Associations between neck plumage and beak darkness, as well as comb size measurements and scores with ranging frequency of Sasso and Green-legged Partridge chickens

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ABSTRACT Despite the intensive genetic selection in modern poultry, variability of domestic fowl phenotypes has remained, especially in breeds adapted to local conditions. The relevance of this variability to the chicken outdoor ranging activities remains unknown. The aim of this study was to investigate if external features were associated with the ranging frequency of the 48 female chickens from each of the 2 breeds: Sasso and Green-legged Partridge. In each of 6 single-breed pens, 8 hens and 2 roosters were housed under conditions of EU requirements for organic meat chicken production, including free access to an outdoor range, from wk 5 to 10 of age. The birds were video-recorded during the experiment to obtain frequencies of individual birds' use of the ranges. Comb size (length and height) was measured using a digital ruler, while the sizes of the dark area of neck plumage and beak were processed and analyzed using ImageJ software. The same traits were scored using direct visual assessment by a trained observer on a scale of 1-3. In addition, the eye color of the bird was recorded. Statistical analysis was conducted independently for each breed using regression models, ANOVAs and Spearman correlations. Significant positive associations between neck plumage ($P < 0.01$), beak darkness ($P = 0.03$) measurements, comb length ($P < 0.01$) and comb height ($P < 0.01$) and frequency of range use were identified for Sasso. Sasso hens scored with darkest neck plumage ($P = 0.03$) and biggest comb size ($P = 0.04$) ranged the most, while their external features were significantly and positively correlated between each other, except beak darkness and comb length. No significant associations between ranging and external features were found in Green-legged Partridge birds, except that their comb height was significantly and positively correlated with neck plumage and beak darkness ($r = 0.39$ and 0.33, respectively). In some genetic strains, better understanding of the associations between chickens' external features with ranging behavior could contribute to improve selection programs and bird welfare, assuring production of breeding stock suitable for outdoor conditions.

Key words: external traits, organic, phenotype, broiler, ranging behavior

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INTRODUCTION

In wild animals, phenotype traits are prominent characteristics of an individual that are essential for its survival due to for example, aposematism (Ruxton et al., 2018), species recognition (Santana et al., 2012), and sexual selection (Andersson, 1994). Farm animals have been genetically selected for productivity. The strong selection pressure has affected their phenotypes (Johnsson et al., 2012), and resulted in animals within the same breed or genetic strain being largely homogenous (FAO - Food and Agriculture Organization, 2000). However, some variability of external features in the production animals' phenotypes has remained.

In conventional broiler production systems, birds are reared in strictly controlled indoor conditions (Lima and Nääs, 2005). Increased public concern of animal welfare in those systems (Marchewka et al., 2013), including decreased ability of the birds to express natural behaviors, has increased consumers' demand for meat from poultry reared in less intense systems (Erian and Phillips, 2017). Those systems are characterized by longer production cycles, where the chickens from slow-growing

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breeds or hybrids may develop sexual dimorphism, including adult comb size, plumage and beak coloration. Moreover, in some less intensive systems, for example, organic systems in EU, birds are provided with ranging area (EU, 2007, 2008). Use of ranging area by broiler chickens is not always optimal, and differences exist not only on the flock or breed level, but also between individual birds in the same flock, even if equal opportunity of access to the range is provided (Dawkins et al., 2003; Taylor et al., 2017).

Domestic chickens are particularly interesting for studies testing the links between phenotype and behavior because, as although maintained in captivity, they have retained many of the behavioral characteristics and social structure of their Asian ancestor, red jungle fowl (*Gallus gallus*) (Navara et al., 2012). The majority of studies have focused on roosters, as they exhibit dramatic and conspicuous sexual signals (Sheldon, 1994) and hens are known to choose males based on a composite assessment of multiple secondary sexual characteristics, including bright red combs and wattles, hackle color, and mating behaviors, such as waltzing (Zuk et al., 1990; Johnsen et al., 1995). However, a variability in phenotypic external features can be observed in hens as well. Although it has not been investigated as frequent as in males, the feather distribution and color of the beak or eyes have been shown to be linked to ranging propensity, particularly in hens of some breeds (Al-Atiyat et al., 2017). Nevertheless, such associations have previously not been investigated, neither in Sasso nor in Green-legged Partridge birds.

One of variable phenotypic external features is iris color, often referred to in animals, including chicken, as eye color (Nelson, 1947). The iris of the eye primarily controls the amount of light that enters the eye, by varying the size of the pupil opening. However, variation in iris color is caused by either presence or absence of different types of pigmentation such as melanin, pteridines, and purines, as well as superficial blood vessels and/or eye structure, irrespective of pigmentation (Waldvogel, 1990). Phenotypic eye color has been suggested as an indicator of genetic predisposition toward certain behaviors, where dark-eyed subjects would tend to display behaviors requiring sensitivity, speed, and reactive responses, while with ones with light-colored eyes, behaviors requiring hesitation, inhibition, and self-paced responses, both in humans and in animals (Elias et al., 2008). Furthermore, it has been proposed that eye coloration in various species may be related to social ranks, aggression, mate recognition, and sexual selection (e.g., Volpato et al., 2003, Amat et al., 2013). Chicken eye color is largely determined by genetics, but age, diet, and disease can affect it as well (Nelson, 1947). However, to our knowledge no studies have investigated the link of the eye color with the behavior of chickens in free-range systems.

Some chicken behaviors have been found to be associated with external features like plumage coloration (Volpato et al., 2003; Keeling et al. 2004; Nätt et al. 2007). Individuals with the dark colored wild-type i/i PMEL17 gene version showed higher level of vocal-based social reinstatement behavior under open-field conditions than white colored I/I gene birds (Nätt et al. 2007), which was suggested to be associated with prelaying anxiety (Freire et al. 1997), suggesting that I/I females are more motivated to find a nesting place or they are more uncomfortable in the prelaying phase. Keeling et al. (2004) observed that wild type coloration birds victimization to feather pecking increased in flocks with increased numbers of wild-type homozygous (i/i) relative to white homozygous mutant (I/I) individuals. Thus, results have already demonstrated that PMEL17 genotype responsible for plumage coloration affects several behavioral patterns but further studies are needed to explore a wider spectrum, including ranging behavior.

As an alternative to dominance establishment by aggression, some studies have found that chickens use comb sizes as a signal of status or fighting ability in the formation of hierarchies, avoiding costly and stressful contests (Cloutier et al., 1996; Pagel And Dawkins, 1997; Campo et al., 2009). In broilers reared in conventional production systems, the comb is involved in heat regulation, and therefore may also assist in survival in crowded intensive production conditions (van Kampen, 1971). However, in broiler chickens selected for extensive production systems, including those with range access, survival in crowded conditions is not a prioritized selection trait. Therefore, it is of interest how the comb size is associated to a prioritized trait in the rearing systems with outdoor access, which is range use.

Indigenous or free-range chickens have variable plumage and biometrical traits representing genes of adaptation to their own environment (Al-Atiyat et al., 2017). Free-range chicken breeds are often classified as gene reservoirs reflecting unique adaptation to their agro-ecological environments (Horst, 1989). The adaptive genes of chickens to the use of free-range can be either precisely measured or visually recognized and scored. Time consuming and manpower demanding measurements of such traits could be included in selection programs for improvement of bird welfare, as it could assure production of breeding stock adapted for the outdoor environmental conditions. Qualitative and subjective scoring are additional approaches to assess animal visual traits. Such indicators can be collected on a large scale and incorporated into livestock breeding schemes to enhance animal welfare and overall resilience (Marchant-Forde, 2015). Moreover, practical on-farm scoring of external features could help producers identifying individuals, which potentially do not use the outdoor ranges to the extent expected, such that corrective appropriate flock management strategies can be implemented.

The current study aimed to investigate, if neck plumage and beak darkness, as well as comb size were associated with the ranging frequency of the hens from 2 breeds: Sasso hybrid and heritage Green-legged Partridge chickens. We hypothesized that in Sasso and in Green-legged Partridge hens comb size, proportion of dark feathers on the neck and beak darkness will be positively associated to their range use. We furthermore aimed to confirm potential associations of ranging frequency of Sasso and Green-legged Partridge hens with the above listed external features evaluated by practical scoring based on visual assessment and determination of eye color. We hypothesized more range use in birds of both breeds scored highest with regard to the external features. If the visual traits proved to be associated to the birds' range use, the correlations between measured external features in each breed would allow identifying the set of the visual characteristics of the birds frequently using the range. Therefore, we aimed to identify correlations between measurements of the hens comb size, proportion of the dark feathers on the neck, and beak darkness.

MATERIALS AND METHODS

The experiment took place in the Mazovian region of Poland, at the experimental farm of Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, in August and September of 2018. The experimental procedures followed standard production methods in the EU organic broiler chicken production system. No invasive manipulations requiring local ethical commission permission were applied to the birds in the current study.

Animals, Housing and Management

Forty-eight, nonbeak trimmed chickens, of each of 2 breeds (total n = 96 birds), Green-legged Partridge and Sasso line C44 were used in the experiment. Green-legged Partridge chickens are the indigenous Polish breed of heritage chicken (Siwek et al., 2013), available only in the partridge color-variety, in which the hens are buff-brown. The slow growing, multicolored broiler chicken hybrid Sasso is widely and successfully used in commercial production across the globe (Hendrix Genetics BV and Sasso, Boxmeer, The Netherlands) and has been especially well adapted to European continental climate.

Until wk 5 of age, 200 birds were reared only indoors in the experimental facility in one common littered pen (5 m × 10 m) with 17 cm/bird perching space provided, automatic feeders and drinkers, providing feed and water ad libitum, and natural light. The climate conditions were controlled automatically and infrared heating lamps were used. At the age of 5 wk, 60 individuals with similar body weight within each breed (on average 2030.6 ± 68.9 g for Sasso and 705.9 ± 8.5 g for Green-legged Partridge), were selected and relocated from their rearing facilities to the experimental house, both at the same farm location. Eight female and 2 male chickens were assigned to each single breed group housed in 12 pens (6/breed) until 10 wk of age. In the current study, only hens were investigated. The size of the indoor pens was 2.5 m × 3.5 m, resulting in a stocking density at slaughter age of 1.4 kg/m^2 for Green-legged Partridge and 2.7 kg/m^2 for Sasso. Birds were housed on sawdust litter, while in each pen, next to the wall, there was a 0.5 m strip covered with sand. Pens were cleaned according to need. Each pen contained two 80 cm long wooden perches with 2 perching levels, one at the height of 15 cm and the second at 40 cm. The perching poles were 50 × 50 mm thick and had rounded edges. Each pen had direct access through the pophole (H × W: 45 cm × 50 cm) to an individual outdoor range (3.5 m × 30 m) providing 10.5 m^2/chicken. All the outdoor ranges had equal vegetation coverage regarding botanical composition, while no trees or shelters were present. The grass was mowed 1 wk before the onset of the experiment. Each ranging area was provided a semiautomatic bell drinker and a wooden box (1 m × 1 m) filled with sand. Additional information about the experimental facilities can be found in Marchewka et al. (2020).

The birds were habituated for 48 h to the new housing after relocation from the rearing facilities to the experimental house. After the habituation period, the popholes were opened (daily from 7.00 until 19.00 h). To allow for individual birds' recognition, all birds were fitted with a laminated paper mark of the size of 9 cm high and 7 cm wide attached to the birds' back by fitting 2 elastic bands around its wings. Ten different colors of the marks were assigned in each pen randomly to the individual birds. Birds were equipped with their color mark during the entire experiment. Birds were inspected twice a day. Commercial pelleted feed (Marchewka et al., 2020) and water were available ad libitum. No coccidiostats or other medications were used. No birds died during the experiment.

Birds were provided only natural light through uncovered windows. Light hours during the experimental period ranged from 12.7 h to 15.7 h/d. There was natural ventilation in the building.

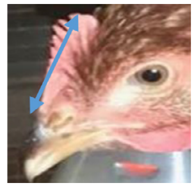
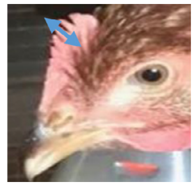
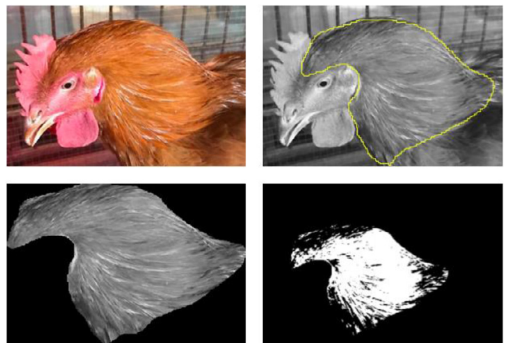
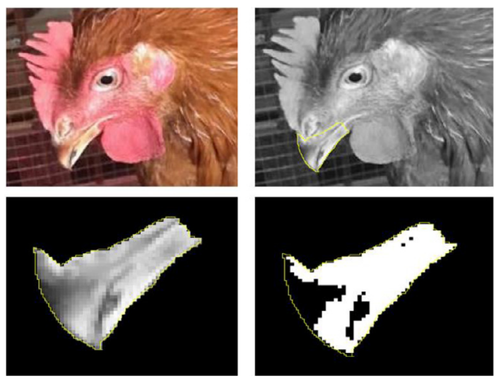
Observations of Ranging Behavior

Ranging behavior of the birds was recorded using video cameras. The 12 outdoor pens were video-recorded simultaneously and continuously using 6 cameras (BCS-DMIP2401IR-M-IV IP 4 Mpix), each covering 2 ranging areas. The films were automatically saved on the network recorder (BCS-NVR0401-IP 4 channel BC). Video material was analyzed and bird behaviors were recorded by the same trained and experienced person, using scan sampling and the Chickitizer program (Sanchez and Estevez, 1998). From the recorded videos, 3 d were chosen per week of experiment (5 wk). On each of those days, 3 times of the day (morning [starting at 8:00], noon [starting at 13:00], and evening [starting at 18:00]), a 3-min period with 10-s sampling intervals was set and repeated after 10 min. The observer recorded each of the experimental birds' absence as "0" or presence as "1″ in the outdoor area.

Measurements (Quantitative Assessment)

The direct measurements of the external features (Table 1) of each individual bird were taken the day

Table 1. Methods and pictorial examples of measurements of the comb length and height, neck plumage, and beak darkness conducted both in Sasso and Green-legged Partridge chickens.

Measurement	Method	Example
Comb length (mm)	Digital ruller	
Comb height (mm)		
Neck plumage darkness (%)	Photograph and ImageJ software	
Beak darkness (%)		

before the end of the experiment. There were 3 persons involved in the measurements, each assigned with a different task: 1) identifying (indicated by the color tag) and catching the birds, 2) measuring the comb size using the method described below, and 3) noting the collected information in a spreadsheet and taking a digital picture of the whole body of each bird from the left side. Comb size was measured, using a digital ruler LCD (Kraft&-Dele, Koteze, Poland), in the highest (from where the comb met the head to the top of the highest spike) and longest place (from end to end) for each individual bird. From the photos taken, the beak coloring was calculated using ImageJ software (Schneider et al., 2012). Each image of an individual bird was imported to ImageJ software, where the area of the beak was contoured and cropped from the whole image. The cropped-out area was binarized, collapsing the 256 color levels to 2 color levels, while adjusting the grayscale using the automatic thresholding method "AutoLocalThreshold", as a plugin to ImageJ software. This plugin binarised 8-bit image using thresholding method that can deal with unevenly illuminated images. The threshold was computed for each pixel according to the image characterizing within a window of radius r (in pixel units) around it. The segmented phase was always shown as white (255, as the maximum gray level). After thresholding, the dark area was calculated and deduced from the total area of interest providing white area size. The proportion of black to white area measurements ratio was calculated and expressed as a percentage. The same method using ImageJ software was applied to the second identical copy of the individual chicken photo to calculate neck plumage coloring, that is, the percentage of dark plumage on the neck, which was defined as the area between the head and the trunk of the bird (Table 1).

Scores (Qualitative Assessment)

After taking the comb measurements and a bird photograph, each bird was handed into a 2-person team, where one person held the bird and the other, based on visual assessment, scored the bird for 3 external features: comb size, neck plumage darkness, and beak darkness, all on a 3-point scale (1-3) within breed. Definitions and examples for each score of each feature in either of the breeds are presented in Table 2.

Statistical Analysis

All the statistical analyses were conducted using software package SAS version 9.4 (SAS, Cary, NC). In each of the simple regression models, the variable describing either the individual Green-legged Partridge or Sasso chicken range use (summed over all observations frequencies of the presences in the outdoor area) was considered as the dependent outcome variable, while each chicken external feature measurement was considered as the independent variable. The outcome variable was analyzed for its association with each independent variable. The outcome variable was normally distributed across the sample population, thus linear univariate regression was used. Furthermore, the residuals were predicted and checked for normality. Residuals were predicted and plotted in normal quantile plots and coefficients of determination (R^2) were calculated.

Independent one-way ANOVAs were performed, separately for Sasso and Green-legged Partridges, using the PROC GLIMMIX procedure. Each model included different chicken external feature scored as “1”, “2”, or “3” as a fixed factor. However, an independent two-way ANOVA was conducted in the same software package for the model including eye color, as both eye color and breed were added as fixed factors as well as their interaction. Pen was included in the model as the random factor. Least Square Means (**LSM**) differences were adjusted for multiple comparisons using the posthoc Tukey test.

Spearman correlations were calculated using the PROC CORR script for each breed separately to test the relationships between measured external features.

RESULTS

Measurements

The results of the simple regression models showing associations between range use by either Sasso or Green-legged Partridge hens and their external features are presented in Table 3. In Sasso hens, significant and positive associations between the range use frequency and comb length and height as well as neck plumage darkness and beak darkness were identified. The proportion of explained variance of the response variable ranged from 18% for beak darkness up to 33% in case of neck plumage darkness. No significant associations between the range use frequency and external features were identified for Green-legged Partridge hens.

Visual Assessment

Significant effects of external features as assessed by scoring were identified in Sasso hens for neck plumage darkness ($P = 0.03$) and comb size ($P = 0.04$), as presented in Table 4. For both features, birds scored the highest used the ranging areas more frequently as compared to birds presenting the lowest score. Moreover, a trend ($P = 0.06$) for an effect of beak darkness on the range areas use frequency was identified. No significant effect of any of the external features on the range use was identified for Green-legged Partridges.

Eye Color

There was a significant breed by eye color interaction effect on the range use of the hens (F = 4.40; $P = 0.04$) in the current study (Figure 1). Sasso hens with gray eye color used the ranges significantly less frequently, as compared to Green-legged Partridges with either brown or gray eyes.

Correlations

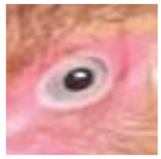
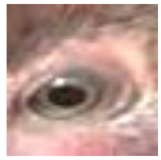
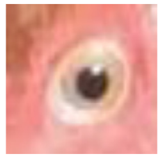
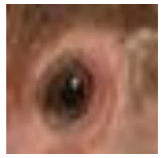
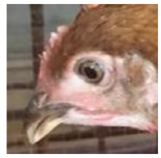
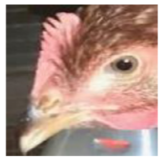
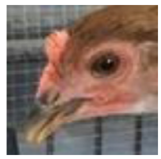
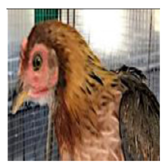
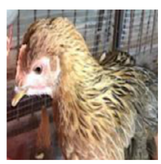
Correlations between external features were identified within each breed (Table 5). In Sasso hens, all external features were significantly and positively correlated between each other, with the exception that no significant correlation was identified between beak darkness and comb length. The strongest positive correlation ($r = 0.85$) was identified between comb length and comb height.

Among Green-legged Partridges, fewer and weaker correlations were identified as compared to Sasso hens (Table 5). Similarly to Sasso hens, the strongest positive correlation was identified between comb length and comb height ($r = 0.55$). Moreover, comb height was significantly and positively correlated with neck plumage darkness and beak darkness ($r = 0.39$ and 0.33, respectively).

DISCUSSION

The current study aimed to investigate, if neck plumage and beak darkness, as well as comb size measurements were associated with the ranging frequency of the female chickens of 2 breeds: Sasso hybrid and heritage Green-legged Partridge. Moreover, we aimed to test if

Table 2. Definitions and pictorial examples for each score of visually assessed external features: eye color, comb size, neck plumage, and beak darkness in Sasso and Green-legged Partridge chickens.

External feature		Breed	
Eye color	Definition	Sasso	Green-legged Partridge
Grey			
Brown			
Comb size score			
1	Very small comb, not much raised from the head		
2	Medium size comb, raised from the head, the height of the tallest spike was not larger than the distance from the eye to the middle of the comb base		
3	Large, marked comb, raised from the head, the height of the tallest spike was larger than the distance from the eye to the middle of the comb base		
Neck plumage coloration score			
1	No or very few dark feathers present (0−5 dark feathers)		
2	Some dark feathers present (6−10 dark feathers)		
3	Dark feathers present (more than 10 dark feathers)		

(continued)

Table 2 (*Continued*)

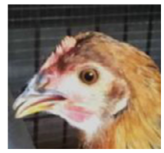
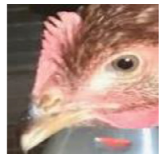
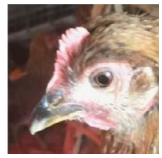
External feature		Breed	
Eye color	Definition	Sasso	Green-legged Partridge
Beak coloration score			
1	No or very small dark area on the beak (<10%)		
2	From 10% up to 50% of the beak area was dark		
3	Majority of the beak was dark (>50%)		

Table 3. Associations between range use and comb length, comb height, neck plumage darkness, and beak darkness measurements in Sasso and Green-legged Partridge, respectively.

		Ranging activity						
Breed	External feature (measurement)	R^2	Parameter estimate (r)	SE	t value	Pr > |t|	95% confidence Limits	
Sasso								
	Comb length (mm)	0.27	13.35	4.52	2.95	0.0069	4.02	22.69
	Comb height (mm)	0.28	21.31	7.05	3.02	0.0059	6.77	35.86
	Neck plumage darkness (%)	0.33	12.76	3.72	3.43	0.0022	5.08	20.44
	Beak darkness (%)	0.18	3.03	1.33	2.28	0.032	0.28	5.77
Green-legged Partridge								
	Comb length (mm)	0.02	3.00	3.53	0.85	0.3989	-4.10	10.12
	Comb height (mm)	0.001	1.09	5.20	0.21	0.8352	-9.38	11.56
	Neck plumage darkness (%)	0.0006	0.15	0.88	0.17	0.8658	-1.63	1.93
	Beak darkness (%)	0.0085	1.00	1.59	0.63	0.5335	-2.21	4.20

such potential associations could be identified by more practical visual assessment, suitable under production conditions, of the comb size and darkness of neck plumage and beak. We also investigated the eye color and breed effect on the range use frequency of the birds. This is important, as increased use of the range area has been found to be positively associated with welfare of the ranging chickens (Taylor et al., 2020). Furthermore, birds that more often used outdoor areas had potentially greater access to its vegetation, providing a larger variety of food sources. In the current study, the analysis was conducted separately for each of the breeds, due to the differences between them in their body sizes and coloration patterns, except for the eye color, which is possible to compare between breeds qualitative trait.

Significant effect of the interaction between eye color and breed on ranging frequency was identified. In chicken, 5 main iris colors: gold, red, brown, black, and pink (albino) can be distinguished (Nelson, 1947). In some colored breeds like Barred Plymouth Rock, green-gray irises are common, for simplicity called gray, and they were correlated with quantity of black feathers in this breed (Slinger and McIlraith, 1944). In the current study, only 2 eye colors, gray and brown, were observed in both breeds. Variation in eye color depends to a large extent on the pigmentation and blood supply to a number of structures within the eye (Crawford, 1990). Wild birds may have intraspecific eye color variability, which seems to be due to the developmental stage of the individual, its breeding status, and/or sexual dimorphism (Negro et al., 2017). Furthermore, eye coloration may be related to visual needs, as the pigments involved capture different light wavelengths (Oliphant et al., 1992). Nonetheless, the origin and functions of eye colors are still poorly understood (Davidson et al. 2017). In this study, Sasso birds with gray eye color used the ranges significantly less frequently, as compared to Green-legged Partridges with either brown or gray eyes. In the

Table 4. Dependency of ranging activity on the scores (1-3) of comb size, neck plumage darkness, and beak darkness of Sasso and Green-legged Partridges, respectively.

Breed	External feature (score)	Ranging activity (mean ± SEM)				
		Score 1	Score 2	Score 3	F value	P value
Sasso						
	Comb size	180.8 ± 40.9^{b}	222.7 ± 53.3^{ab}	382.0 ± 55.7^{a}	3.6	0.0435
	Neck plumage darkness	200.8 ± 34.2^{b}	259.2 ± 62.5^{ab}	475.3 ± 64.3^{a}	4.14	0.0291
	Beak darkness	190.5 ± 36.3	288.1 ± 54.0	462.0 ± 116.0	3.15	0.0619
Green-legged Partridge						
	Comb size	344.4±23.3	386.5±31.0	302.3±42.3	0.96	0.3909
	Neck plumage darkness	377.1±41.1	348.9±33.0	357.8±25.3	0.17	0.8434
	Beak darkness	not present	324.75±66.9	364.3±19.2	0.35	0.5572

Different letters (a, b) next to mean ± SEM values in the same row indicate statistically significant differences ($P < 0.05$).

past, it was a standard procedure to eliminate chickens with gray eyes from the production, to avoid potential risk of introducing pathological lymphomatosis (Nelson, 1947). Nevertheless, chicken can have gray eyes unrelated to any pathology, as was the case for the birds in the current study (Marchewka et al., 2020), while lymphomatosis cannot be accurately diagnosed on the basis of color alone (Nelson and Thorp 1943). Further research into iris color and its associations with chicken health, welfare and productivity would be valuable.

We confirmed the stated hypothesis concerning the birds' feather coloration, but only for Sasso chickens. Sasso have been selected for performance including efficient growth rates, but also for suitability to the ranging systems. Moreover, they are described by the producer as "colored chickens," where it is characteristic for this hybrid to have some degree of dark feathering. In chicken, the α melano-cyte stimulating hormone (**MSH**), as part of the avian melanocortin system, controlled pigment regulation and was directly related to energy homeostasis by regulating food intake (Takeuchi et al., 2003). Chickens expressing any black pigment, eumelanin, carry at least one copy of the wild-type PMEL17 allele (Kerje et al., 2004). Interestingly, in a study focusing on chicken behavior and brain gene expression, Karlsson et al. (2010) identified plumage color genotypes PMEL17 to have a pleiotropic effect on social and explorative behavior in chickens, where wild type birds (i/i) were more active in socializing and exploring, as compared to white chickens homozygous for the mutant allele (I/I). Animals explore their environment or novel stimuli and approach them in order to, for example, find food or water, making this explorative behavior essential for survival (Powell et al., 2004; Nicol, 2015). Exploration is thought to counterbalance fear (Meuser et al., 2021). High fear responses to objects indicated low exploration and foraging of the entire environment (Campbell et al., 2019), indicating reduced range use of the chickens and low adaptation of the animal to the husbandry system. Furthermore, melanin-based color traits in birds often act as honest signals of their quality, as signalers with larger or more intense color patches are perceived by conspecifics as bearers of a superior underlying genotypic quality and as a consequence achieve higher fitness benefits than others displaying smaller or less intense color patches (McGraw, 2008; Guindre-Parker and Love, 2014). To our knowledge, no previous study identified such associations between pigmentation and more frequent range use. To further support underlying mechanism behind the dark pigmentation in Sasso chickens, we identified a positive correlation between neck plumage and beak darkness. If confirmed by further investigations using molecular genetics methods, neck darkness score could be a valuable and practical trait, which could help to select birds in breeding programs suitable for rearing systems with outdoor access, although only in genetic strains with dark pigmentation present.

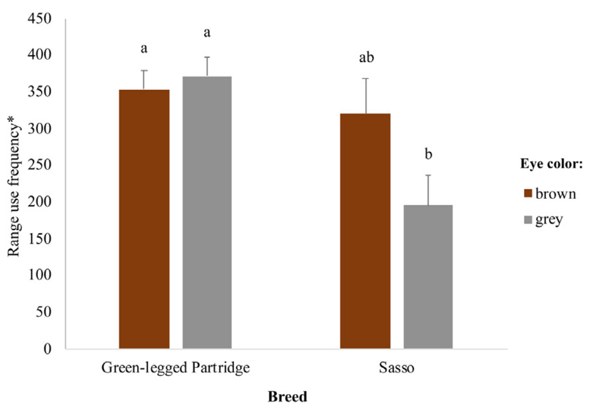


Figure 1. Range use frequency of birds with different eye colors (brown and gray) across breeds (Sasso and Green-legged Partridge). Different letters indicate significant differences in the model including the interaction between eye color and breed.

Relationships between comb size, behavioral characteristics and social structure in chickens (Johnsen, et al. 1995; Cornwallis and Birkhead, 2007; Navara et al. 2012) or their fitness traits (Johnsson et al., 2012) have previously been investigated. In chickens, the primary role of the comb is in heat regulation (Van Kampern, 1971). The comb in adult chickens also plays an important role in reproduction, as it is used for making mating decisions both by male and female birds (Pizzari et al., 2003). Comb size is affected by hormonal status in both hens and cockerels (Eitan et al., 1998; Joseph et al., 2003). In roosters, it is an indicator of social rank, with females actively soliciting matings from males with larger combs (Zuk et al., 1990; Parker and Ligon, 2003). Sexual

Table 5. Correlations between comb length, comb height, neck plumage darkness, and beak darkness measurements presented for Sasso and Green-legged Partridge, respectively.

Breed		Neck plumage darkness (%)	Beak darkness (%)	Comb height (mm)	Comb length (mm)
Sasso					
	Neck plumage darkness (%)	1	0.41*	0.44*	0.43*
	Beak darkness (%)		1	0.43*	0.25
	Comb height (mm)			1	0.85**
	Comb length (mm)				1
Green-legged Partridge					
	Neck plumage darkness (%)	1	0.01	0.39*	0.07
	Beak darkness (%)		1	0.33*	0.22
	Comb height (mm)			1	0.55**
	Comb length (mm)				1

*$P < 0.01$.
**$P > 0.001$.

maturation also promotes the development of comb and wattles on chicken (Joseph et al., 2003), so the more developed chickens have larger combs. In hens, it is indicative of greater reproductive potential (Cornwallis and Birkhead, 2007; Wright et al., 2008). Furthermore, correlations of comb size with bone mass have been identified (Wright et al., 2008). Therefore, a larger comb is an indicator of higher fitness of a chicken. In laying hens, combs have been found to be darker in flocks that used the range area more intensively, while more fearful flocks had lighter combs (Bestmaan and Wagenaar, 2014). Since several diseases and health problems can cause a paler comb, as well as a smaller comb, farmers regard bright red combs as a practical indicator of current hen health. However, the association between comb size and ranging frequencies, as found for Sasso in the present study, remain to the best of the authors' knowledge unexplored. Based on the current results, comb size of Sasso could serve as an indicator of their ranging frequency, although comb size to some degree is affected by reproductive status (Eitan et al., 1998; Joseph et al., 2003).

Only in Sasso, the majority of the visual traits, found to be associated to the range use, also correlated between each other within breed. Therefore, a visual profile of a female bird of this breed with a higher range use could be suggested. Such correlations have been identified for males of other bird species (Yang et al., 2013). However, we are not aware of any studies providing such information in broiler hens. Our findings allow us to suggest not only the individual traits but also the set of the visual characteristics of the Sasso hens with higher range use.

No significant associations between any of the measured or visually assessed external features and ranging activity were identified for Green-legged Partridges. Considering the very good adaptation to the ranging conditions of Green-legged Partridges (Siwek et al., 2013), it is possible that their overall high ranging activity diminished differentiation of range use based on their external features. Therefore, even though the correlations were also identified between external features in Green-legged Partridges, no similar set of traits of the birds, which frequently use the range, could be identified in this breed.

To conclude, we found significant associations between measurements of the external features and ranging activity only for Sasso chickens. Visual assessment of those features, a more practical way of evaluating birds' phenotype than measurements, confirmed the findings obtained by measurements for comb size and neck plumage darkness and tended to do so for beak darkness of Sasso. However, no significant associations between external features and ranging activity were found in Green-legged Partridges. Better understanding of the associations between chickens' external features with their ranging behavior could profitably be included in selection programs and contribute to improvement of bird welfare, as it could assure production of breeding stock adapted for the outdoor environmental conditions.

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DISCLOSURES

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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