly balanced diets and long-term breeding work on farms caused differences between the populations of farmed and wild foxes in many

metric traits of the gastrointestinal and integumentary systems. Compared to the population of farmed foxes, the wild foxes were characterized by poorer hair coat quality, as reflected in considerable felting, absence of down, and deficiency of elements needed for proper hair development.

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