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Effect of rearing system and season on the performance and egg characteristics of Ancona laying hens

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ABSTRACT

Productive performance and egg characteristics of Ancona laying hens reared under three different rearing systems (conventional, organic and organic-plus) were compared during an experimental period of one year. Three-hundred-sixty Ancona female chicks at 28 days of age were divided in three groups and assigned to different rearing systems. The organic group had 4m² pasture/hen according to the requirements imposed by the EC Regulation 1804/99, whereas the organic-plus group had a larger grass paddock (10m²/hen). The Control group was reared in cages under standard housing conditions. The following egg characteristics were recorded and analysed during the year-long cycle: egg weight and egg mass laid/d, weight of egg components, shell thickness, Haugh index and yolk colour. Egg quality was affected by the pasture available. The hens that ingested grass (organic-plus), produced eggs with higher shell weight and percentage, darker yolk colour and higher α -tocopherol, carotenoid and polyphenol contents. The other egg traits were not affected by rearing system.

Key words: Ancona hens, Egg characteristics, Organic production, Grass availability.

RIASSUNTO

EFFETTO DEL SISTEMA DI ALLEVAMENTO E DELLA STAGIONE SULLE PERFORMANCE E SULLA QUALITÀ DELLE UOVA DI GALLINE ANCONA

Le prestazioni produttive e le caratteristiche qualitative di uova ottenute da ovaiole di razza Ancona allevate con tre sistemi di allevamento (Convenzionale, Biologico e Bio-Plus) sono state confrontate durante un periodo sperimentale di un anno. Trecentosessanta pulcini femmine di Ancona a 28 giorni di età sono stati assegnati a tre sistemi di allevamento differenti. Il Bio-Plus aveva a disposizione una maggiore disponibilità di pascolo (10m²/gallina) rispetto ai requisiti imposti dal regolamento EC 1804/99 (4m²/gallina) per la produzione biologica di uova (gruppo Biologico). Il terzo gruppo di galline è stato allevato in gabbia in condizioni standard (Controllo). Sono state registrate ed analizzate le seguenti caratteristiche dell'uovo: peso e percentuale di deposizione, peso delle componenti dell'uovo, spessore del guscio, indice di Haugh, colore del tuorlo e contenuti di α -tocoferolo, carotenoidi e polifenoli. La qualità delle uova è stata influenzata dalla disponibilità del pascolo ed infatti le galline del gruppo Bio-plus hanno prodotto uova con maggior peso e percentuale di guscio, superiori contenuti di tocoferolo, carotenoidi e polifenoli. Le altre caratteristiche dell'uovo non hanno presentato differenze significative.

Parole chiave: Ovaiole Ancona, Qualità uova, Metodo biologico, Pascolo.

Introduction

Recently more attention has been given to animal welfare and consequently more extensive rearing systems have been proposed.

The EC Directive 74/99 establishes the minimal requirements for hen protection and specifies new guidelines that provide for the progressive replacement of conventional cages that are to be completely eliminated by January 2012.

Among the different alternative systems organic egg production has increased during the last 15 years. In 2006, the market share in Italy of organically-produced hen eggs was 7.6% of all the organic products in retail; over a two-year period, the request for organic eggs increased by 4.6% (Ismea, 2007).

To date, there has been no clear evidence that egg quality is improved when hens are raised under the organic production system. Few studies have been published on this subject and the results are conflicting, due to the great variation in the breeds used, production methods, rations used, pasture availability and conservation times of eggs before analysis. Some studies (Casagrande *et al.*, 2001; Minelli *et al.*, 2007; Rossi, 2007) and reviews (Sauveur, 1991; Kouba, 2003) concerning the effect of conventional and *free-range* raising systems on productivity and product quality, did not establish one system as being better than the other. There are advantages and disadvantages in both systems.

In an overview, Hönikel (1998) concluded that the nutritional, hygienic, sensorial and technological qualities of products of animal-origin (including eggs) are not very different between those raised under organic and conventional methods. For some aspects organic foods get better marks, while for others, conventional ones scored higher.

Regarding the genotype of the hens to be used in order to assure a good welfare sta-

tus, the EC Regulation 1804/99 and the final recommendation of Network for Animal Health and Welfare in Organic Agriculture (Hovi *et al.*, 2003), suggest the utilisation of pure breeds for their higher rusticity. Furthermore, these strains selected to produce under highly controlled conditions, seem to be quite unsuitable for more extensive systems, such as the organic system, because the environment is less controlled and the rations are less equilibrated.

The use of less selected strains, which still have natural behaviours, could also be a valuable alternative, particularly if they are in danger of extinction. Sundrum (2001) also ascribes added values such as biodiversity, species preservation, and environmental sustainability to Organic Agriculture.

Italy is the country of origin of some egg-type chicken strains that have seen a drastic decline in number (Sponenberg and Christman, 1995); the Ancona breed which was widespread throughout Europe is an example. It has good productivity (about 280 eggs/year); the eggs are white and weigh 54-56g (Castellini *et al.*, 1990). The Ancona breed was obtained from crosses with the Leghorn breed, so its morphological and productive traits are similar. The main difference is the colour of the plumage, which is black with evenly white-tipped feathers. Analogous to other pure breeds, Ancona has been progressively replaced by hybrids, which are expressly selected for intensive production.

Little information can be found in the literature regarding the effect of alternative rearing systems on the productivity and quality traits of eggs laid by pure breed hens (Kovacs, 1998; Lopez-Bote *et al.*, 1998).

The aim of the present work was to compare the effect of organic production systems with two different areas of available pasture and that of a standard cage system, on the performance of Ancona hens and on some egg quality traits.

Material and methods

Animals, housing, pasture and feeding

Eight hundred 1-day-old chicks of the Ancona breed were reared during the first four weeks under identical conditions in floored pens, covered with wood shavings. The floored pens were located indoors in an environmentally-controlled building at the farm of the Department of Applied Biology (University of Perugia).

All animals were vaccinated against Marek and Newcastle diseases; the beaks were not trimmed and no other pharmacological treatments were given.

At 4-weeks of age, the females were divided in three homogenous groups of 120 birds each.

Two groups of them were transferred to a farm with building labelled for the requirements of the Council Directive 1999/74 and Directive 1804/99 regarding organic production. One group was assigned to each of the following conditions:

Organic (O): reared in three covered, straw-bedded houses (6bird/m²) with access to three pens with natural grazing (4m²/bird; three replications);

Organic-plus (OP): reared in three, identical covered houses (6birds/m²), but each pen was provided with natural grazing in a larger natural pasture area (10m²/bird; three replications).

Feed and water were provided indoors with manual bell feeders and automatic drinkers, respectively. Inside the paddocks, there was a small hut with nests (1 per 6 hens) and perches. Environmental temperature, humidity and photoperiod were the natural ones. A conveyor belt running along the back of the nests collected the eggs.

According to EC Regulation (2091/92), the pasture lands were not treated with pesticides or herbicides three years prior to the onset of organic production. The pasture

area had mature trees, bushes and hedges. The plant species were numerous; their physiological stages were different and graduated. The main floristic species found in pastures in the different seasons are reported in Table 2 (the first three species shown in the columns represent 72% of the pasture floristic composition), replicated 4 times in 10 different sampling areas (1m²).

The third group of birds, the Control (C), was kept at the farm of Department of Applied Biology, Animal Science section (University of Perugia) and reared in cages, under standard housing conditions (single bird cages, 0.75m² of three tier batteries that were provided with a linear automatic feeder and drinker); at 17 weeks-of-age an artificial photoperiod of 16h per day of light was applied. The building was under a controlled ventilation regime (10m³/hen/h), the temperature ranged from 15 to 29°C (mean/average daily temperatures, winter 13-17°C, and summer, 23-35°C, extremes) and the relative humidity ranged from 50 to 80%.

A layer standard feed was given *ad libitum* to all groups; for organic groups more than 90% of the ingredients (maize, wheat and whole soybean) were organically grown, as established by EC Regulation 2092/91. The nutritive characteristics of the feed were similar for all the groups, with as principal differences the quantity and quality of vitamins that, for organic groups were lower quantities and from natural fonts, whereas for conventional were greater quantities and synthetics (Table 1).

Sample collection

The floristic and chemical composition in each pasture pen was estimated by cutting a 1m² fenced area by means of a garden scissors (at 5cm above soil) before the onset of the trial. Samples of fresh grass (3pensx4 seasons per group) were taken throughout the period of egg collection.

Table 1. Composition of feed.

Control	Organic
Maize	Maize
Extracted soybean meal	Full fat soybean seed
Wheat flour	Wheat flour
Calcium carbonate	Calcium carbonate
	Pea seed protein
Maize gluten	Maize gluten
Soybean oil	Fava seed
Dried brewer's yeast	Alfalfa meal
Di-calcium phosphate	Di-calcium phosphate
Sodium bicarbonate	Sodium bicarbonate
Sodium chloride	Sodium chloride
*Vitamin premix	**Vitamin premix

*per kg of feed: Vitamin A, 12,500 U; Vitamin D, 3000 U; Vitamin K, 2 mg; Vitamin B₁, 2 mg; Vitamin B₂, 6 mg; Vitamin B₆, 4 mg; Vitamin B₁₂, 0.02 mg; Vitamin PP 30 mg; α -tocoferyl acetate, 60 mg; Folic acid, 0.50 mg; Pantothenic Acid, 8 mg; Coline, 750 mg; Luthein+Zeaxanthin+Violaxanthin, 75 mg; Fe, 35 mg; Zn, 42 mg; I, 0.5 mg; Co, 0.5 mg.

**per kg of feed: Vitamin A, 10,000 U; Vitamin D₃, 1500 U; α -tocopherol, 30 mg; Mn, 80 mg; Cu, 15 mg; Zn, 80 mg; Luthein+Zeaxanthin, 34 mg. From natural fonts.

The plants in the mixture were manually separated into groups and the species were identified by macroscopic examinations. The characterisation was carried out in the laboratory of the Department of Applied Biology where voucher specimens were stored.

To estimate grass quantity during the rearing cycle, samples were taken in a 1m² not fenced in the same pens.

Eggs were collected for analyses during the four seasons, in all the different phases of the productive activity, from 21 December 2006 to 20 November 2007.

Forty-five eggs per group were gathered (at 07.30h) on 3 consecutive Tuesdays in Winter, Spring, Summer and Autumn (Figure 1). All eggs (135 per group/season) were stored at 5°C until the analyses (maximum 2 days after) were carried out in the laboratory of the Department of Applied Biology.

Productive performance

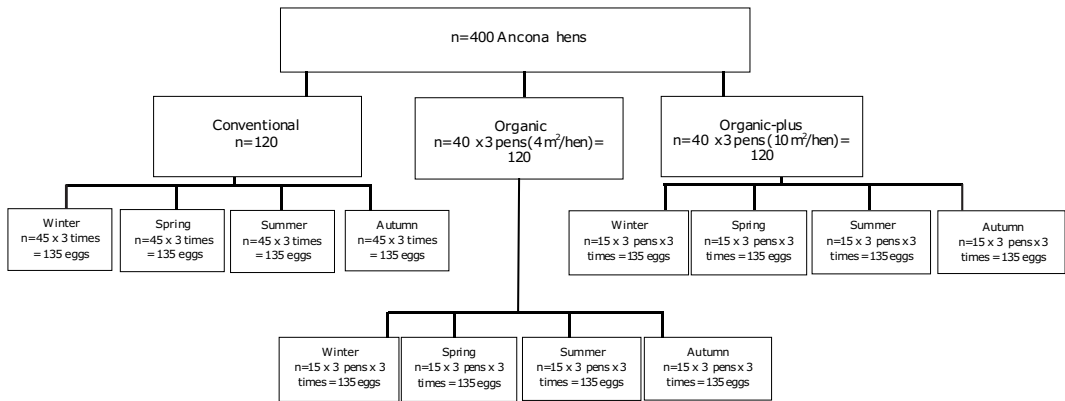
Data for calculating the egg mass, % deposition, feed intake and bird mortality were recorded per pen or cage/group throughout the productive cycle by the farm workers. Feed consumption per pen/cage was recorded weekly. Mortality was recorded daily, while hen body weight (BW) was recorded at the beginning and at the end of the experimental period.

Analytical determination

Feed, pasture and eggs - The chemical composition of the feed and pasture (OP pens) were determined according to AOAC (1995).

The tocopherol level was measured on 1 g of homogenised for feed, grass and yolk, in 10mL 0.054M PBS (pH=7.0). After mixing the mixture with ethanol and hexane (1:1), the supernatant was evaporated, and then redissolved in ethanol. The α -tocopherol

Figure 1. Scheme of experimental protocol



content was measured by HPLC, using the method of Zaspel and Csallany (1983).

The quantitative determination of carotenoids was carried out on the aerial portions of the different plant species that made up the pasture as well as on the feed and egg yolks. The acetone extracts were filtered through Millipore filters (0.2 μ m), and then analysed in HPLC (Jasco 880-PU) equipped with reverse-phase column (Hypersil ODS, 5mm, 250x4.6mm; Tracer) in line with a C18 pre-column (Luna, Phenomenex). The solvent system consisted of a solution A (methane/water/acetonitrile 5/10/85) and solution B (methanol/ethyl-acetate 70/30). The flow was 1mL/min and the elution program was a gradient starting from 90% A in a 20 min step to 100% B and then a second isocratic step of 10min. The detector was an UV-VIS spectrophotometer (Jasco 875-UV) set at λ 436nm. The different carotenoids were identified and quantified by comparing the sample with pure commercial standards (Sigma-Aldrich, Steinheim, Germany; Extrasynthese, Genay, France).

The total polyphenol content was also determined on the aerial parts of the plants and in the feed and egg yolks, using the colori-

metric method of Singleton and Rossi (1965) after extraction in methanol. The methanol extracts (200 μ L) were mixed with 800 μ L of distilled water and 10mL of 0.2N Folin-Ciocalteu reagent (Sigma[®]); 8mL of saturated sodium carbonate (75g/L) were added and the mixture was shaken. After 2 hours the absorbency of the solution at 765nm was measured with a spectrophotometer. Quantification was based on the standard curve of 50, 100, 200, 300 and 500mg/L gallic acid prepared in the same way.

Physical analysis of eggs - Data recorded were:

- whole egg weight;
- integrity, weight and thickness of shell (Mueller and Scott, 1940);
- weight and colour of yolk (Roche scale);
- albumen height (Haugh unit) using an electronic gauge (Bukley *et al.*, 1981).

Statistical analyses

A linear model (STATA[®] corp., 2005) was used to analyse data to assess the effect of rearing system along the different seasons.

Significance of the differences was assessed by the multiple t-test and X-square was used for the non-parametric variables.

Results and discussion

The analysis of the floristic composition of the pasture (Table 2) showed a mixture of different species in all the seasons; in particular, *Lotus corniculatus*, *Sorghum halepense*, *Trifolium pratense*, *Lolium perenne*, *Diplotaxis erucoides*, *Malva moscheta*, *Coniza Canadensis* and *Amarantus retroflexus*. The most common species were *Lolium perenne*, *Lotus corniculatus* and *Trifolium pratense*; in summer and autumn *Sorghum halepense* replaced *Lolium perenne* due to its greater resistance to dry periods.

Grazed pens showed differences depending on housing system and season. In particular, O pens always had lower quantities of grass with the lowest values recorded during the summer period (0.05kg of fresh matter/m²); in contrast OP pens had greater quantities of grass throughout the trial with the highest values recorded in the spring (1.44kg of fresh matter/m²)

The chemical characteristics of the feed and pasture (Table 3), as expected, showed that the grass had low dry matter (DM) values and high amounts (on DM basis) of fibre, tocopherol, carotenoids and polyphenols. The content of these last components, according to our previous studies (Castellini *et al.*, 2006a), confirmed the notable contribution of antioxidant substances from the grass, especially during the spring. These antioxidants from grass are crucial for the organic rearing system since the use of synthetic vitamins in feed has been banned (EC Regulation 1804/99). A seasonal affect on grass composition was observed with an increase in DM in summer with a consequent variation of the other components in this dry period, typical of the Mediterranean area.

Laying hen performance was significantly affected by the rearing system (Table 4) and by season when age was also considered. As expected, the organic hens, espe-

cially the OP group, produced less egg mass; the egg mass of these hens reached higher levels only in spring, in correspondence to a higher deposition. Regarding percentage of deposition, all of the hens showed an effect of age with a decrease in values throughout the experimental period. Organic hens (of both groups) showed the minimum values for the deposition in the summer and autumn (P<0.01). Moreover, OP hens showed a lower feed intake (P<0.05). Organic hens showed higher feed conversion (P<0.01), this was probably due to the higher motor activity (Castellini *et al.*, 2004) of the hens of these two groups (behavioural data not shown).

Between the organic groups, OP hens had a higher percentages of eggs laid outside the nest (P<0.01) and consequently non-marketable eggs (dirty, cracked or broken; P<0.01). This situation was due to the collection system and probably to disturbances by dominant hens as observed by Lundeberg and Keeling (1999).

The average mortality was lower in the organic groups (P<0.01); the highest percentage occurred during the Spring period in correspondence to the egg-deposition peak. The causes could have been due to ovarian diseases that affected the hens that showed the highest rates of deposition. Lewis *et al.* (1996) reported that mortality increased with photoperiod, which corresponds to increases in deposition rate. In the cage system, the mortality was still high in the Summer season probably due to the higher temperatures reached during this period. The organic hens that could find shade in the outdoor paddock, had a very low mortality rate in the summertime (P<0.01). In accord with this finding, Mashaly *et al.* (2004) reported a higher mortality in heat-stressed hens.

In this trial, the whole egg weight and component weight were not affected by the rearing system and season (Table 5). Data

Table 2. Floristic composition of pastures and productivity (kg fresh matter/m²) of pens in different seasons.

Fenced pens							
Winter		Spring		Summer		Autumn	
<i>Lolium perenne</i>		<i>Lolium perenne</i>		<i>Lotus corniculatus</i>		<i>Sorghum halepense</i>	
<i>Lotus corniculatus</i>		<i>Lotus corniculatus</i>		<i>Sorghum halepensis</i>		<i>Lotus corniculatus</i>	
<i>Trifolium pratense</i>		<i>Trifolium pratense</i>		<i>Trifolium pratense</i>		<i>Trifolium pratense</i>	
<i>Daucus carota</i>		<i>Dactylis glomerata</i>		<i>Lolium perenne</i>		<i>Lolium perenne</i>	
<i>Diptotaxis eruroides</i>		<i>Diptotaxis eruroides</i>		<i>Diptotaxis eruroides</i>		<i>Diptotaxis eruroides</i>	
<i>Malva moscheta</i>		<i>Malva moscheta</i>		<i>Malva moschea</i>		<i>Malva moschea</i>	
<i>Coniza canadensis</i>		<i>Coniza canadensis</i>		<i>Coniza canadensis</i>		<i>Coniza canadensis</i>	
<i>Amaranthus retroflexus</i>		<i>Amarantus retroflexus</i>		<i>Amarantus retroflexus</i>		<i>Amarantus retroflexus</i>	
<i>Convolvulus arvensis</i>		<i>Convolvulus arvensis</i>		<i>Convolvulus arvensis</i>		<i>Convolvulus arvensis</i>	
<i>Ranunculus bulbosus</i>		<i>Brassica oleracea</i>		<i>Kickia spuria</i>		-	
Grazed pens							
Winter		Spring		Summer		Autumn	
O	OP	O	OP	O	OP	O	OP
0.12	1.04	0.18	1.44	0.05	0.56	0.15	0.96

from the literature are conflicting (Saveur, 1991) and show some interaction with the breed, season and age.

Eggs from hens reared under the organic systems always had a higher shell weight ($P < 0.05$) than the control eggs except in the autumn when values were similar. This result could be explained by the lower deposition percentage that Control hens showed in this period; in fact as egg production rate decreases, the egg weight increases as age advances (Summers and Leeson, 1983). Also Roland (1979) observed that in older hens, an increase in egg size increased shell deposition. The higher shell weights recorded in both organic groups could have been due to the ingestion of tiny stones from the ground and to a higher synthesis of vitamin D₃ (Bar *et al.*, 1999) as a result of a greater exposure to sunlight. Vitamin D is formed in the skin of animals through the following processes:

provitamin D, previtamin D, Vitamin D; the conversion of provitamin D to previtamin D is a photochemical reaction requiring ultraviolet B photons (Wang *et al.*, 2001). Analysing results as percentages (yolk, albumen and shell) differences lowered.

Differences in Haugh units were found between groups only in the Autumn-sampled OP eggs that also showed the highest Roche value ($P < 0.001$). This increasing trend in the Haugh unit is not in accord with reports by many authors; indeed it has been widely demonstrated (Williams, 1992) that as age increases this index decreases. This hypothesis is confirmed by the Haugh unit trend in the C group and is attributed to a higher incidence of ovarian disorders that occurs in hens that produce more eggs as age increases. In contrast, Spackman (1987) found no age or inverse effects in "pathogen free" hens thanks to their better health status; this could also be

Table 3. Chemical composition of feed and pasture in the different seasons (n=4).

Chemical composition		Feed		OP Pasture			
		Control	Organic	Winter	Spring	Summer	Autumn
Dry matter	%	87.2	88.4	25.8	21.5	35.6	29.9
Crude protein	% DM	18.4	17.3	18.1	17.8	12.8	13.8
Lipid	"	4.4	4.7	2.6	1.8	2.6	5.5
Crude fibre	"	3.7	3.8	17.3	15.4	23.1	20.8
Ash	"	15.3	15.7	9.8	14.8	8.9	7.4
ME	MJ kg ⁻¹ DM	12.7	13.0	9.0	11.7	9.7	12.7
Carotenoids	µg g ⁻¹ DM	68.0	33.0	69.6	77.4	28.1	36.2
Polyphenols	"	158.9	172.4	448.4	570.7	354.5	591.0
α-tocopherol	mg kg ⁻¹ DM	95.1	62.9	132.0	224.8	172.3	149.8

ME: Metabolized Energy, by Carré *et al.*, 1989.

the case for the OP hens (Mugnai, 2008). Regarding the increase of the Haugh unit in the OP eggs in the summer and autumn, many authors (Benabdeljelil and Jensen, 1990; Franchini *et al.*, 2002) have observed that this index is positively influenced by the Vitamin C content of albumen. This water-soluble vitamin is widely distributed throughout plant species (USDA). Presumably after the OP hens have ingested fresh grass from the pastures, this vitamin is accumulated in albumen of their eggs.

Moreover, Minelli *et al.* (2007) observed that eggs from conventionally raised hens had a significant reduction of this index and attributes the reduction to the high ammonia concentration in the housing environment that increases the pH of the albumen which decreases the Haugh unit (Sauveur, 1991).

The colour differences were attributed to the level of carotenoid in the feed and pasture. Regarding the carotenoid profile in the yolk of organic eggs, the effect of pasture was very strong, considering the absence of synthetic vitamins (Castellini *et al.*, 2006b). This value reflects perfectly the carotenoid trend registered

in grass during the four seasons: e.g. O eggs had lower Roche values in the summer when pasture was almost absent in the grazed pens (0.05kg of fresh grass/m²). Another further explanation for the lower Roche value observed in the summer for the organic group could be that during this period solar irradiation is the highest, and the biological protective action of these compounds against sunlight is well known (Garmyn *et al.*, 1995; Stahl *et al.*, 1998; Stahl and Sies, 2002). It is probable that these hens metabolised more carotenoids. Besides affecting the colour, carotenoids, together with tocopherol, are important antioxidants. They play a key role in the healthy development of chick embryos (Haq *et al.*, 1996; Surai *et al.*, 1996), assure higher lipid stability in the eggs (Lopez-Bote *et al.*, 1998) and provide a stronger immune response (Tengerdy *et al.*, 1990; Moller *et al.*, 2000).

On the contrary, OP eggs showed the highest Roche value (P<0.01) in autumn when egg deposition decreased and the vegetative phases resumed (0.96kg of fresh matter/m²), which resulted in a higher accumulation of carotenoids in the yolk.

Table 4. Effect of housing system and season on laying hen performance.

Housing system	Control				Organic				Organic-plus				Pooled SEM/ X^2
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	
Egg mass g egg hen day ⁻¹	42.0 ^C	42.1 ^C	40.1 ^C	41.9 ^C	26.2 ^A	32.4 ^B	29.1 ^{AB}	31.4 ^B	23.5 ^A	34.8 ^B	26.9 ^A	25.0 ^A	4.8
Deposition %	80.6 ^D	78.2 ^D	62.1 ^C	62.8 ^C	68.1 ^{CD}	77.0 ^D	52.0 ^B	45.3 ^A	70.1 ^C	78.1 ^D	50.0 ^B	40.2 ^A	9.2
Feed intake g d ⁻¹	113.0 ^b	114.2 ^b	102.1 ^a	113.1 ^b	114.0 ^b	113.2 ^b	100.2 ^a	114.8 ^b	105.0 ^a	100.0 ^a	95.1 ^a	104.5 ^a	29.1
ICA feed ingested egg laid ⁻¹	2.7 ^A	2.7 ^A	2.5 ^A	2.7 ^A	4.4 ^C	3.5 ^B	3.4 ^B	3.6 ^B	4.5 ^C	2.9 ^A	3.5 ^B	4.2 ^C	1.0
Eggs laid out nest %	-	-	-	-	2.0 ^A	2.5 ^A	2.8 ^A	2.5 ^A	5.3 ^B	4.9 ^B	6.6 ^B	5.3 ^B	0.9*
Broken or cracked eggs "	0.6 ^A	0.7 ^A	0.5 ^A	0.5 ^A	2.3 ^B	3.5 ^C	2.0 ^B	3.2 ^C	2.4 ^B	2.5 ^B	2.4 ^B	2.6 ^B	4.0*
Mortality "	3.0 ^B	6.1 ^D	4.0 ^C	3.3 ^B	2.6 ^B	3.0 ^B	1.7 ^A	2.2 ^B	2.3 ^B	2.6 ^B	0.9 ^A	1.3 ^A	1.9*

N=135 per group/season; A...D: P<0.001; a,b: P<0.05.

Table 5. Effect of housing system and season on main characteristics of eggs.

Housing system	Control				Organic				Organic-plus				Pooled SEM	
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn		
Egg weight (EW)	g	56.9	56.7	56.4	57.1	56.5	57.0	55.4	56.8	56.4	57.4	55.9	57.4	7.6
Yolk weight	"	17.1	17.4	17.9	17.9	16.8	17.0	16.4	16.9	17.6	18.5	18.4	18.3	3.4
Albumen weight	"	31.6	32.2	31.2	31.4	32.3	32.9	31.7	32.9	31.6	31.6	31.1	31.4	4.8
Shell weight	"	6.2 ^a	6.0 ^a	6.3 ^a	6.8 ^b	7.4 ^{bc}	7.1 ^b	7.3 ^{bc}	6.9 ^b	7.2 ^b	6.8 ^b	7.1 ^b	7.8 ^c	1.8
Shell thickness	mm	0.34	0.35	0.36	0.35	0.36	0.38	0.43	0.42	0.40	0.37	0.41	0.40	0.21
Yolk	% EW	31.2	31.3	32.4	31.9	29.7	29.9	29.7	29.9	31.1	32.8	32.6	31.9	3.0
Albumen	"	57.6	57.9	56.2	55.9	57.1	57.7	57.2	57.8	56.1	56.1	54.9	55.1	6.1
Shell	"	11.2 ^a	10.8 ^a	11.3 ^a	12.2 ^{ab}	13.2 ^b	12.5 ^b	13.1 ^b	12.3 ^{ab}	12.7 ^{ab}	12.1 ^{ab}	12.5 ^{ab}	12.9 ^{ab}	2.0
Haugh unit		86.7 ^A	87.4 ^A	87.9 ^A	85.7 ^A	86.0 ^A	86.1 ^A	88.6 ^A	90.6 ^A	86.9 ^A	88.4 ^A	93.1 ^A	101.1 ^B	15.0
Yolk colour	Roche scale	9.9 ^B	9.7 ^B	9.8 ^B	9.5 ^B	10.0 ^B	10.2 ^B	7.4 ^A	11.3 ^B	10.5 ^B	11.4 ^B	9.9 ^B	13.1 ^C	1.5
Carotenoids	$\mu\text{g g}^{-1}$ yolk	7.6 ^A	7.6 ^A	7.6 ^A	7.4 ^A	6.8 ^A	7.7 ^A	5.8 ^A	7.7 ^A	14.4 ^B	17.7 ^C	11.5 ^B	19.5 ^D	0.8
Polyphenols	mg g^{-1} yolk	0.15 ^A	0.21 ^A	0.09 ^A	0.11 ^A	0.14 ^A	0.20 ^A	0.10 ^A	0.12 ^A	0.52 ^B	0.56 ^B	0.23 ^A	0.47 ^B	0.05
α -tocopherol	$\mu\text{g g}^{-1}$ yolk	90.0 ^{BC}	102.0 ^C	94.5 ^{BC}	100.0 ^C	54.0 ^A	51.3 ^A	50.5 ^A	59.0 ^A	81.7 ^B	116.1 ^C	71.3 ^B	98.2 ^C	19.9

N=135 per group/season; A,B:P<0.001; a,b: P<0.05.

Control group had stable Roche colour and carotenoid contents of yolk in agreement with Rossi (2007) who showed that caged hens produced eggs that had darker coloured yolks than those of organic eggs; and she attributed this difference to the presence of synthetic colourants in the conventional feed.

The apparent discrepancy between carotenoid contents and colour could be explained by the different carotenoids profile (data not shown).

According to the carotenoid level, the concentration of polyphenols in the yolk was the highest in the OP group ($P < 0.01$), except that in summer as observed in the grass pasture.

The α -tocopherol content of the OP eggs was similar to that of the C group, and was higher than that of the O eggs in all season, due to the large intake of grass, which is rich in α -tocopherol (Lopez-Bote *et al.*, 1998). The C group values were correlated with dietary supplementation of D-L- α -tocopheryl acetate (Gebert *et al.*, 1998; Meluzzi *et al.*, 2000; Franchini *et al.*, 2002); hens have the capacity to transfer α -tocopherol from feed to the egg which has a strong antioxidant effect (Cherian *et al.*, 1996a, 1996b; Galobart *et al.*, 2001a, 2001b).

Conclusions

Raising hens according to a standard organic production system has a slight effect on the production and qualitative characteristics of the eggs also using "low productive"

genotype. Only organic-plus hens produced eggs that were markedly different from the conventional ones; this difference was due to the availability of green pasture throughout the year. The eggs from these hens had higher carotenoid, polyphenol and tocopherol contents which are bioactive compounds that have a relevant effect on human health. The colour of the yolk showed that the Roche value suggested for the European market (Nardone and Valfrè, 1999) can be attained without adding any supplemental colouring agent only if grass is available for the hens.

These findings highlight the importance of having more grassed pasture land available to the hens than is currently recommended by the Regulations, in Mediterranean areas, for the hens raised on the organic system.

The reduction of the number of eggs produced by the organic-plus group was associated with an increase in the egg characteristics. The egg production could also be managed by increasing protein and energy contents in the diet of the hens.

Further research on pasture composition and pasture rotation in the different seasons is needed to define precise protocols for producing more homogeneous organic-plus eggs.

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