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Abstract

BACKGROUND: The aim of this study was to determine the effect of husbandry system and season on the grass intake and egg quality in laying hens considering that characteristics of organic eggs obtained in a system with no grass in the external area are similar conventional ones.

RESULTS: Three hundred and sixty Ancona hens were randomly assigned to three groups: a control group (C), with the hens kept in cages under standard housing conditions; the organic group (O), with the hens kept under an organic production system (4 m^2 per hen) and the organic-plus group (OP), with the hens maintained under organic conditions but with a larger grass paddock availability (10 m^2 per hen). The estimated intake of forage, carotenoids and flavonoids of the OP hens was very high as the amounts deposited in eggs, mainly in the spring season. The fatty acid composition of the yolk was significantly affected by pasture. The OP eggs showed lower concentrations of polyunsaturated fatty acids (PUFA) *n*-6 and a higher percentage of PUFA *n*-3.

CONCLUSIONS: The results showed that the grass intake was largely affected by the husbandry system and highlighted the seasonal effect of grass availability on the nutritional quality of eggs produced in organic systems.

Keywords: hens; grass intake; season; egg quality; organic production

INTRODUCTION

It is widely known that the welfare of the animal and the characteristics of the products largely depend on the adaptation of the genetic strain to extensive farming systems (foraging behaviour and resistance to environmental stresses). Indeed, the characteristics of organic eggs obtained in a system with no grass in the external area are rather similar to those produced in a conventional system,[1–5] whereas some egg characteristics are affected when laying hens have sufficient grass in their paddock.[2,6–8] Lopez-Bote *et al*.[6] showed that the egg yolk of Leghorn hens kept in a free-range system had increases in ***α***-tocopherol and ***α***-linolenic acid levels. However, there are many difficulties in determining forage intake, which itself has an unknown nutritive value; therefore, very little is known about the ability of layers to take advantage of forage to cover their nutritional needs.

In organic production systems, the potential contribution of vegetation, earthworms, insects and other food items from the outdoor area have been overlooked, presumably because the current production systems do not emphasise the utilisation of outdoor areas by poultry.[9] However, grass could be considered a dietary source of energy, protein and vitamins and can also reduce the feed consumption to different degrees (5 – 10%,[8] 30%[10]).

Gustafson and Antell[11] indicate that hens foraging on oilseed, sunflower and wheat cropping systems are capable of supporting their entire nutritional needs. This observation is supported by studies on the crop content, indicating that free-range hens have a considerable intake of herbage and other accessible food items.[12 –14]

It has been reported that the most relevant role of grass in organic poultry is represented by the intake of several bioactive compounds (i.e. polyunsaturated fatty acids, vitamins and pigments[15]) that have a direct effect on the quality of the meat and eggs yet cannot be added in synthetic forms to organic diets. For these reasons, it is important to determine the intake and nutritional relevance of pasture to develop rations for free-range birds and to investigate the ability to transfer the above-mentioned compounds into the poultry products.

Considering that the effects of grass ingestion and seasonal variations on the qualitative characteristics of free-range poultry remain largely unknown, the aim of the present work is to analyse the effect of pasture availability in different seasons on the egg characteristics of laying hens.

MATERIAL AND METHODS

Experimental design

One hundred and twenty-six Ancona hens were randomly assigned to one of the following conditions:

- *Control (C) group*: The hens were maintained at the experimental farm of Department of Applied Biology, Animal Science section (University of Perugia) and kept in cages under standard housing conditions (single-bird cages, 0.75 m^2) of three-tier batteries that were provided with a linear automatic feeder and drinker; an artificial photoperiod of 16 h per day of light was applied at 17 weeks of age. The building was under a controlled ventilation regime (10 m^3 hen^{-1} h^{-1}): the temperature ranged from 15 to 29° C (mean/average daily temperatures, extremes of 13 – 17° C in winter and 23 – 35° C in summer), and the relative humidity ranged from 50 to 80%.
- *Organic (O) group*: The hens were kept in four (one per season) adjacent covered, straw-bedded houses (6 hens m^{-2}) with access to four separate pens (one per season) with natural pasture; each pens was divided into three sub-pens (4 m^2 per hen; 12 replications).
- *Organic-plus (OP) group*: The hens were kept in four identical (one per season) adjacent covered, straw-bedded houses (6 hens m^{-2}) with access to four separate pens (one per season) with natural pasture; each pen was divided into three sub-pens (10 m^2 per hen; 12 replications).

Feed and water were provided indoors with manual bell feeders and automatic drinkers, respectively. Perches were also provided inside the paddocks. The environmental temperature, humidity and photoperiod were natural. The pasture lands were not treated with pesticides or herbicides for 3 years prior to the onset of organic production. The pasture area contained mature trees, bushes and hedges. The same organic feed, obtained from organic crops, was provided *ad libitum* (Table 1) to all the animals.

Pasture composition and grass intake

The floristic and chemical composition in each pasture pen was estimated by sampling a 1-m^2 fenced area by cutting with garden scissors (at 2 cm above the soil) before the onset of

the trial. Samples of fresh grass (three pens × four seasons per group) were collected throughout the period of egg collection.

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

The grass intake was estimated according to the method of Lantinga *et al*.[16] using a metallic frame (0.50 m × 0.50 m) per period with a fixed cutting height of 4 cm. The herbage samples were collected before and after the outdoor period in each of the pens. The first batch of samples was collected the day before the hens were transferred to the outdoor fields at the beginning of every season, and the second batch was collected shortly after the hens were moved to the next pen (in the next season).

The grass intake (GI) was estimated using the following equation:

$$GI = (GM_s - GM_e) + \left[1 - (GM_e/GM_s) \right] / -\ln (GM_e/GM_s) \times (GM_u - GM_s)$$

where GM_s was the herbage mass present at the introduction of the hens in each pen; GM_e was the grass that remained at the end of the sub-period, and GM_u was the undisturbed mass of grass from nearby ungrazed areas inside every sub-pen.

Productive performance

Data for calculating the deposition rate and feed intake were recorded per pen or cage/group throughout the productive cycle by the farm workers. Feed consumption per pen/cage was recorded weekly.

Egg sampling

Eggs were collected for analyses during the four seasons in each of the different phases of productive activity. Forty eggs per group were gathered (at 07.50 h) on three consecutive Tuesdays in winter, spring, summer and autumn. All the eggs (120 per group/season) were stored at 5° C until the analyses (maximum 2 days after) were performed in the laboratory of the Department of Applied Biology.

Analytical determinations

The chemical composition of the feed was determined according to AOAC (1995). The fatty acid profile of the feed, pasture and yolk was determined by gas chromatography after lipid extraction according to Folch *et al*.[17] In particular, 1 mL of lipid extract was evaporated under a stream of nitrogen, and the residue was derivatised by adding 3 mL of sulfuric acid (3% in methanol). After incubating at 80° C for 1 h, the methyl esters were extracted with petroleum ether, and 1 mL was injected into the gas chromatograph (Mega 2 - model HRGC; Carlo Erba, Milan, Italy), which was equipped with a flame ionisation detector. The separation of the fatty acid methyl esters (FAMEs) was performed using an Agilent (J&W) capillary column (30 m × 0.25 mm I.D; CPS Analitica, Milan, Italy) coated with a DB-wax stationary phase (film thickness of 0.25 mm). The operating conditions upon column

injection of the 1 mL sample volume were as follows: the temperatures of the injector and detector were set at 270° C and 280° C, respectively, and the detector gas flows were H_2 at 50 mL min^{-1} and air at 100 mL min^{-1}. The oven temperature was programmed to provide a good peak separation, as follows: the initial oven temperature was set at 130° C; this temperature increased at a rate of 4.0° C min^{-1} to 180° C and was held for 5 min; the temperature was then increased at a rate of 5.0° C min^{-1} to 230° C; and the oven was held at the final temperature for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.1 mL min^{-1}. Individual fatty acid methyl esters were identified by reference to the retention time of FAME authentic standards. The relative proportion of each fatty acid in the fatty acid pattern of the egg yolks was expressed as a percentage.

The mean value of each fatty acid was used to calculate the sum of the saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and long-chain polyunsaturated fatty acids (LCPs; ≥ 20C) of different series (*n*-3 and *n*-6).

The peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai:[18]

$$PI = (\%\ \text{monoenoic} \times 0.025) + (\%\ \text{dienoic} \times 1) + (\%\ \text{trienoic} \times 2) + (\%\ \text{tetraenoic} \times 4) + (\%\ \text{pentaenoic} \times 6) + (\%\ \text{hexaenoic} \times 8)$$

The amount of each fatty acid was used to calculate the indexes of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate,[19] and the hypocholesterolaemic/hypercholesterolaemic ratio (HH), as suggested by Santos-Silva *et al*.:[20]

$$AI = \frac{(C12:0 + 4 \times C14:0 + C16:0)}{MUFA + (n-6) + (n-3)}$$

$$TI = \frac{(C14:0 + C16:0 + C18:0)}{(0.5 \times MUFA + 0.5 \times (n-6) + 3 \times (n-3) + (n-3)/(n-6)}$$

$$HH = [(C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)/(C14:0 + C16:0)$$

The ***α***-tocopherol level was assessed according to Hewavitha-rana *et al*.[21] using a high-performance liquid chromatogrphy (HPLC) method (pump model PU-1580, equipped with an autosam-pler system, model AS 950 – 10; Jasco, Tokyo, Japan) and an Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 □m particle size; CPS Analitica). The tocopherols were identified using a fluorescence detector (Jasco model FP-1520) set at excitation and emission wavelengths of 295 nm and 328 nm, respectively, and were

quantified using external calibration curves prepared with increasing amounts of pure tocopherols in ethanol.

The extraction of individual carotenoids was performed using the aerial portions of the different plant species of the pasture and in the feed and yolk.[22] Acetone extracts of the plants and feed were filtered through Millipore filters (0.2 mm). The carotenoid contents were quantitatively determined by HPLC (Jasco, pump model PU-1580, equipped with an autosampler system, model AS 950 – 10, Tokyo, Japan) and a Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 □m particle size; CPS Analitica). The solvent system consisted of solution A (methanol – water – acetonitrile, 10:20:70) and solution B (methanol – ethyl acetate, 70:30). The flow rate was 1 mL min^{-1}, 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 10 min. The detector was a UV – visible spectrophotometer (Jasco UV2075 Plus) set at a wavelength of 450 nm. The different carotenoids were identified and quantified by comparing the sample with pure commercial standards (Sigma-Aldrich, Steinheim, Germany; and Extrasynthese, Genay, France).

The total polyphenols were quantified using the colorimetric method described by Singleton and Rossi[23] after methanol extraction. An 800 mL aliquot of Millipore water was added to 200 mL of the methanol extract, and 10 mL of 0.2 mol L^{-1} Folin-Ciocalteu reagent (Sigma) was added after saturation with 8 mL sodium carbonate (75 g L^{-1}). After 2 h, the solution was analysed spectrophotometrically (UV-2550; Shimadzu Corporation, Kyoto, Japan) at 765 nm by a comparison with standard curves for different levels of gallic acid (50, 100, 200, 300 and 500 mg L^{-1}).

The chlorophyll content was determined according to an AOAC[24] procedure, with the modification of Della Torre *et al*.[25]

Statistical analysis

A linear model[26] was used to assess the effect of the husbandry system during the different seasons. The significance of the differences was assessed by a multiple *t*-test. All the statements of significance are based on a probability ≤ 0.05.

RESULTS AND DISCUSSION

Feed and grass intake

With regard to the floristic composition of the pasture pens in the different seasons, the plant species were numerous, and their physiological stages were different, varying according to the season. The main floristic species found in the pastures during the different seasons were *Lolium perenne*, *Lotus corniculatus*, *Sorghum halepense* and *Trifolium pratense* representing about 72% of the pasture floristic composition (data not shown).

The fatty acid profiles of the feed and grass (Table 2) showed significant differences ($P \leq 0.05$). The feed had a higher *n*-6 content due to the inclusion of soybean, sunflower and corn (rich in linoleic acid, C18:2*n*-6[27]), whereas the pasture showed a greater ($P \leq 0.05$) content of *n*-3 (particularly **α**-linolenic acid, C18:3*n*-3). This fatty acid profile resulted in a higher *n*-6/*n*-3 ratio in the feed than the pasture, which, in turn, showed a lower value in spring (0.2). These findings agree with those of Ponte,[28] who reported that the fatty acid composition of green forages was characterised by a high percentage of PUFAs, mainly

represented by ***α***-linolenic acid (60 – 75%) and linoleic acid (10 – 20%), with an *n*-6/*n*-3 ratio of approximately 0.20.

The fatty acid profile of grass varies with different factors, with the vegetative status being the most important. According to Morand-Fehr and Tran,[15] plant mixtures show higher contents of ***α***-linolenic acid and lower SFAs in spring, with respect to the same plants in a more advanced vegetative state.

The grass sampled had different ($P \leq 0.05$) carotenoid, ***α***-tocopherols, chlorophyll, and flavonoid contents due to the different vegetative stages in the four seasons, and this amount was always superior to the values in the feed (Table 3).

In the grass, violaxanthine was the most represented carotenoid, followed by ***β***-carotene and lutein, whereas zeaxanthin and lutein represented approximately 90% of the carotenoids in the control feed.

In our previous study,[2,7] we observed that grass had low dry matter values and high amounts of fibre, tocopherol, carotenoids and flavonoids compared to a control feed, and the intake grass provided a notable amount of bioactive substances, particularly in spring. According to Lopez-Bote *et al*.,[6] there was approximately six times more ***α***-tocopherol and two to eight times more carotenoids in grass than in commercial feed.[29] Furthermore, this source of antioxidants from grass is crucial for organic chickens because the use of synthetic vitamins in feed is banned.[30]

Walker and Gordon[8] observed that the nutritive value of grass was affected by the season and growth stage; in spring, grass had high sugar and protein contents and a relatively low fibre content, whereas the sugar and protein contents decline in summer prior to resurgence in early autumn.

The data reported in Table 4 confirmed that the presence of grass was affected by the animal density and season. The OP pens always showed higher quantities of grass, mainly in spring (1.64 kg FM m^{-2}); in contrast, the O pens had lower quantities of grass throughout the year, with the lowest values recorded during the summer period (0.06 kg DM m^{-2} FM m^{-2}; where DM is dry matter and FM is fresh matter). Based on these data, the estimated intake of carotenoids, flavonoids, ***α***-tocopherol and C18:3*n*-3 increased according to the pasture area per bird.

The estimated grass intake varied ($P \leq 0.05$) according to the bird density, confirming the grazing attitude of Ancona hens. In particular, the OP hens reached a maximum level of intake in spring (59.2 g FM day^{-1}). Some authors[31] have confirmed the importance of establishing the amount of ingested herbage and the nutritional value of forage to develop rations for free-range chickens or hens and to better understand its effects on product quality.

Hughes and Dun,[32] in systems in which grass was available, estimated a high consumption of approximately 30 – 40 g DM grass/layer per day, in addition to more than 100 g of standard feed. Bassler[33] found that a 15% reduction in the concentrate fed to layers had no detrimental effect on egg production, whereas individual herbage consumption was as high as 30 g DM per day. Ponte *et al*.[28,34] observed that grass represents between 2.5 and 4.5%, on a DM basis, of the total feed intake in grazing birds. Our results indicated a grass intake that was intermediate between the above evaluations.

Egg quality

The egg deposition rate in the organic groups (O and OP) was more dependent ($P \leq 0.05$) on the season, with a value lower than 50% in autumn. In contrast, the egg weight was not affected ($P \geq 0.05$) by the husbandry system or season (Table 5).

Regarding bioactive compounds, the OP eggs showed amounts of carotenoids and tocopherols in the yolk that were remarkably high, resemble values for the eggs of wild animal species.[35] The contents of total carotenoids (lutein accounted for 81 – 85% of the total content) and flavonoids reflected the trend registered in the grass samples during the four seasons. Indeed, the higher ***α***-tocopherol, carotenoid and flavonoid contents in egg yolks were observed in the OP hens during spring and autumn.

These results confirm the high capacity of hens to transfer ***α***-tocopherol[36,37] and other bioactive compounds from pasture/feed to their eggs.[6]

The total concentration of flavonoids in egg yolk was the highest ($P \leq 0.05$) in the OP group, independently of the season, whereas the concentration in the albumen was less dependent on the husbandry system and season.

In addition to affecting the yolk colour,[7] carotenoids, tocopherols and flavonoids play a key role as antioxidants in the healthy development of chick embryos,[38 –40] assuring a stronger immune response,[41,42] and result in a higher egg lipid stability.[39]

Even chlorophyll and its metabolite (chlorophyllin) are known to have antioxidant activity. Studies in animals have shown that
chlorophyllin exhibits an antioxidant activity that was at least as good as that of vitamin C. The *in vivo* and *in vitro* roles of chlorophyllin were related to the inhibition of lipid peroxidation and mitochondrial protection from the oxidative damage induced by free radicals and other reactive oxygen species.[43]

At the peak egg production, overlapping with springtime, the paddock of the hens kept under the standard organic system (4 m^2 hen^{-1}) was almost devoid of grass. Therefore, only the hens with the greater space allowance had a sufficient amount of grass, which had a relevant effect on the qualitative traits of the eggs.

Fatty acid profile of yolk

The fatty acid composition of the yolk was significantly affected ($P \leq 0.05$) by the pasture availability (Table 6). The eggs from hens of the OP group showed a lower concentration of PUFA *n*-6 (17.32% vs. 21.85% control) due to less C18:2 *n*-6 (12.86% vs. 17.92%) and a higher percentage of PUFA *n*-3 (3.64% vs. 2.46%) due mainly to a higher percentage of C18:3, C21:5 and C22:6 *n*-3. Therefore, the *n*-6/*n*-3 PUFA ratio was lower (4.75 vs. 8.88).

The total SFA value was not affected ($P \geq 0.05$) because the increased amount of C16:0 was compensated for by the decrease in C18:0.

The different levels of PUFA *n*-3 and *n*-6 precursors determined the modifications of their specific derivatives: a high level of linoleic acid resulted in an increase in arachidonic acid (C and O groups). In the OP group, the higher presence of ***α***-linolenic acid (ALA) favoured the synthesis of DPA, and DHA; in spring, 100 g of OP egg yolk had 3.3 g of PUFA, with 446 mg of DHA. The *n*-3 profile confirmed that DHA was the major LCP *n*-3 and that egg is one of the most important terrestrial feed sources of DHA for human. Indeed, DHA in standard or enriched meat of several animal species (pork, chicken, rabbit) is poorly represented whereas EPA is found much more.

Increases in the DHA value were reported to be higher in eggs produced from hens fed diets with 5% extruded linseed[44] or *Sativa camelina* (a plant rich in linolenic acid).[45]

Moreover, these results confirmed the efficiency of the hens' enzymatic capability[7,46,47] to elongate and desaturate LNA into *n*-3 LCP and to transfer these fatty acids to their eggs where they play a crucial role during chick development.[46] Simopoulos and Salem[4] also observed high levels of *n*-3 LCP and, consequently, a lower *n*-6/*n*-3 ratio in eggs produced by free-range hens in comparison to standard eggs.

The dietary strategies for increasing the nutritional properties of animal products[48] mainly consist of enriching diets with ingredients rich in LNA (e.g. linseed, colza[49 –51]) or directly with LCP (e.g. fish oils, algae, water plants[52 –54]). The supplementation of LNA produces less dramatic LCP increases, the levels of which are also affected by the *n*-6/*n*-3 ratio of the diet and hen metabolism; however, such a strategy is physiological and dependent on the oxidative status of the animals and products.[53] Indeed, high levels of PUFA, if not adequately protected, can transform the dietetic advantages of an LCP enrichment into risks upon the formation of unstable and extremely injurious by-products.[55]

The atherogenic and thrombogenic indexes in all the groups were optimal under a nutritional point of view (Table 7) and were consistently lower that the results of Gonzalez´-Munoz˜ *et al*.[56] in layers fed different dietary fats. Because the atherogenic and thrombogenic ratios are considered markers of fat quality,[57] the changes observed in these indexes suggested a better fat quality for the eggs produced by the organic hens.

The high level of egg fat unsaturation resulted in a decline in the peroxidability index, particularly in spring. These results agree with those of Pike and Peng[58] with regard to the oxidability of fresh eggs.

The Index of Nutritional Quality (INQ), which considers the amount (mg 100 g^{-1}) of EPA and DHA with respect to the total energy (kcal 100 g^{-1}) of the product, was consistently (exception in winter) higher ($P \leq 0.05$) in the OP eggs. In spring, the INQ values of these eggs reached levels approximately four times higher than those of the O eggs in the same season. The HH index, calculated as the ratio between hypo- and hypercholesterolaemic fatty acids, was similar in all the eggs confirming the optimal nutritional value of the eggs. Contrary to past reports, recent epidemiological studies have indicated that eggs are not a predisposing factor for the risk of cardiovascular pathologies.[54,59,60]

Dietary recommendations[61] have underlined the necessity for humans to consume products with an *n*-6/*n*-3 ratio equal to or less than 6, though Simopoulos[62] indicated 4:1 the as optimal ratio. The increase in *n*-6 fatty acids observed in modern human diets can represent a risk factor for tumours, diabetes and cardiovascular disease. Interestingly, the OP eggs consistently showed an *n*-6/*n*-3 value smaller than 4, and the eggs produced in spring showed values equal to 1.9.

Regarding the linoleic/linolenic ratio, this parameter is very important because it affects the efficacy of desatura-tion/elongation. When hens are fed diets rich in linolenic and/or *n*-3 fatty acids, the conversion of linoleic acid to arachidonic acid drastically decreases,[51,63] with linoleic acid favouring platelet aggregation and immuno-suppression.[64]

The content of DHA per 100 kcal of egg was consistently higher than the ideal contribution of the food values recommended by LSRO,[65] which are equal to 8 mg 100 $kcal^{-1}$. The OP eggs showed higher ($P \leq 0.05$) values in spring, summer and autumn.

In spring, the OP eggs exhibited a value of 325 mg 100 g^{-1} egg of *n*-3 PUFAs, which was higher than the EFSA[66] recommendations for enriched eggs (300 mg 100 g^{-1} egg).

Many factors affect the cholesterol content of eggs. In our study, the cholesterol level showed a significant effect for the husbandry system. Some authors[67,68] found higher levels of cholesterol in eggs produced from hens kept under free-range conditions.

According to Minelli *et al*.,[69] the cholesterol level in organic versus conventional eggs was higher, evidencing that the cholesterol in yolk was negatively correlated with the productivity of the hens.[70] Our TBARS results showed that the organic eggs had higher TBARS values with respect to the control group, with the exception of the beginning of deposition time period. Sirri *et al*.[71] observed a similar trend and attributed this phenomenon to the absence of synthetic vitamins in organic feeds and to the higher unsaturation level of organic eggs (see the peroxidability index). Based on their different fatty acid profiles, the low TBARS value of the C group eggs was attributable to a higher amount of dietary synthetic vitamin E and to the lower oxidative metabolism of hens kept in cages in which motor activity was very limited.

It is important to emphasise that, even if the TBARS values of the OP eggs were significantly higher than the control, the *n*-3 amount in these eggs remained higher, most likely due to the greater antioxidant content in these eggs. In agreement with this state-ment, Lai *et al*.[72] observed a reduction of oxidative processes of the cholesterol in egg lipids enriched with vitamin E and carotenoids, suggesting a synergic action between these molecules.[73]

CONCLUSIONS

Raising hens according to an organic production system modified the qualitative characteristics and nutritional quality (tocopherol, carotenoids, *n*-3 content) of the eggs produced, mainly when the pasture is widely available.

These findings highlight the relevance of grass pasture for organically kept hens with regard to egg quality. The improvements in egg characteristics could counter-balance the lower deposition rate, which could also be improved by modifying the diet. Higher ***α***-tocopherol, carotenoid and flavonoid contents in the egg yolks were observed in the OP hens during spring and autumn. All the bioactive compounds of 'natural' functional food obtained without any forced dietary strategy.

Moreover, rearing an autochthonous genotype under organic conditions, with wide pasture availability, matches the expectations of consumers regarding the higher quality of organic eggs and the maintenance of biodiversity. Further research on pasture composition and turned pasture is necessary to define suitable protocols for producing organic eggs.

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Table 1. Mean ingredients (g kg^{-1} of diets) and nutrient composition (g kg^{-1}) of diet used in conventional, organic and organic-plus systems

Ingredients and nutrient composition	Concentration (g kg^{-1})
Ingredient	
Maize	450
Extruded soybean flakes	200
Maize gluten feed	160
Sunflower meal	88
Alfalfa meal	30
Vitamin mineral premix*	10
Calcium carbonate	50
Dicalcium phosphate	5
Sodium bicarbonate	5
Salt	2
Nutrient composition	
Crude protein	178
Ether extract	53
Crude fibre	56
Ash	111
Metabolisable energy (MJ kg^{-1})	11.8

*Vitamins in the organic diet were provided by cod liver oil and malt yeast.

Table 2. Fatty acid profile of feed and grass in different season (g kg^{-1} feed or grass)

		Grass			
Fatty acid	Feed	Winter	Spring	Summer	Autumn
C16:0	6.01	4.26	2.99	3.62	4.46
C18:0	3.34	0.57	0.40	0.49	0.60
Others	0.47	0.23	0.16	0.19	0.24
∑ SFA	9.83	5.06	3.55	4.31	5.30
C16:1*n*-7	0.17	0.11	0.06	0.08	0.10
C18:1*n*-9	11.1	2.59	1.81	2.20	2.71
Others	0.62	0.24	0.17	0.21	0.25
∑ MUFA	11.9	2.94	2.06	2.49	3.07
C18:2*n*-6	13.4	3.65	2.56	3.11	3.82
Others	0.15	0.05	0.03	0.04	0.05
∑ *n*-6	13.6	3.70	2.60	3.15	3.87
C18:3*n*-3	1.45	11.3	13.4	9.64	11.9
Others	0.22	0.02	0.05	0.02	0.02
∑ *n*-3	1.67	11.36	13.48	9.66	11.9
∑ PUFA	15.3	15.0	16.0	12.8	15.0
n-6/*n*-3	0.08	0.03	0.02	0.03	0.03

Each value represents the mean value of three determinations.

Table 3. Carotenoids (mg kg^{-1} DM), α-tocopherol (mg kg^{-1} DM), chlorophyll (mg kg^{-1} DM) and flavonoids (mg kg^{-1} DM) amounts in feed and grass during different seasons

Compound	Feed	Winter	Spring	Summer	Autumn	SEM
Violaxanthin	0.40	27.4	34.0	10.3	9.75	2.01
Lutein	3.73	18.2	13.4	5.10	4.50	1.11
β-Carotene	0.53	13.6	23.6	11.9	1.45	2.35
Neoxanthin	0.06	5.56	3.62	ND	18.7	0.58
Zeaxanthin	6.02	1.40	1.65	0.72	0.62	0.25
Anteroxanthin	ND	1.48	1.19	0.68	0.36	0.14
Cryptoxanthin	0.17	1.49	1.12	ND	0.06	0.19
Total carotenoids	10.9	69.1	78.6	28.79	35.4	3.45
α-Tocopherol	35.5	130.6	229.9	175.2	149.1	15.1
Chlorophyll	ND	15.2	45.0	19.5	12.1	2.28
Flavonoids	170.1	440.1	575.1	360.8	597.6	48.4

Each value represents the mean value of three determinations.
DM, dry matter; ND, not detected.

Table 4. Estimated pasture availability of every season and estimated daily intake of bioactive compounds

	Organic				Organic-plus				
Availability and intake	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	SEM
Availability									
Grass (kg FM m^{-2})	0.12	0.17^{a}	0.06^{a}	0.13^{a}	1.07^{c}	1.64^{d}	0.55^{b}	1.01^{c}	0.11
Moisture (%)	74.2	76.5	64.4	70.1	74.8	76.2	63.9	71.2	3.2
Carotenoids (mg m^{-2})	2.21^{a}	3.42^{a}	0.54^{a}	1.33^{a}	18.7^{c}	24.0^{d}	5.30^{a}	10.4^{b}	2.1
α-Tocopherol (mg m^{-2})	4.10^{a}	8.73^{a}	3.14^{a}	6.72^{a}	35.4^{b}	69.7^{d}	34.4^{b}	43.0^{c}	3.5
C18:3*n*-3 (mg m^{-2})	350.1^{ab}	519.0^{b}	171.7^{a}	532.4^{ab}	3041.1^{cd}	4157.6^{d}	1922.6^{c}	3407.2^{cd}	102.4
Flavonoids (mg m^{-2})	13.9°	22.1^{a}	6.3^{a}	26.6^{a}	120.3^{c}	176.8^{d}	70.7^{b}	169.6^{d}	5.7
Intake									
Grass (g FM hen^{-1} day^{-1})	13.2^{ab}	17.8^{b}	5.7^{a}	13.8^{ab}	29.1^{c}	59.2^{d}	14.3^{ab}	32.1^{c}	2.9
Carotenoids (µg hen^{-1} day^{-1})	235.5^{b}	300.9^{b}	58.0^{a}	146.4^{ab}	519.1^{c}	1001.4	146.1^{ab}	329.4^{cb}	25.6
α-Tocopherol (µg hen^{-1} day^{-1})	444.7^{a}	880.6^{b}	355.6^{a}	614.1^{ab}	980.3^{bc}	2927.9^{d}	891.1^{b}	1385.8^{c}	87.5
C18:3*n*-3 (mg hen^{-1} day^{-1})	38.5^{b}	51.0^{c}	19.6^{a}	48.5^{b}	85.0^{cd}	170.9^{d}	49.0^{c}	110.4^{d}	25.7
Flavonoids (mg hen^{-1} day^{-1})	1498.7^{b}	2200.3^{c}	730.4^{a}	2463.6^{c}	3303.3^{c}	7318.9^{d}	1832.7^{b}	5551.4^{cd}	142.5

a..d: $P < 0.05$.

Table 5. Rate of deposition, feed intake and weight, and bioactive compounds of eggs

	Control				Organic				Organic-plus				
Parameter	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	SEM
Deposition rate (%)	84.6	81.9	65.3	64.7	71.9	79.6	55.2	47.8	72.0	80.0	52.2	43.8	4.30
Feed intake (g day^{-1})	110.1^{b}	113.0^{b}	100.6^{a}	115.3^{b}	112.0^{b}	111.2^{b}	102.3^{a}	116.0^{b}	103.5^{a}	101.2^{a}	95.8^{a}	102.85^{a}	24.5
Egg weight (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
Carotenoids (mg kg^{-1} yolk)	7.02^{a}	7.61^{a}	6.85^{a}	7.38^{a}	7.85^{a}	8.73^{a}	5.95^{a}	7.81^{a}	15.10^{ab}	17.88^{bc}	11.07^{ab}	19.86^{c}	0.87
Lutein (mg kg^{-1} yolk)	5.56^{a}	6.01^{a}	5.62^{a}	5.55^{a}	5.41^{a}	6.20^{a}	4.41^{a}	6.09^{a}	12.45^{b}	15.24^{c}	10.35^{b}	17.45^{c}	0.85
Zeaxanthin (mg kg^{-1} yolk)	0.91^{a}	0.95^{a}	0.82^{a}	1.09^{ab}	0.90^{a}	0.91^{a}	0.85^{a}	0.99^{a}	1.38^{b}	1.75^{c}	1.09^{ab}	1.24^{b}	0.21
Antheroxanthin (mg kg^{-1} yolk)	0.27	0.36	0.21	0.39	0.29	0.30	0.25	0.32	0.27	0.36	0.21	0.39	0.05
Cryptoxanthin (mg kg^{-1} yolk)	0.28	0.29	0.20	0.35	0.25	0.32	0.28	0.32	0.30	0.32	0.22	0.38	0.07
α-Tocopherol (mg kg^{-1} yolk)	50.5^{a}	52.2^{a}	51.2^{a}	51.4^{a}	52.4^{a}	55.1^{a}	51.4^{a}	51.8^{a}	54.0^{a}	96.4^{c}	61.3^{b}	82.0^{bc}	4.43
Flavonoids (g kg^{-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^{ab}	0.47^{b}	0.05

$N = 8$ pool of 5 yolk per group/season.
a..c: $P < 0.05$.

Table 6. Fatty acid composition of egg yolk (g kg^{-1} yolk)

	Control				Organic				Organic-plus				
Fatty acid	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	SEM
C14:0	1.03	0.98	1.12	1.14	0.90	0.89	1.01	0.92	0.89	0.95	0.96	0.81	0.25
C16:0	54.9	53.0	55.4	53.6	42.7	38.8	42.7	43.5	51.6	47.0	54.0	52.0	1.90
C18:0	28.5	26.1	27.4	28.0	22.0	19.0	17.2	18.5	23.0	18.2	19.6	19.9	1.11
Others	1.87	1.85	1.90	1.75	1.56	1.74	1.56	1.41	1.90	1.68	1.98	2.09	0.52
SFA	85.7^{b}	82.1^{b}	85.0^{b}	85.0^{b}	68.1^{ab}	60.1^{a}	63.5^{a}	62.4^{a}	73.6^{ab}	68.1^{ab}	75.5^{ab}	75.0^{ab}	2.04
C16:1*n*-7	9.22	8.15	9.75	10.6	9.35	11.5	11.9	9.69	7.87	7.25	7.36	7.35	0.59
C18:1*n*-9	92.6^{a}	100.6^{a}	97.0^{a}	94.6^{a}	103.6^{b}	104.7^{b}	101.6^{ab}	104.0^{ab}	111.8^{b}	114.4^{b}	108.1^{b}	111.2^{b}	1.18
Others	0.78	0.65	0.60	0.81	0.94	0.93	0.85	0.80	0.57	0.61	0.54	0.59	0.12
MUFA	105.7^{a}	112.0^{a}	108.1^{a}	108.0^{a}	112.5^{B}	114.9^{b}	115.5^{b}	115.2^{b}	117.9^{b}	125.3^{b}	115.7^{b}	118.0^{b}	3.42
C18:2*n*-6	27.5^{b}	26.1^{b}	25.8^{b}	26.9^{b}	24.1^{b}	27.1^{b}	25.6^{b}	27.0^{b}	20.7^{a}	18.8^{a}	19.0^{a}	19.2^{a}	1.45
C20:4*n*-6	4.14^{b}	5.88^{b}	5.14^{b}	5.15^{b}	4.47^{b}	5.04^{b}	4.74^{b}	4.52^{b}	0.78^{a}	1.10^{a}	0.70^{a}	0.88^{a}	1.14
Others	1.22^{a}	1.14^{a}	1.24^{a}	1.28^{a}	1.24^{a}	1.47^{a}	1.36^{a}	1.41^{a}	1.30^{a}	2.71^{b}	1.47^{a}	1.15^{a}	0.50
C18:3*n*-3	1.25^{a}	1.30^{a}	1.84^{ab}	1.74^{a}	1.50^{a}	1.74^{ab}	1.20^{a}	1.41^{a}	2.48^{b}	3.34^{c}	2.92^{bc}	2.89^{bc}	0.38
C20:5*n*-3	0.11	0.09	0.14	0.12	0.28	0.29	0.25	0.23	0.25	0.31	0.23	0.28	0.15
C22:5*n*-3	0.08	0.09	0.08	0.07	0.12	0.15	0.13	0.14	0.15	0.15	0.13	0.12	0.16
C22:6*n*-3	0.95^{a}	0.87^{a}	1.05^{a}	0.64^{a}	1.10^{a}	1.11^{a}	1.08^{a}	1.09^{a}	1.66^{a}	5.01^{c}	2.70^{b}	3.45^{b}	0.50
Others	0.70	0.75	0.76	0.69	0.85	0.83	0.85	0.88	0.84	1.28	1.11	1.18	0.84
PUFA	38.1^{b}	34.1^{ab}	36.1^{ab}	35.1^{ab}	32.9^{a}	37.5^{b}	35.0^{ab}	35.5^{ab}	28.1^{a}	33.3^{a}	29.9^{a}	30.0^{a}	1.87

$N = 8$ pool of 5 yolk per group/season.
a..c: $P < 0.05$.

Table 7. Nutritional indexes of eggs

Index	Control				Organic				Organic-plus				SEM
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	
Atherogenic index	0.43^{b}	0.39^{b}	0.42^{b}	0.41^{b}	0.31^{a}	0.27^{a}	0.31^{a}	0.31^{a}	0.37^{ab}	0.33^{a}	0.40^{b}	0.37^{ab}	0.34
Trombogenic index	1.10^{b}	0.99^{b}	1.03^{b}	1.04^{b}	0.79^{ab}	0.67^{a}	0.73^{a}	0.74^{a}	0.86^{ab}	0.64^{a}	0.83^{ab}	0.77^{a}	0.62
Peroxidability index	25.55^{b}	27.38^{b}	27.01^{b}	26.14^{b}	26.95^{b}	29.64^{bc}	28.04^{bc}	28.03^{bc}	21.51^{a}	34.62^{c}	24.98^{b}	28.14^{b}	4.15
INQ	1.09^{a}	0.94^{a}	1.11^{a}	0.68^{a}	1.24^{a}	1.34^{a}	1.27^{a}	1.32^{a}	1.43^{a}	4.79^{c}	2.43^{b}	3.37^{bc}	1.97
HH	2.26	2.50	2.32	2.36	3.10	3.53	3.08	3.11	2.62	2.97	2.42	2.60	0.94
$\sum$ *n*-3 (g kg^{-1} egg)	5.02^{a}	5.61^{a}	6.63^{a}	4.97^{a}	6.76^{a}	7.77^{a}	6.22^{a}	7.15^{a}	11.2^{b}	20.7^{c}	12.6^{b}	15.4^{b}	1.87
$\sum$ *n*-6 (g kg^{-1} egg)	57.7^{b}	58.4^{b}	57.0^{b}	58.3^{B}	52.7^{b}	59.0^{b}	57.2^{b}	57.9^{b}	40.42^{a}	39.4^{a}	37.9^{a}	37.0^{a}	2.15
n-6/*n*-3	11.5^{a}	10.4^{c}	8.6^{b}	10.6^{C}	7.8^{b}	7.6^{b}	9.2^{bc}	8.1^{b}	3.6^{a}	1.9^{a}	3.0^{a}	2.4^{a}	1.8
C18:2*n*-6/C18:3*n*-3	21.9^{c}	20.0^{c}	14.0^{b}	15.4^{b}	16.0^{b}	15.6^{b}	21.3^{c}	19.1^{b}	8.3^{a}	5.6^{a}	6.5^{a}	6.6^{a}	4.8
DHA (mg 100 $kcal^{-1}$ egg)	68.3^{a}	63.3^{a}	69.9^{a}	43.5^{a}	76.8^{a}	85.5^{a}	73.8^{a}	80.9^{a}	92.9^{a}	342.2^{d}	162.2^{b}	233.8^{c}	45.5
Cholesterol (g kg^{-1} yolk)	10.3^{a}	10.7^{a}	10.4^{a}	10.8^{a}	10.9^{a}	11.1^{ab}	11.0^{ab}	11.7^{ab}	12.4^{b}	12.9^{b}	11.9^{b}	11.9^{ab}	1.00
TBARS (mg MDA kg^{-1} yolk)	0.20^{a}	0.18^{a}	0.22^{a}	0.20^{a}	0.35^{ab}	0.40^{b}	0.41^{b}	0.37^{b}	0.25^{a}	0.44^{b}	0.38^{b}	0.40^{b}	0.09

$N = 8$ pool of 5 yolk per group/season.
a..d: $P < 0.05$.
HH, hypocholesterolaemic/hypercholesterolaemic ratio; INQ, Index of Nutritional Quality.