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Free range characteristics and use by chickens from two genetic lines

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The free-range system improves chicken's welfare and enriches their diet. There is little information about influence of chickens on the sward quality. The aim of the study was to identify differences in the chemical and botanical composition of the pasture sward used by chickens and to assess if frequency of chicken exits outdoors is associated with the botanical composition of the ranging areas. One hundred twenty non-beak trimmed mixed sex birds of Green-legged Partridge (GP) and Sasso line C44 (S) were used. Each pen had direct access to an individual outdoor range providing 10.5 m^2 per chicken, video-recorded continuously. Vegetation coverage regarding botanical composition was determined based on botanical-weight analysis from three control plots, while range use was calculated from the video material. The data were analysed by ANOVA model in SAS software (v 9.4). Regarding chemical composition, fiber content was higher in dry matter from the control pastures. Significantly more *Dactylis glomerata* (L.) and *Alopecurus pratensis* (*L.*) were observed on ranges used by Sasso. The higher share (%)

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of weeds and grasses was at the ranges used by chickens (weeds: S:8.66 (1.42) and GP:7.61 (0.59) and grasses: S:10.36 (0.96) and GP:12.10 (0.65)) as compared to control plots (1.45 (1.34)) and 3.86 (2.97), respectively) in contrast to legumes ((S:0.01 (0.01) and GP:0.32(0.26)) and sedges (S:0.64 (0.48) and GP: 0.09 (0.06)) most present in the control plots (10.88 (1.55)). Positive association between range use frequency and proportion of *Heracleum sphondylium* (L.), *Rumex acetosa* (L.), *Festuca rubra* (L.), and *Lolium perenne* (L.) was observed for Sasso, while foe Green-legged Partridges with proportion of *Armoracia rusticana* (L.), *Stellaria media* (L.), *Ranunculus repens* (L.), *Cirsium arvense* (L.) and *Rumex crispus* (L.). Concluding, the sward quality of the ranging areas was affected by the presence of the chickens and it differed between genotypes. Unknown remain if birds used the pasture more frequently depending on its botanical composition or if the pasture composition depended on the frequency of its uses by the birds.

KEY WORDS: botanical composition / broiler / extensive free-range / organic

Outdoor access has many beneficial effects on poultry including chickens and hence is required by some higher welfare and pro-sustainability production schemes [Cooper, Horbańczuk 2004, Horbańczuk *et al*. 2008] for instance organic system regulated by EU legislation. In extensive rearing systems pasture intake was reported as 2.5% (4 g) dry matter (DM) per chick daily [Ponte *et al.* 2008], while grazing accounted even for 10-15% of the feed intake (2-5 g DM/chick/day) [Lorenz *et al*. 2013]. It complemented standard concentrate diet with fresh grass, insects, and worms leading to enhanced product quality [Sossidou *et al.* 2015]. Pasture constitutes a source of energy and proteins for chickens, while providing a range of bioactive compounds such as antioxidants, hypocholesterolemic and anticarcinogenic compounds [Ponte *et al.* 2008]. Fresh grass supplies vitamins, minerals and other compounds like carotenoids or omega-3 fatty acids necessary for metabolic functions [Spencer, 2013].

Organically reared broilers overcame growth impairments associated with methionine deficiency through foraging [Tufarelli *et al*. 2018]. Chickens grazing mixed-grass pastures exhibited decreased mortality and improved immune responses, by better regulation of the intestinal microbiota by increasing the prevalence of beneficial bacteria [Zheng *et al*. 2021]. Legume-based pasture intake promoted bird performance while contributing to broiler meat production with preferred sensory attributes.

The key aspects of proper chicken range management are the sward quality, including the height and density of it and appropriate plant species selection. As known from practice, chickens prefer the sward with a height of around 5-10 cm as higher may cause their legs and feathers to get wet. Moreover, too tall and mature sward contains more fiber and becomes less digestible for animals. The low permanent grass species, along with legumes and deep rooting herbs, can increase the potential intake of protein, minerals, and nutrients from the forage. The botanical composition of ranging areas for chickens is affected by many factors including soil type, its moisture, mineral components, and their availability for plants, as well as environmental factors like weather [Shakhane *et al.* 2013, Głowacz and Niżnikowski 2018]. On the other hand, range use by chickens may modify soil fertility or its moisture [Elbe *et al*. 2004, Jondreville *et al.* 2011], which in consequence could affect botanical composition and the sward of the ranging areas.

In the free-range systems choice of appropriate chicken genotype is very important, while slow and medium-growing breeds appear to be most suitable [Sossidou *et al.* 2011]. We have previously shown that weather is associated with the frequency of pasture use by individual birds and is breed-specific [Sztandarski *et al*. 2021ab]. We have also found that in some breeds, the chickens which are more frequently seen on the ranges, have more pasture matter in their digestive tract, as compared to the indoor-preferring individuals, while this is not the case in other breeds [Marchewka *et al*. 2021, Sztandarski *et al.* 2022]. It remains unknown how various genotypes of chickens reared for meat purposes with access to the range affect its chemical and botanical composition as well as sward quality.

The aim of the study was to identify differences in the chemical and botanical composition of the pastures sward used by two breeds of chickens. Moreover, we were interested if there are potential parameters related to pasture sward, chemical and botanical composition associated with more frequent use of the ranging areas by meat chickens of the two genotypes.

Material and methods

The experiment took place in the Mazovian region of Poland, at the experimental farm of the Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, in August and September of 2018.

Animals, housing and management

Sixty non-beak trimmed mixed sex birds of each of two breeds (total n = 120 birds), Green-legged Partridge and Sasso line C44 were used in the experiment. Sasso C44 is a commercially available hybrid, colored slow-growing broiler (Hendrix Genetics BV, The Netherlands). Sasso birds are well skilled to forage on the outdoor ranges, having high resistance to low temperatures and diseases, while the meat is characterized by a very good taste and quality [Getiso *et al.* 2017]. Sasso birds reach their slaughter weight of 2.3-2.8 kg at about 2 months of age. The Green-legged Partridge is a traditional, dual-purpose Polish breed of chickens, well adapted to extensive management due to their high disease resistance and good foraging. The meat and eggs of these birds are characterized by good flavor [Krawczyk 2009, Siwek *et al.* 2013].

Until week 5 of age, 120 birds were reared in the experimental facility without outdoor access in two pens, divided by the breed into two groups (1 group per pen) of 60 birds. At the age of 5 wk., all individuals were relocated from the rearing facility to the experimental house, both at the same location. Eight female and two male chickens were assigned to each single breed group housed in 12 pens until 10 wk of age. In each pen, 6 birds (5 females and 1 male) 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. To make the recognition of individuals possible all birds had fitted with a laminated paper mark of the size of 9 cm high and 7 cm wide attached to the birds' back by 2 elastic bands around its wings. Ten different colors of the marks were assigned in each pen randomly to the individual birds. Birds were wearing color mark during the entire experiment. They were inspected twice a day. No birds died during the experiment.

The outline of the experimental facility was presented in Marchewka *et al.* 2020 and Sztandarski *et al.* 2021. In short, the size of the indoor pens was 2.5 m x 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 the sawdust litter, while in each pen, next to the wall there was a 0.5 m stripe covered with sand. Pens were cleaned when needed. In each pen, there were two 80 cm long wooden perches at 2 perching levels, one at the height of 15 cm and the second at 40 cm. The perching poles were 50 x 50 mm thick and had rounded edges. Each pen had direct access through the pophole (45 cm high x 50 cm wide) to an individual outdoor range (3.5 m x 30 m) providing 10.5 m^2/chicken. All the outdoor ranges had the same vegetation coverage regarding botanical composition, no trees or shelters were present. The grass was mowed 1 wk. before the onset of the experiment. Each free-range area was provided with a semi-automatic bell drinker and a wooden box (1 m x 1 m) filled with sand. Three additional control ranges of the same size and shape were located in the row of chicken ranging areas as first before, middle and as the last outdoor range but no birds had access to them.

The birds were habituated for 48 h to the new housing and social situation. Popholes were opened daily from 7.00 until 19.00 h. Commercial pelleted poultry feed was used to nourish the birds. Feed and water were available *ad libitum*. The feed was composed of wheat, maize, sunflower expeller, pea, soybean expeller, legumes mix, gruel corn, monocalcium phosphate, soybean oil and calcium carbonate with supplements [Marchewka *et al.* 2020]. The chemical composition of the feed was intended to meet the birds' nutritional requirements [Classen 2017]. No coccidiostats or other medication were used.

Birds were provided only natural light through uncovered windows. Light hours during the experimental period ranged from 12.7 h to 15.7 h/day. There was natural ventilation in the building. Indoor climate parameters were continuously collected by a device of the weather measuring device (Davis Instruments Vantage® Pro 2 DAV-6152EU, California, USA) placed in the middle of the chicken rearing house at a height of 1 m. The associations between weather conditions and individual range use by were previously described in Marchewka *et al*. [2021].

Observations of ranging behavior

The behavioral data collection of range use in the current study has previously been described [Marchewka *et al*. 2020]. Range use 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 free-range areas. The cameras were attached to the wall of the experimental facility at a height of 3 m from the ground. The video material was recorded with the network recorder BCS-NVR0401-IP4 channel BC. After that it was analyzed by one trained and experienced person, using the Chickitizer program [Sanchez and Estevez, 1998]. From the recorded videos, 3 days were chosen per week of the experiment (5 wk.). On each of those days, 3 times of the day (at 8:00, at 13:00, at 18:00) a 3-min-period with 10 s sampling intervals was set and repeated after 10 min. The observer registered the absence or presence of each of the experimental birds' in the outdoor area.

Sward cover, chemical and botanical composition of the pasture

Due to the length of the research pasture area (30 m), it was divided into 3 equal parts (A, B, C) on which the cover and botanical composition of the sward were assessed. Sward density (cover of the soil surface with plants, %) was measured in 3 randomly chosen places on range area (one per separated area A, B, C) using a frame (50cm x 50cm), divided into 25 squares one day after the end of the experiment. Samples of forage were dried at room temperature to a constant weight and then ground in a laboratory cutting mill SM 100 (RETSCH). Aliquots of the dried samples were washed at 550°C. The samples collected at the same date were mixed together. Cell wall constituents (crude fibre, NDF, ADF, ADL) were analysed in ANKOM fibre bag analyser. Dry matter, protein and fat content were determined by Weende analysis with a default laboratory procedure. The organic matter (OM) digestibility (OMD) was measured in vitro by an enzymatic method based on sequential sample treatment with ND solution followed by incubation with a cellulase [Aufrere, 1982]. Total soluble sugars were determined according to the method of Yem & Willis (1954). The botanical composition of the sward (%) was determined on the basis of botanical-weight analysis. The green matter samples of 500 g were taken in from the same area. Plant material was separated into groups of plants (grasses, legumes, herbs and weeds) and individual species (detailed analysis). Dry matter of each species was used to calculate the share of the separated species and fractions in sward [Hodgson *et al.* 1993].

Statistical analysis

Birds of both breeds were divided into three ranging profiles using rank-frequency distribution (a discrete form of a quantile function in reverse order, giving the size of the element at a given rank) of their range use frequency summed over all the observation periods – i.e., between 0 and 1620 times. All the birds within a breed were assigned a rank based on their individual frequency of outdoor use. We segmented the rank distribution of the birds into three ranges: outdoor-preferring ranging profile, with the mean value of 506.1±47.9 outdoor uses count per bird during all observation periods in Sasso and 502.6±22.5 for Green-legged Partridge; moderate-outdoor ranging profile, with the mean value of 219.6±18.8 outdoor uses count per bird during all observation periods for Sasso and 332.4±13 outdoor uses count per bird for Green-legged Partridge; and indoor-preferring ranging profile, with the mean value of 89.8±11.7 outdoor uses count per bird for during all observation periods Sasso and 223.9±12.1 outdoor uses count per bird for Green-legged Partridge. The rank intervals were equal (modified from Campbell *et al.* 2016) [Campbell *et al*. 2016].

Statistical analyses were performed with SAS 9.4. The GLIMMIX procedure was used to perform generalized linear mixed models for the using either normal or gamma distribution where appropriate, applying the type of pasture (range used by Sasso, range used by Green-legged Partridge and control range) as fixed effect in the model. The pen was included in the model as a random effect. The assumptions of homogeneity of variance and normally distributed residuals were examined visually using the conditional Studentized residuals plots. The results are shown as means with standard errors, and *P*-values below 0.05 were considered significant, while between 0.05 and 0.06 were considered a significant trend. Tukey's post hoc test was performed to investigate significant differences between test groups. The outcome variables were analyzed for associations with any of the independent variables.

The outcome variables: exits (sum/pen) were normally distributed across the sample population, thus linear univariate regression was used. Residuals were predicted and checked for normality. Associations with P-value <0.2 were further analyzed in a multivariate linear regression analysis. Models were obtained by backward exclusion until all associations obtained reached P-value <0.05. Interactions between independent variables were tested in the final models and were not detected. Residuals were predicted and plotted in normal quantile plots, and coefficients of determination (R^2) were calculated and used to choose the model that explains the variability of the response data. The likelihood ratio test was used to observe the improvement of the multiple regression models by inclusion and exclusion of independent variables. Akaike's information criterion and Schwarz's Bayesian information criterion were used to compare maximum likelihood of reduced and full models. The selection of the final models was based on the smaller values of the information criterion.

Results and discussion

Effect of breed on the sward cover, chemical and botanical composition of the pasture

The effect of breed on the sward cover, chemical and botanical composition of the pasture is presented in Table 1. Regarding chemical composition, fiber content was higher in dry matter from the control pastures as compared to those used by either of the breeds. Ash levels were higher in dry matter from the ranges used by the Green-legged Partridges, as compared to the control plots. The higher ADF proportion was identified in the control plots, as compared to ranges used by Green-legged Partridges.

Considering the percentage of sward cover, overall, it was highest on the control ranges, as compared to ranges used by the birds, while differences also existed between breeds. The sward cover of the pasture used by the Green-legged Partridge breed was higher compared to those used by Sasso. Dicotyledonous plants covered more space of the range area of the control plots, as compared to ranges used by either of the breeds. Opposite trend was observed for monocotyledonous plants.

Analyzing the botanical composition of the pasture, statistically significant differences were observed in seven representatives of grasses. The greatest numerical

Table 1. Chemical composition, sword cover, plant species and plant groups on the free ranges used by Sasso or Green-legged Partridges and on the control ranges

	Variables	Mean (SEM)			F value	*p*
		Sasso	Green-legged	Control		
Chemical composition (g/100g)	Moisture	7.48 (0.20)	7.46 (0.18)	8.61 (0.24)	2.92	0.07
	Protein	18.14 (0.71)	19.45 (0.82)	15.18 (1.62)	2.51	0.10
	Fiber	19.96^{B} (0.68)	19.61^{B} (0.55)	24.61^{A} (1.05)	4.95	0.01
	Ash	12.49^{AB} (0.38)	12.95^{A} (0.35)	10.47^{B} (0.88)	3.34	0.05
	NDF	43.08 (0.87)	42.31 (0.89)	46.98 (2.00)	2.03	0.15
	ADF	27.94^{BA} (0.45)	27.64^{B} (0.45)	30.64^{A} (0.83)	3.27	0.05
	ADL	4.16 (0.08)	4.17 (0.09)	4.34 (0.04)	0.35	0.71
	OMDigestibility	55.21 (1.41)	56.62 (1.52)	55.71 (2.31)	0.24	0.79
	DMDigestibility	53.24 (1.52)	54.75 (1.62)	54.49 (2.80)	0.25	0.78
	TotalSolubleSugars	8.18 (0.36)	7.31 (0.53)	8.85 (0.91)	1.41	0.26
Sword cover (%)	sodding	65.28^{C} (3.39)	73.06^{B} (2.22)	91.65^{A} (3.33)	13.64	<.0001
	Dicotyledonous	37.50^{B} (5.00)	33.06^{B} (4.75)	50.00^{A} (5.77)	10.58	<.001
	Monocotyledonous	62.50^{A} (5.0)	66.94^{A} (4.75)	50.00^{B} (5.77)	6.09	0.01
Plant species (weighed %)	Grasses					
	*Festuca rubra (*L.*)*	0.02 (0.02)	0 (0)	0 (0)	0.00	1.00
	*Festuca pratensis (*Huds.*)*	0.10^{B} (0.08)	1.59^{A} (0.74)	0^{B} (0)	6.51	<.001
	*Festuca arundinacea (*Schreb.*)*	6.13 (1.79)	5.86 (2.65)	0 (0)	0.06	0.95
	*Dactylis glomerata (*L.*)*	26.39^{A} (4.77)	23.78^{A} (3.66)	0.75^{B} (0.75)	15.09	<.0001
	*Elymus repens (*L.*)*	1.07^{B} (0.27)	0.63^{B} (0.22)	5.81^{A} (5.81)	20.81	<.0001
	*Arrhenatherum elatius (*L.*)*	2.03^{B} (1.08)	2.34^{B} (1.40)	14.29^{A} (8.20)	49.02	<.0001
	*Phleum pratense (*L.*)*	3.06^{B} (1.30)	5.56^{A} (2.20)	0.66^{B} (0.55)	9.91	<.001
	*Poa pratensis (*L.*)*	0.75 (0.17)	1.18 (0.55)	0 (0)	0.84	0.44
	*Alopecurus pratensis (*L.*)*	16.51^{A} (3.64)	15.23^{A} (2.58)	2.25^{B} (0.76)	13.21	<.0001
	*Lolium perenne (*L.*)*	5.83^{B} (2.07)	2.06^{C} (1.03)	21.59^{A} (3.24)	70.42	<.0001
	Legumes					
	*Trifolium repens (*L.*)*	0.03 (0.02)	0.02 (0.02)	0 (0)	0.02	0.98
	*Trifolium pratense (*L.*)*	0.03 (0.02)	0.26 (0.26)	0.05 (0.05)	1.27	0.29
	Herbs					
	*Plantago lanceolata (*L.*)*	0.66^{B} (0.37)	0.02^{B} (0.02)	2.60^{A} (0.49)	7.16	<.001
	*Plantago major (*L.*)*	0.03^{B} (0.03)	0^{B} (0)	2.27^{A} (2.27)	4.78	0.01
	*Achillea millefolium (*L.*)*	1.16 (0.33)	0.63 (0.27)	0.16 (0.16)	2.12	0.14
	*Taraxacum officinale (*F.H. Wigg.)	9.84 (1.77)	8.87 (1.64)	5.62 (4.11)	2.55	0.09
	*Potentilla anserina (*L.*)*	0 (0)	0 (0)	20.42 (4.39)	<.001	1.00
	Dicotyledonous weeds					
	Heracleum sphondylium (L.)	13.51 (2.60)^{B}	19.63 (2.53)^{A}	3.17 (2.04)^{C}	23.10	<.0001
	*Geranium pratense (*L.*)*	0.11 (0.09)	0.43 (0.26)	0 (0)	1.44	0.25
	*Artemisia vulgaris (*L.*)*	0.10 (0.09)	0.02 (0.02)	0 (0)	0.39	0.68
	*Armoracia rusticana (*L.*)*	1.40^{B} (1.40)	3.82^{A} (1.89)	0^{C} (0)	9.28	<.001
	*Stellaria media (*L.*)*	0.44 (0.24)	0.44 (0.20)	0 (0)	0.00	1.00
	*Erodium cicutarium (*L.*)*	0.08 (0.08)	0 (0)	0.05 (0.05)	0.01	0.99
	*Ranunculus repens (*L.*)*	0 (0)	0.05 (0.05)	0 (0)	0.00	1.00
	*Lamium purpureum (*L.*)*	0.36 (0.25)	0.25 (0.14)	0 (0)	0.16	0.85
	*Anchusa arvensis (*L.*)*	0 (0)	0.24 (0.24)	0 (0)	0.00	1.00
	*Tripleurospermum maritimum (*L.*)*	0.24 (0.24)	0.74 (0.60)	0 (0)	2.04	0.14
	*Cirsium arvense (*L.*)*	0.67 (0.25)	0.49 (0.30)	0 (0)	0.25	0.78
	*Raphanus raphanistrum (*L.*)*	2.54 (1.38)	2.55 (1.14)	0.13 (0.07)	1.72	0.19
	*Senecio vulgaris (*L.*)*	0 (0)	0.21 (0.19)	0.08 (0.08)	0.10	0.90
	*Amaranthus retroflexus (*L.*)*	1.14 (1.03)	0.56 (0.38)	0 (0)	1.75	0.19
	*Amaranthus caudatus (*L.*)*	0.11 (0.08)	0 (0)	0 (0)	0.00	1.00
	*Rumex crispus (*L.*)*	2.12^{A} (1.08)	0.35^{B} (0.27)	0^{B} (0)	8.75	<.001
	*Rumex acetosa (*L.*)*	0.19^{B} (0.17)	0.20^{B} (0.20)	4.19^{A} (4.19)	21.17	<.0001
	*Capsella bursa-pastoris (*L.*)*	0.06 (0.05)	0.42 (0.40)	0 (0)	1.79	0.18
	*Thlaspi arvense (*L.*)*	0.07 (0.07)	0 (0)	0 (0)	0.00	1.00
	*Tanacetum vulgare (*L.*)*	0.27 (0.16)	0.47 (0.30)	0 (0)	0.48	0.62
	*Galinsoga parviflora (*Cav.*)*	1.18^{A} (0.62)	0.36^{B} (0.25)	0^{B} (0)	3.50	0.04
	Monocotyledonous weeds					
	*Echinochloa crus-galli (*L.*)*	1.05^{B} (0.84)	0.50^{B} (0.42)	4.43^{A} (2.83)	14.41	<.0001
	Sedges					
	*Carex hirta (*L.*)*	0.64^{B} (0.48)	0.09^{B} (0.05)	10.01^{A} (4.93)	43.32	<.0001
Plant group (weighed %)	Weeds	8.66^{A} (1.42)	7.61^{A} (0.59)	1.45^{B} (1.34)	7.00	<.001
	Legumes	0.01^{B} (0.01)	0.32^{B} (0.26)	2.59^{A} (1.41)	9.60	<.001
	Grasses	10.36^{A} (0.96)	12.10^{A} (0.65)	3.86^{B} (2.97)	7.67	<.001
	Sedges	0.64^{B} (0.48)	0.09^{B} (0.06)	10.88^{A} (1.55)	47.24	<.0001
	Herbs	5.81^{B} (1.34)	8.76^{A} (1.67)	2.60^{B} (0.49)	9.50	0.00

^{ABC}Means bearing different superscripts differ significantly atP<0.05.

differences were in *Dactylis glomerata* (L.) and *Alopecurus pratensis* (*L.*) where the highest percentage share of those plants in the pasture composition was observed on ranges used by both breeds of chickens as compared to the control group. *Phleum pratense* (L.) has the highest share on the pastures used by Green-legged Partridge as compared to Sasso, and on the control plots. The highest values in the control group were found for *Elymus repens* (L.), *Arrhenatherum elatius* (L.), and *Lolium perenne* (L*.)*. In the case of the first two species, no significant differences were observed between the breeds. However, in the case of *Lolium perenne* (L.), the share on pastures used by Sasso was higher than on those used by Green-legged Partridge. *Festuca pratensis* (Huds.) showed higher share on the Green-legged Partridge plots compared to Sasso and control plots. In herbs, differences were in two plant species *Plantago lanceolata* (L.) and *Plantago major* (L.). For both plants, the highest value was on the control plots, while lower on ranges used by chickens of either breed. The highest share of weeds: *Heracleum sphondylium* (L.) and *Armoracia rusticana* (L.) was on the Green-legged Partridge ranges, and the lowest was in the control group. *Galinsoga parviflora* (Cav.) and *Rumex crispus* (L.) had the highest value on the Sasso ranges, while *Rumex acetosa* (L.) and *Echinochloa crus-galli* (L.) had the highest value in the control group. *Carex hirta* (L.), the only sedge specimen found in the pasture, had the highest share on the control plot.

Weeds and grasses were more present on the ranges used by the birds as compared to the control plots. Legumes plants and sedges were mostly represented in the control plots. Herbs were most present on the ranges used by Green-legged Partridges.

Associations between sodding, chemical and botanical composition of the pastures and their use by Sasso and Green-legged Partridges

The significant regression models and their results are presented in Table 2. More frequent exits to the outdoor areas were associated with higher protein and ash content in the green matter collected from the pasture in both breeds, explaining 76% of exits frequency variability of Green-legged Partridges and 70% in Sasso chickens. Pastures composed of: *Heracleum sphondylium* (L.), *Rumex acetosa* (L.), *Festuca rubra* (L*.)*, *Lolium perenne* (L.) were positively associated with the frequency of ranging areas use by Sasso, while of: *Armoracia rusticana* (L.), *Stellaria media (*L*.)*, *Ranunculus repens* (L.), *Cirsium arvense* (L.), *Rumex crispus* (L.) by Green-legged Partridges. In case of both breeds the pastures', botanical composition explained more than 90% of the range use frequency variability. Only in Sasso a positive association between the frequency of range use and the weighted percentage of plants belonging to the weed group on the range was identified.

Optimization of the range use by the chickens improves their health and welfare [Marchewka *et al.* 2020]. On the other hand, overuse of the ranges by the chickens can bring a variety of negative effects, such as vegetation destruction, soil compaction, and even acidification[(Hilimire *et al.* 2013, Spencer 2013]. We hypothesized that free-ranging chickens can affect the characteristics of the free range areas they have access to.

Table 2. Significant regression models of the range use frequency depending on the genotype with the chemical and botanical composition of ranges

Parameter		Breed	Response variable	R-Square	Coefficient (r)	SEM	t Value	Pr>t	95% Confidence limits	
Exits (sum/pen)	Chemical composition (g/100g)	Sasso	Ash	0.7003	233.4	47.2	4.94	<.0001	169.6	281.9
			Protein		293.8	88.6	3.31	<.0001	172.3	409.3
		Greenlegged partridge	Ash	0.7559	229.6	36.5	6.28	<.0001	151.6	307.5
			Protein		305.3	84.6	3.61	0.0026	124.8	485.8
	Plant species (weighed %)	Sasso	*Heracleum sphondylium (*L.)	0.9325	38.2	9.6	3.96	0.0016	17.3	59.1
			*Rumex acetosa (*L.)		513.7	142.5	3.60	0.0032	205.7	821.7
			*Festuca rubra (*L.)		3982.4	892.0	4.46	0.0006	2054.9	5909.1
			*Lolium perenne (*L.)		68.7	8.5	8.05	<.0001	50.3	87.2
		Greenlegged partridge	*Armoracia rusticana (*L.)	0.9550	79.7	7.8	10.22	<.0001	62.7	96.7
			*Stellaria media (*L.)		374.4	74.6	5.02	0.0003	211.8	536.9
			*Ranunculus repens (*L.)		1964.7	297.6	6.60	<.0001	1316.2	2613.2
			*Cirsium arvense (*L.)		207.1	49.1	4.22	0.0012	100.1	314.2
			*Rumex crispus (*L.)		584.4	57.4	10.17	<.0001	459.1	709.6
	Plant group (weighed %)	Sasso	Weed	0.2391	105.5	32.2	3.27	0.0025	39.9	171.1

Our hypothesis was confirmed, as in the current experiment the percentage of sward cover was the highest in the control group, as compared to ranges used by the birds, however differences existed between breeds. In the Green-legged Partridge breed, the percentage of sward cover was higher than in the Sasso group. Sasso are larger than Green-legged Partridges, therefore they not only consume larger amounts of plants but potentially also affect more the range area by scratching the soil or excretions. It may be necessary to consider outdoor stocking density limits individualized per breed and not only as it is currently done by the poultry species.

The botanical composition of a pasture is critically important for extensive poultry farms as it contributes, together with soil fertility and climate, to the productivity of birds [Kosmidou *et al*. 2006, Singh and Cowieson, 2013, El Jeni *et al.* 2021]. Moreover, plant diversity in grasslands affects greater ecosystem stability[Shakhane *et al.* 2013]. No study exists to the authors knowledge where qualitative analysis of the plants botanical composition has been compared between chicken breeds and those not used as birds ranges.

With regard to qualitative composition of the ranges we have identified some plant species which were present on the plots which birds used more frequently. The set of those plants were however not equal for Sasso and Green-legged Partridges. Sasso were seen outdoors more frequently on the plots where higher percentage of: *Heracleum sphondylium* (L.), *Rumex acetosa* (L.), *Festuca rubra* (L.) and *Lolium perenne* (L.) were found after the experiment. On the other hand, Green-legged Partridges were more frequently using ranges on which *Armoracia rusticana* (L.), *Stellaria media* (L.), *Ranunculus repens* (L.), *Cirsium arvense* (L.) and *Rumex crispus* (L.) were observed. In the case of selected plant species, if their presence on the control plots was higher, as compared to the birds' ranges, we assumed that the birds had eaten them or that the birds' presence affected their disappearance. This could be through either changing the soil parameters due to its fecal contamination, changing its moisture or mechanical damage by stepping on them. On the other hand, if there was more of a particular plant on the bird's ranges, as compared to the control plots we suspect that birds' presence enhanced their growth.

As the European Union regulations on organic agriculture do not permit the use of in-feed growth stimulators or synthetic amino acids it is especially important to realize which chicken health promoting plants are consumed by the birds when available on ranges. The favorable impact of narrowleaf plantain (*Plantago lanceolata L.*), used as a fortifying and fodder herb for poultry, has been demonstrated [Camy *et al.* 2020, Redoy *et al.* 2021]. This herb boasts a broad phytochemical profile: acteoside, aucubin and catalpol, which have a favourable impact on the health and performance of animals [Yap *et al*. 2019]. In the current study we were able to confirm that on ranges used by both genotypes of chickens the share of this plant species was lower as compared to the control plot which may suggest that birds ingested this plant during the experiment. In the current study we were able to prove the differences between chicken genotypes in their preferences for the particular plants. It is known that chickens are sensitive to taste stimuli, which is consistent to the well- developed taste system indicated by the recent studies and indicates a higher than previously thought impact on feeding behaviors [Roura *et al.* 2013] and possibly also on the selection of the plants on the ranges. It may be possible that the taste preferences depend on the genetic background of the chickens and could influence their dietary preferences, which may have further application in optimization of chicken management strategies.

Identified fiber content was higher in dry matter from the control pastures as compared to those used by either of the breeds. Moreover, higher ADF proportion was identified in the control plots, as compared to ranges used by Green-legged Partridges. In the current study we found that the fiber levels were reduced by around 5% by the presence of chickens of either of the breeds on the ranges, as compared to control plots, however remained at around 20%. The exact mechanism as to how this reduction happened if it was through ingestion of the plants or through other factors remains unknown. Although the general opinion of broiler producers and feed manufacturers is that the fiber content of the rations should be kept below 7%, production does not appear to be influenced much by some increase to 8-10%. Excess feeding of fiber sources to chickens may lead to enlargement of the intestinal villi arising from physical stimulation of villous growth. The increased size of the villi is often coupled with about a two-fold increase in goblet cell numbers which adversely affects absorption. The excessive use of fiber in the diet may also increase viscosity of the intestinal content, with a resulting decrease in bioavailability of vitamin A and utilization of dietary fats, which adversely affects body weight gain and carcass quality. This agrees with our previous finding in Sasso, that villi in the small intestines were significantly higher in the outdoor-preferring compared to indoor-preferring profiled birds, while their area was larger in the outdoor-preferring Sasso birds [Marchewka *et al.* 2021]. Moreover, amino acids in fibrous feed ingredients are typically less digestible than those in low-fiber ingredients, requiring consumption of larger amounts of amino acids to satisfy the requirement for digestible amino acids. This may be especially important in the EU organic type of production system where the main dietary challenge is to fulfill the protein balance requirements, especially the methionine deficiency [Van Krimpen *et al.* 2016]. Therefore, further research should focus on through what mechanism the chickens reduced the fiber content in the green matter collected from their ranges and what was the fiber intake from the pasture matter by chickens. This could allow for better formulated diets for the ranging chickens.

Ash levels were higher in green matter from the ranges used by the Green-legged Partridges, as compared to the control plots. Ash in forages is composed of minerals contained within the plant (for example, potassium, calcium, magnesium, and copper) and soil contamination that was either splashed onto the surface of the plant. The sward cover higher at the ranges used by Green-legged Partridges could either contain more ash coming from the soiling or birds of this breed potentially left the higher plants which contained more internally sourced ash uneaten.

Conclusion

Our results showed that the sward of the pasture plots are affected by the chickens with range on them and that this effect is different depending on the reared breed. We found significant differences between selected plant species, sward cover and chemical composition of the dry matter from the pastures used by chickens of two breeds and the control plots. Moreover, there was a significant positive relationship between protein and ash content of the dry matter and frequency of the pasture use in both breeds. We have also found a significant positive relationship between selected plant species and frequency of the pasture use in both breeds. The sets of the plants in the identified model were different between breeds. Nevertheless, the causal relationship could not be identified, and it remains unknown if birds used the pasture more frequently depending on its botanical and chemical composition or if the pasture compositions were dependent on the frequency of its use by the birds.

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