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Selenium and vitamin E diet inclusion for optimal reproduction performances of red-legged partridge (*Alectoris rufa*)

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ABSTRACT

To date there is little knowledge regarding the requirements of Se and vitamin E of red-legged partridges (*Alectoris rufa*). For this reason, in the present study four different Se and vitamin E diet inclusions have been tested. A total of 360 parents were used and randomly divided into four groups; diets were supplemented with 0.2, 0.3, 0.4 and 0.5 mg/kg of Se and Se to vitamin E ratio was kept approximately constant in all groups. The effects of the diets on parents' reproduction performances and on embryos visceral organs were investigated. The best laying rate was reached with 0.4 mg/kg Se diet supplementation while the best hatching rate was reached with 0.3 mg/kg ($p < 0.05$). The relative weight of duodenum, jejunum and ileum in embryo was higher ($p < 0.05$) in the groups fed 0.4 and 0.5 mg/kg Se compared to the other groups. Significant differences ($p < 0.05$) were also observed for jejunum and ileum length as animals were fed the highest Se to vitamin E ratios. The number and height of villi and goblet cells density of jejunum were higher ($p < 0.05$) in the groups fed 0.4 and 0.5 mg/kg of Se than in the group fed 0.2 mg/kg. Epithelial buds density in the Bursa of Fabricius of embryos was significantly higher ($p < 0.05$) for 0.4 and 0.5 mg/kg Se supplemented groups than in the others. In conclusion our results suggest that 0.4 mg/kg of selenium and 100 mg/kg vitamin E should be included in the parents' diet in order to optimise red-legged partridges performances.

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Alectoris rufa; nutrition; reproduction; selenium; vitamin E

Introduction

Pheasants and red-legged partridges are game-bird species widely farmed in the Mediterranean area; the aim of breeding is the releasing into the wild, either for increasing wildlife populations or for hunting activities. Their farm breeding performances as well as their survival rate after releasing need to be improved. For these reasons, studies are still carried out on nutrition, reproduction, breeding and releasing techniques (Bagliacca et al. 2008; Fronte et al. 2008).

Regarding nutrition, selenium content of grain is generally low in most European countries and domestic animals are routinely supplied with extra dietary selenium in order to avoid the consequences of selenium deficiency (Kenny & Kemp 2005). Selenomethionine (Se-Met) is the major form of naturally occurring selenium in feedstuffs. According to the European rules, total Se in feeds may not exceed 0.5 ppm to guarantee safety of consumer who may eat tissues and/or products of animals-fed diets integrated with selenium (EFSA 2012). Egg laying performances, growth and viability of chicks increase with selenium supplementation (Scheideler et al. 2010; Baylan et al. 2011) and NRC (1994) recommends a selenium content in diets for broiler of 0.15 mg/kg. It is also very well known that several factors may affect gut traits, e.g. diet composition, feed particle size, organic acids diet inclusion (Gauthier et al. 2007; Yang et al. 2009; Fronte et al. 2013). Soto-Navarro et al. (2004) demonstrated that diet selenium content also may affects the morphological and histological traits in the small intestine. Regarding growth performances, Swain et al. (2010) reported the maximum daily weight gain and feed conversion rate when chickens were fed a diet containing 0.50 mg/kg of selenium. Pappas et al. (2005) stated that the egg selenium concentration might be increased with an increasing selenium amount in broiler breeder diets. Moreover, the experiments carried out to evaluate the effects of dietary vitamin E, selenium and a combination of the two, on productive performance, serum metabolites and

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immune responses of poultry, showed that there is an optimal constant ratio which must be maintained between vitamin E and selenium (Jenkins & Hidiroglou 1972; Rotruck et al. 1980; Bartholomew et al. 1997; Surai et al. 1998; Singh et al. 2006; Habibian et al. 2014).

Despite Se and vitamin E diet content has been studied on several domestic species, still there is a lack of knowledge in relation to game-birds such as red-legged partridges and both nutritionists and farmers normally refer to the indication given for broilers. For this reason, in the present work we investigated the effects of different levels of Se and vitamin E supplementation, with an approximately constant Se/vitamin E ratio, on some reproductive traits of red-legged partridges.

Materials and methods

Birds and breeding system

A total of 360 pairs, 2 years old, red-legged partridges were raised in outdoor pairs cages (size 45 cm ×80 cm ×35 cm, 1 cm ×1 cm wire mesh floor) within a game-bird farm located in Grosseto province, Tuscany, central Italy (648218E, 4750479N; 65 m above sea level). The partridges were initially subjected to natural lighting but since 29th January (when the photo-period was 10 h and 37 min of light) 1-h artificial lighting was added each week until 4th March, when a complete photo-period (natural light + artificial light) of 16 h was reached (35 lux artificial light intensity minimum). This lighting program was maintained until 21st May, when artificial light was removed since natural photo-period reached the complete lighting and the partridges laying rate starts declining.

Egg deposition started during the second week of March. From 12 March onward, the eggs were collected daily and kept at 14 °C and 70% RH, 1 through 7 days before being loaded in the incubator (weekly loading, 14 weeks total, March through June). The eggs were pre-warmed for 6 h, by maintaining them in the room where the incubator itself was located (room temperature 22–24 °C and 55% RH). Incubation parameters were 99.7 °F (37.61 °C) and 47% RH (82 °F wet bulb), and hatching parameters 99 °F (37.2 °C) and variable RH 38%→86%→43% (78-56-80 °F wet bulb). After day 8th of incubation, the eggs were candled to determine their apparent fertility.

Diets

Parent couples were randomly distributed into four different sets differing just on Se and vitamin E supplementation. In particular, four different experimental diets were supplemented with 0.2, 0.3, 0.4 and 0.5 mg/kg of selenium; to keep constant the Se to vitamin E ratio, vitamin E was supplemented with 66, 75, 100 and 125 mg/kg. The groups were named on the base of the diet Se content fed (0.2, 0.3, 0.4 and 0.5 mg/kg, respectively). The ingredients and composition of diets are shown in Table 1. Since the positive effects of Se and vitamin E supplementation on animal physiology, as well as their interactions, are fully documented and nowadays considered a 'normal' practice, the experiment was designed with a control group fed a diet covering the minimum requirements of selenium and vitamin E (NRC 1994). The diets were supplied starting from 2 weeks before the onset of egg laying period. As a selenium source, a specific inactivated whole selenised yeast (*Saccharomyces cerevisiae*) was used (Alkosel® R397, Lallemand©, Blagnac, France) to reach the target concentration; hence, the added Se was a mixture of organic seleno-compounds with L(+) selenomethionine as the predominant source of selenium, <36% of total selenium in the form of unspecified Se-compounds and <2% of residual inorganic selenium.

Se content determination

Feed and egg Se content was assessed according to the AOAC (2006) procedures; 0.8–1.0 g of sample (feed, yolk and albumen) were digested with 5 ml nitric acid and 2 ml percloric acid until the solution cleared; afterward, the solution was diluted up to 10 or 25 ml with deionised water and samples analysed using inductively coupled plasma atomic emission spectroscopy with sodium selenite (Sigma-Aldrich©, St. Louis, MO) as standard.

Embryos histological analysis

Just before hatching, on day 24th, two eggs from three settings from each group were randomly selected and weighed. The embryos were sacrificed by cervical dislocation and the digestive tract, heart, brain, bursa of Fabricius, liver and lung were carefully excised, weighed and measured. After removing the intestinal content, a portion of ~5 cm of duodenum (mid-point of the pancreatic loop), jejunum (mid-point of jejunum) and ileum (after Meckel's diverticulum) were removed for gut morphological measurements. Samples of bursa of Fabricius and jejunum were gently flushed with phosphate buffer 0.1 M pH 7.1 (PB) and fixed in PB with 4% formaldehyde. After 1 day in the fixative, samples routinely dehydrated in alcohol (70% −> 80% −> 95%

Table 1. Diet ingredients and analysed nutrient composition (as-fed basis).

	Diets			
Ingredients and composition	0.2 mg/kg	0.3 mg/kg	0.4 mg/kg	0.5 mg/kg
Soybean meal solv extr 44, %	24.550	24.530	24.495	24.460
Barley, %	13.50	13.50	13.50	13.50
Corn, %	27.00	27.00	27.00	27.00
Corn gluten meal, %	12.00	12.00	12.00	12.00
Sunflower-seed meal solv extr, %	5.00	5.00	5.00	5.00
Soft wheat white shorts, %	5.00	5.00	5.00	5.00
$CaCO_3$, %	6.00	6.00	6.00	6.00
Linseed extruded, %	1.60	1.60	1.60	1.60
$CaHPO_4$, %	1.00	1.00	1.00	1.00
Soybean oil, %	1.00	1.00	1.00	1.00
Canola molasse, %	2.00	2.00	2.00	2.00
Vitamin and mineral premix[a], %	0.50	0.50	0.50	0.50
NaCl, %	0.22	0.22	0.22	0.22
L-lysina HCL, %	0.22	0.22	0.22	0.22
DL-methionin, %	0.21	0.21	0.21	0.21
$NaHCO_3$, %	0.19	0.19	0.19	0.19
Alkosel® 1000 (R397), %	0.01	0.02	0.03	0.04
Vitamin E (10% Dl-a-tocopherol), %	–	0.01	0.04	0.06
TOTAL, %	100	100	100	100
Moisture, %	11.02	11.02	11.02	11.01
Crude protein, %	19.57	19.57	19.56	19.55
Crude fiber, %	5.39	5.39	5.38	5.38
Fat, %	3.77	3.77	3.77	3.78
Ash, %	11.94	11.95	11.98	12.00
Se, mg/kg	0.24	0.32	0.42	0.53
Vitamin E, mg/kg	66	75	100	125
EM (calculated), MJ/kg	10.58	10.58	10.58	10.57

[a]Supplied (mg/kg diet): retinol 4.5, dl-a-tocopherol 30, cholecalciferol 0.075, menadione 3, thiamin 2, riboflavin 8, pyridoxin 5, cyanocobalamin 0,03, d-biotin 0.1, nicotinic acid 40, pantothenic acid 15, folic acid 1.25, choline chloride 600, Mn 150, Zn 60, Fe 35, Co 0.5, Cu 10, J 0.5, Se 0.1 and ethoxyquin 2.5.

−> 100%), and embedded in resin (JB-4, Polyscience, Warrington, PA). A series of 4 μm sections were cut with a microtome (Reichert-Jung. Mod. 1140yAutocut, San Diego, CA) and collected onto gelatin-coated slides. For morphological measurements, intestinal sections were stained with hematoxylin (Mayer's hematoxyline, code n. 46051501, CARLO ERBA Reagents S.r.l., Italy) and eosin (Eosin solution 1%, code n. 446644, CARLO ERBA Reagents S.r.l., Italy). Bursa Fabricius sections were stained (Fischer et al. 2006) with Giemsa (J.T Baker, ref. 3856, Holland). Sections were examined using a light microscope (Leitz, Diaplan) connected to a PC via a Nikon digital system (Digital Sight DS-U1, Tokyo, Japan). Images were acquired using the NIS-Elements F version 2.10 software per transverse section of small intestine. Determination of goblet cells containing acidic and neutral mucin was done by staining 4 μm sections with alcian blue (AB) pH 2.5 + periodic acid-Schiff reagent (PAS) according to the following protocol. Slides were incubated with AB pH 2.5 solution for 30 min, rinsed in running water for 5 min and placed in 0.5% of periodic acid solution for 10 min, rinsed in running water for 5 min and incubated in Schiff reagent for 10 min. After washing in running water and then in distilled water, the slides were dehydrated and mounted.

Measurements and statistical analysis

Hatching results were recorded weekly. Embryo measurements were made on digital images using ImageJ® 1.37V software (Institute of Health, Bethesda, MD). For bursa of Fabricius, the sections surface area was measured, the number of epithelial buds counted on per transverse section and the density of epithelial buds calculated. Ten well-oriented and intact villus units of each slide of jejunum section were measured in triplicate. The villi height was defined as the distance from villus tip to crypt junction. The villus width was measured from the outside epithelial edge to the outside of the opposite epithelial fringe at the half-height of the villus. The perimeter of the villus was measured at the villus boundary (edge). Villus surface area was calculated from villus height and width at the half-height. The number of villi was counted on per transverse section of small intestine. The number of AB/PAS-positive cells along the villi was determined by light microscopy.

Hatching results were subjected to chi-squared test followed by chi square Yates correction to test

Table 2. Laying performances and Se content of the eggs in relation to selenium supplementation in parents diet (mean ± SE).

Group	Egg laying rate, % $n = 20,383$	Infertility rate, % $n = 2393$	Hatched on fertile, % $n = 9608$	Yolk Se-content, mg/kg $n = 6$	Albumen Se-content, mg/kg $n = 6$
0.2	37.9^{c}	22.4^{a}	93.2^{bc}	0.353 ± 0.0433^{ns}	0.194 ± 0.0238^{ns}
0.3	36.7^{c}	16.3^{b}	95.9^{a}	0.373 ± 0.0452^{ns}	0.261 ± 0.0316^{ns}
0.4	40.3^{a}	16.9^{b}	92.0^{c}	0.379 ± 0.0461^{ns}	0.322 ± 0.0392^{ns}
0.5	38.2^{b}	20.3^{a}	94.1^{b}	0.385 ± 0.0462^{ns}	0.215 ± 0.0258^{ns}
Chi square	>100**	40**	>68**		

Means within the same column bearing different letters differ per $p < 0.05$.
**High significant values of chi square.

Table 3. Eggs and embryo traits in relation to selenium supplementation in parents diet ($n = 6$, mean ± SE).

Group	0.2	0.3	0.4	0.5
Egg, g	15.2 ± 1.24	15.0 ± 0.84	15.8 ± 1.03	16.2 ± 0.91
Egg shell, %	14.2 ± 4.22^{b}	14.1 ± 6.13^{b}	15.3 ± 4.53^{a}	14.9 ± 0.22^{a}
Embryo, %	82.2 ± 8.75^{b}	84.0 ± 5.40^{b}	86.7 ± 6.01^{a}	84.6 ± 5.25^{a}
Heart, %	0.621 ± 0.0422^{c}	0.713 ± 0.0623^{b}	0.694 ± 0.0571^{bc}	0.810 ± 0.0782^{a}
Liver, %	1.92 ± 0.324^{b}	2.11 ± 0.241^{b}	2.10 ± 0.435^{b}	2.34 ± 0.566^{a}
Lung, %	0.689 ± 0.0509	0.673 ± 0.0461	0.711 ± 0.0620	0.70 ± 0.089
Brain, %	4.29 ± 0.433	4.28 ± 0.568	4.15 ± 0.764	4.18 ± 0.342

Means within the same row bearing different letters differ per $p < 0.05$.

the differences among the groups. Relative weight of organs were calculated through the following formula: Relative weight = organ weight/body weight; absolute data were statistically tested for normality and homoscedasticity and after confirmation subjected to analysis of variance followed by Tukey test for group differences; relative weights were submitted to non-parametric Wilcoxon test (SAS Institute 2008).

Finally, all procedures were in compliance with the national laws and regulations for Animal Experimentation and performed in accordance with the Guiding Principles for the Care and Use of Experimental Animals.

Results

Laying performances and eggs Se content

The laying rate observed for all the considered groups were 37.9%, 36.7%, 40.3% and 38.2%, for groups 0.2, 0.3, 0.4 and 0.5, respectively (Table 2); in particular, the differences observed were statistically significant ($p < 0.05$) between the groups 0.4 and 0.5, as well as among the groups 0.4 and 0.5 and the remaining groups 0.2 and 0.3. No difference was observed between groups 0.2 and 0.3.

The infertility rates were 22.4%, 16.3%, 16.9% and 20.3%, for groups 0.2, 0.3, 0.4 and 0.5, respectively (Table 2); differences were statistically significant ($p < 0.05$) between group 0.2 and 0.5 compared to groups 0.4 and 0.3. Regarding the egg hatched on fertile eggs, the highest values were observed for group 0.3 (95.9%) and this rate was significantly different in comparison to all the other groups ($p < 0.05$). No difference was observed for egg selenium content in relationship to any group.

Weight of eggs and embryos

Our results showed significant differences ($p < 0.05$) between egg shell and embryo relative weights of 0.4 and 0.5 groups versus 0.2 and 0.3 groups (Table 3). Also the relative weight of heart was significantly higher for group 0.5 in comparison to the other groups ($p < 0.05$); moreover, a difference was observed between groups 0.3 and 0.2 ($p < 0.05$). In relation to the relative weight of liver, the group fed 0.5 mg/kg of selenium showed a significantly higher index than the other groups ($p < 0.05$). Although, no difference was observed for duodenum lengths, jejunum was significantly longer in 0.4 and 0.5 groups in comparison to the group 0.2 ($p < 0.05$). Also for ileum length (Table 4), differences were observed between groups 0.2 and 0.3, while the highest length value was observed in the group fed the diet with 0.5 mg/kg of selenium ($p < 0.05$). Relative weight of duodenum was significantly lower ($p < 0.05$) for group 0.2 compared to groups 0.3, 0.4 and 0.5. The relative weight of jejunum was significantly higher in groups 0.4 and 0.5 than in 0.2 and 0.3 ($p < 0.05$). The relative weight of ileum was significantly lower for group 0.2 than in 0.4 and 0.5 ($p < 0.05$), but no differences ($p > 0.05$) were

Table 4. Length and relative weight of intestine tracts in relation to selenium supplementation in parents diet ($n = 6$, mean ± SE).

	Length (cm)			Weight (%)		
Group	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
0.2	2.27 ± 0.301	3.63 ± 1.121^{b}	1.88 ± 0.313^{c}	0.48 ± 0.043^{b}	0.64 ± 0.051^{b}	0.18 ± 0.001^{b}
0.3	2.34 ± 0.484	4.13 ± 0.712^{ab}	2.14 ± 0.211^{b}	0.65 ± 0.052^{a}	0.61 ± 0.042^{b}	0.24 ± 0.003^{ab}
0.4	2.59 ± 0.553	4.61 ± 0.563^{a}	2.16 ± 0.494^{ab}	0.66 ± 0.074^{a}	0.72 ± 0.032^{a}	0.28 ± 0.005^{a}
0.5	2.62 ± 0.212	4.42 ± 0.531^{a}	2.24 ± 0.342^{a}	0.62 ± 0.031^{a}	0.71 ± 0.061^{a}	0.27 ± 0.005^{a}

Means within the same column bearing different letters differ per $p < 0.05$.

Table 5. Jejunum morphological and histological traits, in relation to selenium supplementation in parents diet ($n = 6$, mean ± SE).

Group	Villi density (n/mm^2)	Villi height (μm)	Villi width (μm)	Villi surface area (mm^2)	Goblet cell density (cells/mm^2)
0.2	49.0 ± 6.591^{b}	511.21 ± 72.862^{b}	119.35 ± 16.543	0.063 ± 0.014	183.47 ± 75.182^{b}
0.3	54.6 ± 3.642^{b}	528.15 ± 50.091^{ab}	123.53 ± 23.224	0.065 ± 0.015	228.47 ± 80.671^{ab}
0.4	65.0 ± 3.933^{a}	542.89 ± 56.493^{ab}	131.21 ± 25.481	0.073 ± 0.024	248.52 ± 96.723^{a}
0.5	66.2 ± 5.802^{a}	564.53 ± 71.212^{a}	126.31 ± 11.061	0.068 ± 0.008	287.07 ± 99.544^{a}

Means within the same column bearing different letters differ per $p < 0.05$.

Table 6. Morphological and histological traits of the bursa of Fabricius of hatching embryo in relation to selenium supplementation in parents diet ($n = 6$, mean ± SE).

Group	Epithelia buds (*n*)	Bursa surface area (mm^2)	Epithelia buds density (buds/mm^2)
0.2	108.62 ± 23.163^{c}	1.75 ± 0.221^{b}	62.25 ± 15.201^{b}
0.3	99.21 ± 16.332^{c}	1.51 ± 0.194^{b}	66.36 ± 14.223^{a}
0.4	134.33 ± 19.513^{b}	2.35 ± 0.332^{a}	68.32 ± 14.911^{a}
0.5	169.25 ± 5.371^{a}	2.54 ± 0.344^{a}	67.38 ± 7.653^{a}

Means within the same column bearing different letters differ per $p < 0.05$.

observed between group 0.3 and the others (Table 4).

Histomorphological measurements

The number of villi in groups 0.4 and 0.5 were significantly higher (Table 5) than in groups 0.2 and 0.3 ($p < 0.05$). The height of villi was significantly lower for group 0.2 than in group 0.5 ($p < 0.05$), this trend showing a significant linear regression with the Se content of the diet. No differences were observed in relation to width and surface area of villi in jejunum. The density of goblet cell on jejunum was significantly higher in groups 0.4 and 0.5 than in the group 0.2 ($p < 0.05$).

Epithelia buds on Bursa of Fabricius

The number of epithelial buds of bursa of Fabricius was different among groups 0.5, 0.4 and 0.3 ($p < 0.05$), with the highest value observed in group 0.5 and the lowest in group 0.3 (Table 6). Groups 0.4 and 0.5 showed a bigger surface of bursa compared with the other groups ($p < 0.05$). Also, group 0.2 showed significantly lower density of epithelial buds of bursa compared with all the other groups ($p < 0.05$).

Discussion

Our results confirmed quite clearly that the Se and vitamin E content of the diet of red-legged partridge parents, significantly affects reproduction performances and embryo traits. The laying performances observed in the present study are in contrast with the results reported by Bennett and Cheng (2010); these Authors did not observe any effect of the Se diet content on hens egg production, despite the lowest Se diet level used was 0.31 and the highest 5.43 mg/kg and the reason of that might be due to the reduced numerousness of the analysed samples. Also for laying hens Scheideler et al. (2010) refer about an egg production increment only when the Se content rose from 0.5 to 0.75 mg/kg of diet; in this case, the selenium to vitamin E ratio was not kept constant and probably it caused a decrease of absorption of vitamin E and the observed reduction of α-tocopherol in the yolk.

While in general the higher supplemented diets significantly promote the egg laying process, the increase of candled eggs in the group 0.5 seems to suggest that eggs fertility reaches its maximum in correspondence to medium supplementation. Hatchability on

fertile eggs also was positively affected by a medium Se and vitamin E diet supplementation (the best performance was obtained for group 0.3). Regarding egg production, these results only partially confirm those observed by Scheideler et al. (2010) for laying hens since they refer about an increased egg production when the diet Se content rose from 0.55 to 0.75 mg/kg. Contrarily to what observed by Pappas et al. (2006a) on egg fertility and hatchability of laying hens, in the present study differences were observed for both parameters. In particular, fertility (estimated as percentage of candled eggs) increased with intermediate diet contents of Se and vitamin E while hatchability on fertile eggs did not follow a clear trend. Finally, in the present study no statistically significant difference was observed in egg Selenium content, probably due to the reduced number of analysed eggs (n). However the trend showed by the mean values confirmed the observations of other Authors in laying hens (Pappas et al. 2005; Bennett & Cheng 2010; Scheideler et al. 2010; Wang et al. 2010).

Parents' nutrition significantly influenced chick quality; supplementing selenium and vitamin E in partridge parents' diets from 0.2 and 66 mg/kg to 0.5 and 125 mg/kg, increased egg shell and embryo relative weight. The observed results are in accordance with Pappas et al. (2006b), whose study showed that chicks derived from parents-fed selenium enriched diets were heavier at hatch than those fed the low selenium diets. Though, Ševčíková et al. (2006) reported no difference in body weight of broiler chickens-fed diets containing 0 and 0.3 mg/kg Se, Cantor et al. (1982) observed higher body weight at 28-day post-hatch when poults were fed Se supplemented diets (0.04–0.12 mg/kg Se); results observed in the present work confirmed the same trend just from the pre-hatching embryos (24 days old embryos).

The length and relative weight of jejunum and ileum were influenced by the content of selenium of partridge parents diets, even though only the relative weight of duodenum was significantly affected by the level of supplementation in the parent diets. These results are in accordance with the major effect of high-Se wheat on visceral organ mass occurred in jejunal tissue, which is one of the most metabolically active tissue (Soto-Navarro et al. 2004; Read-Snyder et al. 2009). In general, visceral organs are metabolically very active and represent a substantial amount of maintenance energy consumption (Caton et al. 2000). The metabolic activity of an organ is the product of the organ size and metabolic activity per unit of tissue, and jejunum had higher fractional rates of protein synthesis (Soto-Navarro et al. 2004). In our research, the level of selenium and vitamin E in partridge breeder diets seemed positively affecting jejunum and ileum tissues metabolic activity. Higher level of selenium and vitamin E in partridge parent diets, generally produced also a higher number of villi, characterised by higher length and more goblet cells. These observations are in accord with Read-Snyder et al. (2009) who reported increased villus height in the duodenum, jejunum and ileum, were associated with higher selenium diet content in broiler chicken.

The bursa of Fabricius is the primary lymphoid organ responsible for the establishment and maintenance of the B cell compartment in avian species (Peng et al. 2009). The number of epithelial buds and the surface area of bursa of Fabricius, in this study were variable according to partridge parents selenium diets level. Higher diet level of selenium and vitamin E generally increased epithelial buds number and bursa of Fabricius surface area. Our results are in line with Peng et al. (2009) and El-Sheikh et al. (2010), who reported that selenium plays a significant role in the development of bursa of Fabricius. Marsh et al. (1986), Hegazy and Adachi (2000) and Hussain et al. (2004) found that lymphoid organs (bursa of Fabricius, spleen and thymus) size of birds fed lower selenium diet content was significantly lower than birds fed higher selenium diet levels.

As final conclusion, the results observed in the present study suggest that reproduction performances of red-legged partridges may be significantly enhanced by a selenised yeasts diet supplementation (at least with Se to vitamin E constant ratio), especially up to 0.4 mg/kg diet, near to the maximum level allowed by the European rules and at a doubled level in respect to that suggested for domestic bird by the EFSA (2012). This level of supplementation improves also offspring physiological, morphological, histological and immunological traits at hatching, confirming this as an appropriate selenium level in the hen diet since positively affected the size, vigour and, consequently, the immunological *status* of the hatching chicks. For these reasons, when offspring are raised for release into the wild, an increased selenium level in the feed (maintaining constant the Se to vitamin E ratio) may be useful to improve survival rate and performances after release.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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