PROCESSING, PRODUCTS, AND FOOD SAFETY

Chemical composition of meat and egg yolk of hybrid and Italian breed hens reared using an organic production system

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ABSTRACT A trial was done to study the chemical composition of meat and egg yolk of laying hens belonging to different genotypes reared using an organic production system. Two commercial hybrid hens (Hy-Line Brown and Hy-Line White 36) and 2 Italian dual-purpose breed hens (Ermellinata di Rovigo and Robusta Maculata) were reared from 25 to 44 wk of age. During the experimental period (July to December), the environmental temperature decreased from about 25 to 13°C and the RH increased from 62 to 73%. The photoperiod was 16L:8D. Meat and egg yolks of 44-wk-old hens were analyzed. The Italian breeds showed higher meat production and lower egg production when compared with the hybrids. Genotype affected protein, lipid, and ash content of breast and thigh meat. Hy-Line White and Ermellinata di Rovigo showed the lowest ($P < 0.01$) breast lipid and Hy-Line White had the lowest ($P < 0.01$) thigh lipid. Hy-Line White showed higher ($P < 0.05$) breast and thigh cholesterol. Genotype affected egg yolk cholesterol, which was higher ($P < 0.01$) in Italian breeds than in hybrids. The fatty acid profiles of meat and yolk were significantly affected by genotype. The hybrid and Robusta Maculata meat and egg fatty acid composition may be considered to be more favorable to human health. The results indicate that the ability of laying hens to incorporate fatty acids is genetically dependent and a possible interaction between strain and environmental conditions may exist for the absorption and utilization of dietary components.

Key words: hen, organic egg, organic chicken, meat quality, yolk quality

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INTRODUCTION

One of the aims of an organic production system (European Union, 1999) is to enhance the rearing and diffusion of purebreds or local genotypes; in the last 50 yr, these have been replaced by hybrid birds, which, being subjected to intense genetic improvements to obtain a higher productivity, may also be very sensitive to any environmental stressor agents. Commercial hybrid genotypes present homogeneous body characteristics such as plumage color (white or brown), BW, and egg size in comparison to purebreds. Many purebreds reared on farms until 50 yr ago have become extinct and many of the existing breeds have been numerically reduced. Some of the purebred birds are dual-purpose and have production performance parameters lower than those of hybrid chickens and hens (Castellini et al., 2002a; Rizzi et al., 2002). They should, however, be more resistant to pathologies and environmental stressors. These characteristics are very important in an organic production system, which requires outdoor rearing and prohibits the use of allopathic drugs (European Union, 1999). As far as Italian breeds are concerned, some dual-purpose breeds still exist, but indications of their production performance and product quality are scarce (Castellini et al., 2002a; Rizzi et al., 2007).

An increasing consumer interest is being focused on the well-being of animals and on their rearing conditions in addition to the quality of products such as lower lipid content or other factors that may represent risks for human health. The fatty acid composition of lipid from the different body tissues is affected both by hepatic lipogenesis and the dietary chemical composition. In addition to diet, other factors such as age and genotype may influence the lipid metabolism of laying hens (Cherian et al., 1995; Ayerza and Coates, 2000; Bean and Leeson, 2003).

The goal of this study was to compare the productive performance and the chemical composition of the meat and eggs produced by hens belonging to 2 hybrid commercial strains to those of 2 Italian breeds reared under the same organic production system.

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MATERIALS AND METHODS

Birds and Environmental Conditions

Laying hens belonging to 2 hybrid strains, Hy-Line Brown (brown eggshell, **HLB**) and Hy-Line White 36 (white eggshell, **HLW**) (Hy-Line International, West Des Moines, IA), and to 2 Italian dual-purpose and slow-growing breeds, Ermellinata di Rovigo (brown eggshell, **E**) and Robusta Maculata (brown eggshell, **R**) were reared under organic farming production procedures (European Union, 1999). The E and R birds were provided by the regional farm that preserves these breeds with financing from the Veneto regional government (De Marchi et al., 2006). The local genotypes were created in Veneto (Italy) during the 1950s, using Sussex and Rhode Island (E breed) and Brown Orpington and White America (R breed) purebreds. They have been maintained as pure breeds in the conservation program (Zanetti et al., 2007).

The hens (70 birds per each genotype group) were experimentally reared from 25 to 44 wk of age, from July to December. Each genotype had access to outdoor (4 m^2/bird) and indoor (0.20 m^2/bird) spaces, divided by netting. The indoor spaces were provided with wooden nests (8 birds/nest) and perches (22 cm/bird; 100 cm of height off the ground; a width of 3 cm); the floor was covered by a mixture of straw and wood shavings to a depth of 15 cm. The birds were 1 d of age at their arrival at the trial station and it took 2 h to bring them there. During the first 4 wk, the birds were kept indoors, on litter (straw and wood shavings), and under infrared radiation lamps, with a decrease from 32 to 24°C over a 4-wk period. At 8 wk of age, the birds had free access to outdoor spaces.

The birds showed similar behavior regarding the use of their outdoor area, which was initially in good condition (with the presence of grass—average composition: *Lolium* and *Festuca* prevailing with *Agrostis*, *Poa*, *Trifolium*, *Taraxacum*, and other species) until a few weeks before starting the experimental period. Continuous foraging and walking on the same area by the birds and climatic conditions caused modification of the outdoor area: at the start of summer when rain levels are low in Northern Italy, the grass began to die and did not regrow, a situation that lasted until the autumn. In such conditions in the absence of grass, insects and worms were either not present or were not available to the birds in these areas. The birds were subjected to the same prophylaxis procedures (Marek's disease, Newcastle disease, Gumboro disease, infectious bronchitis, coccidiosis, and fowl pox vaccines), rearing conditions (temperature and photoperiod), and feeding from the time of hatching until the end of the experimental period. The feed given to the birds throughout the first 15 wk of life (average composition: CP = 19.5% and ME = 2,830 kcal/kg) contained no additives but did contain some nonorganic ingredients. Six weeks before the start of the trial, the birds were given an organic feed throughout the conversion period (European Union, 1999). Thereafter, throughout the experimental period, the birds were fed ad libitum the organic crumbled feed (Table 1). The hens were weighed both at the start and at the end of the trial; feed consumption and egg production were checked daily during the experimental period. Throughout the trial and twice per week, the egg weight was checked from the whole day's egg production.

Throughout the trial, the environmental temperature and the RH levels were checked by a thermohygrograph (model TIG-ITH, LSI, Milan, Italy). In summer, the mean temperature was 25 ± 5°C and the daily maximum and minimum values were 31 and 19°C, respectively; the mean RH was 62 ± 6% with a daily maximum and minimum value of 68 and 55%, respectively. In autumn, the mean temperature was 13 ± 7°C and the daily maximum and minimum values were 20 and 7°C, respectively; the mean RH was 73 ± 9% and the

Table 1. Formulation, nutrients (as-fed basis, means ± SD), and major fatty acid composition of the diet

Item	Amount
Ingredient, %	
Corn	58.0
Wheat bran	11.0
Soybean	13.0
Sunflower	10.0
Calcium carbonate	7.50
Dicalcium phosphate	0.500
Chemical composition, %	
DM	89.8 ± 0.181
CP	16.9 ± 0.483
Ether extract	6.28 ± 0.255
Crude fiber	3.65 ± 0.654
Ash	10.1 ± 0.750
Ca	3.30 ± 0.441
P	0.791 ± 0.030
Na	0.161 ± 0.012
K	0.874 ± 0.003
Fe	0.040 ± 0.012
ME, kcal/kg	2,856
Fatty acid[1]	
C14:0	0.461
C16:0	16.2
C16:1	0.464
C18:0	3.90
C18:1n-9	23.9
C18:1n-7	1.58
C18:2n-6	46.4
C18:3n-3	3.98
C20:4n-6	0.132
C20:5n-3	0.160
C22:5n-6	0.011
C22:5n-3	0.120
C22:6n-3	0.533
SFA[2]	21.2
MUFA[3]	26.4
PUFA[4]	51.4
n-6:n-3	9.73
SFA:PUFA	0.41
SFA:n-3 without PUFA	4.43

[1]Percentage of total fatty acids.
[2]SFA = saturated fatty acids.
[3]MUFA = monounsaturated fatty acids.
[4]PUFA = polyunsaturated fatty acids.

daily maximum and minimum values were 84 and 65%, respectively.

The indoor and outdoor thermal-hygrometric conditions followed the same trend; for temperature, the indoor values were higher than the outdoor values both in summer and in autumn (about 3 ± 0.8°C), whereas the RH values were very similar. The birds had free access to the outdoor space and used it throughout the day and stayed indoors for laying eggs and during the night; in autumn, on rainy or foggy days, the hens spent less time outdoors.

The photoperiod was initially natural, but when it began to decrease naturally according to the geographical position of the trial station (Northern Italy), it was complimented by artificial light (1 incandescent bulb, 40 W) inside the rooms where the birds spent the night (16L:8D). In indoor spaces, the light intensity ranged from 20 to 90 lx [measured by a Delta Ohm HD8366 luxmeter (Delta Ohm, Padova, Italy) with a silicon sensor] as it varied according to the time of day and to the weather conditions. The minimum values were taken at the beginning and at the end of the day when the light was on.

At 44 wk of age, 12 birds per genotype were weighed and brought to the slaughterhouse. The slaughterhouse was about 70 km from the trial station; feed and water withdrawal times were 12 and 8 h, respectively, before slaughtering. The birds were electronically stunned (300 Hz, 68 to 80 V), killed by mechanical exsanguination (using a mechanical killer, cutting under the lower mandible), mechanically plucked (52°C scald temperature for 90 s), and eviscerated (Rizzi et al., 2007). After being refrigerated for 24 h (air-chilled within 4 h of slaughter with a final temperature of +4°C), carcasses were weighed and dissected, and breast muscle, superficialis major, and all thigh muscles and tendons were removed and frozen at −20°C up to 20 d for further analyses.

At 44 wk of age, 30 eggs from a whole day's production were collected per group, excluding the defective eggs (double yolk and abnormal shell). The yolk was manually separated from the albumen 3 times (by means of a separator composed of the top cup designed to retain the yolk while letting the albumen slide to the bottom cup) and 10 samples (3 yolks/sample) per genotype were obtained and frozen at −20°C up to 20 d for further analyses.

Chemical and Fatty Acid Composition of Diet, Meat, and Egg Yolk

The crumbled feed was ground (1-mm mesh screen, Grindomix ZM 200, Retsch GmbH, Haan, Germany) and meat was homogenized in a homogenizer (Grindomix GM 200, Retsch GmbH) and then chemically analyzed for DM (method 950.46, AOAC, 2000), protein (method 981.10, AOAC, 2000), lipid (method 991.36, AOAC, 2000), and ash (method 920.153, AOAC, 2000). Yolk DM, protein, and ash were determined following the Istituto Superiore di Sanità methods (ISTISAN, 1996). Energy content was calculated based on the diet formulation and based on the chemical composition of each ingredient given by the NRC (1994). From mineralized samples (method 999.10, AOAC, 2000; by means of a microwave digestion system, model Ethos 900, Milestone Inc., Monroe, CT), each mineral (sodium, potassium, and iron) was measured using an inductively coupled plasma-optical emission spectrometry spectrometer (Spectro Ciros^{CCD}, Spectro, Kleve, Germany). Mineral element calibration was done using diluted certified inductively coupled plasma monoelement standards traceable to the National Institute of Standards and Technology (CPI International Europe, Amsterdam, the Netherlands). All solutions were acidified with 3% HNO_3 (suprapure quality—nitric acid 65%, Merck KGaA, Darmstad, Germany). The calibration range was from 0.01 to 100 mg/L for sodium, 0.05 to 500 mg/L for potassium, and 0.005 to 50 mg/L for iron and the wavelength set for the measure was 589.592, 766.490, and 259.940 nm for sodium, potassium, and iron, respectively.

Lipid Extraction. Total lipids were extracted from 1 g of feed, yolk (freeze-dried and ground), and meat (freeze-dried) samples according to the method of Folch et al. (1957). Each sample, placed in a glass tube provided with a sintered glass filter (a pore size of 40 to 60 µm) at the bottom, was homogenized (ART MICCRA D8, Mullheim, Germany) with chloroform:methanol (2:1, vol/vol) and filtered 3 times (30 mL each time); the aliquots were placed into a funnel separator where a saline solution (0.88% KCl, vol/vol) was added (1/4 of the extracted volume). The lipid fraction was separated (after 6 h) and the bottom liquid was discharged from a funnel separator and washed with methanol (1:1, vol/vol); after 30 min, it was filtered through paper filters (fast—ashless 41, Whatman Ltd., Maidstone, UK) topped with anhydrous sodium sulfate. Filters with anhydrous sodium sulfate were washed with 5 mL of chloroform. The bottom liquid and the chloroform wash were put into a rotating evaporator (Büchi Rotavapor, Fawil, Switzerland) kept in a water bath at 37°C until it reached constant weight and then dried in an oven at 105°C for 10 min.

Fatty Acid Analyses. The extracted lipids were then transesterified: samples of 20 to 40 mg were put into 5-mL Pyrex tubes (Corning Inc., Lowell, MA) and 2 mL of internal standard, methylnonadecanoate:hexane (1:1, wt/vol; Fluka, Sigma-Aldrich, Steinheim, Switzerland), and 100 µL of sodium methoxide (1 *M*) were added. The samples were shaken (ASAL 717, ASAL, Milan, Italy) for 10 min, 150 µL of oxalic acid-diethyl ether (1 g/30 mL; Carlo Erba, Milan, Italy) was added, shaken again for 30 s, and then centrifuged (Sigma Laborzentrifugen, 3K 15, Braun Biotech International, Melsungen, Germany) at 800 × g for 10 min. The supernatant was placed in vials for gas chromatography

analyses. Individual fatty acids were separated by a gas chromatograph (model 8000 Series Top, Carlo Erba) equipped with a Omega Wax 250 capillary column (30-m length, 0.25-mm internal diameter, Supelco, Bellefonte, PA) with an injection split ratio of 100:1. The operating conditions of the gas chromatograph were as follows: the initial temperature was 140°C, increasing by 4°C/min to 220°C. The temperature of the injector and the detector remained stable at 250 and 260°C, respectively. Helium was used as the carrier gas at a flow rate of 1.6 mL/min in the column. The fatty acid percentage was calculated with respect to the total fatty acid mass expressed as total area. Each fatty acid was identified in the form of a methyl ester by comparing the retention times with the standard sample (F.A.M.E. mix C4-C24, lipid standard, 18919-1AMP, Sigma-Aldrich, St. Louis, MO).

Cholesterol Determination. Tubes containing freeze-dried yolk (100 mg) or meat (500 mg) samples had 5 mL of ethanol and 2 mL of KOH (50%, vol/vol) added, and then were placed in a water bath at 70°C and shaken (ASAL TRM 750) for 10 min. The tubes were then allowed to cool at room temperature and 1 mL of internal standard [45 mg of pregnenolone (Sigma Chemical Co., St. Louis, MO) in hexane] and 35 mL of hexane:ethyl ether (1:1, vol/vol) were added. After the addition of 20 mL of deionized water, and after being shaken for 1 min, centrifugation (ALC centrifuge 4227, Milan, Italy) took place (450 × g for 20 min). An aliquot, 25 mL, of the supernatant organic phase was taken and dried in a rotating evaporator kept in a water bath at 35°C. The sample obtained and 5 mL of the mobile phase (isopropanol:hexane, 7%, vol/vol) were analyzed by HPLC [LC Pump Series 410, UV spectrometer detector LC90 operating at 208 nm (Perkin Elmer, Waltham, MA; flow rate: 0.8 mL/min) equipped with a Bondclone column (10 m, Silica, 300 × 3.9 mm, Phenomenex, Torrance, CA)].

Statistical Analysis

All data on the productive performance and on the chemical composition of the meat and eggs were subjected to 1-way ANOVA as a completely randomized design, with genotype as the main effect, using the GLM procedure of SAS (SAS Institute, 2001). Significant differences among the means were determined using Duncan's multiple range test (SAS Institute, 2001).

RESULTS AND DISCUSSION

Because these genotypes showed differences in values for BW, feed intake, and egg yield, it is worth analyzing the responses of the birds and the product quality throughout the different phases of the productive cycle. This study presents the productive performance of the hens from 25 to 44 wk of age and the chemical composition of the meat and egg at 44 wk of age.

Production Standards

Figure 1 shows the hen-day egg production throughout the experimental period. Hybrid hens showed a higher ($P < 0.01$) laying rate than the Italian ones. In the first half of the trial, the R hens showed the lowest ($P < 0.01$) hen-day egg production. Hybrid strains showed similar values in the middle of the examined productive cycle; E hens constantly showed lower ($P < 0.01$) values than hybrids, but they were generally higher ($P < 0.01$) than R.

Table 2 shows the production performance of the hens. At 25 wk of age, significant ($P < 0.01$) differences in BW were found between Italian and hybrid hens and between HLB and HLW. At the end of the trial, the dual-purpose strains showed significant ($P < 0.01$) differences in carcass weight. The growth rate:initial BW ratio was different ($P < 0.01$) among all 4 groups, with higher values for R and HLB and lower values for E and HLW. The feed intake and the daily egg mass:metabolic BW (Borgioli, 1985) ratio were considered for the first and last period of the experimental trial because these 2 periods (4 wk each) were related to the initial and final age and to extreme summer and autumn environmental conditions. The feed ingestion:metabolic BW ratio was higher ($P < 0.01$) for the hybrid hens in comparison to the Italian birds and differences ($P < 0.01$) were found both between Italian and between hybrid strains (Table 2) according to the period of the trial.

The daily egg mass:metabolic BW ratio significantly ($P < 0.01$) differed among the 4 strains, with less favorable values for Italian birds. Although the hybrid genotypes had a similar hen-day egg production (Rizzi et al., 2002), different increments of egg yield were observed throughout the entire period. From 25 to 44 wk of age, 24 and 4% increase in hen-day egg production were observed for HLB and HLW, respectively. The E birds showed an increase of 9%, whereas R showed a more marked variation.

The different genetics of hybrid and Italian genotypes were evident in production performance (Rizzi et al., 2002). It should be noted that HLB and HLW are strains selected for high egg production and have better egg yields and lower body growth, whereas the E and R are dual-purpose genotypes and showed higher body gain (Rizzi et al., 2007) and lower egg production.

The results of the feed intake:metabolic BW ratio indicate higher values for hybrids due to their lower BW and higher egg production; as a consequence, these 2 genotypes showed an increase in these values greater than that of Italian breeds in the autumn period.

Chemical Composition of Breast and Thigh Meat and Egg Yolk

Before discussing the chemical composition of meat and yolk, to provide an indication of the differences among genotypes, it is worth remembering that at 44 wk of age, the 2 Italian dual-purpose breeds had sig-

nificantly heavier breasts, thighs, and drumsticks (R = 401 and 677 g, respectively; E = 279 and 569 g, respectively) than hybrid hens (HLB = 201 and 379 g, respectively; HLW = 173 and 302 g, respectively) and significant differences were found both between Italian and between hybrid hens (Rizzi et al., 2007).

The chemical composition of meat and yolk is shown in Figure 2. Breast protein was higher ($P < 0.01$) in HLW when compared with HLB and R. Breast lipid differed among genotypes: the R hens showed the highest ($P < 0.01$) value and the HLW had the lowest ($P < 0.01$) content. Ash was higher ($P < 0.01$) in E breasts with respect to the other genotypes.

Thigh protein differed significantly among genotypes: hybrids showed a higher ($P < 0.01$) protein content than R and E. Lipid showed the opposite trend: E thighs showed the highest ($P < 0.01$) content, followed by R ($P < 0.01$) and HLB and HLW ($P < 0.01$). The local breeds had higher lipid content than the hybrids. Ash was higher in the hybrids, particularly in HLW ($P < 0.01$), when compared with the Italian groups.

The R and HLW yolks had the highest ($P < 0.01$) protein and the lowest lipid contents. The HLW yolks had the highest ($P < 0.01$) ash content when compared with the other groups.

Adult hens differ from immature birds by having a higher adipose tissue content and having their metabolism directed to reproductive activity. At the end of the trial, the hens aged 300 d showed notable variations among strains in breast lipid (HLW 40% lower than R) and in thigh lipid (HLW 22% lower than E). Italian hens had a higher percentage of meat lipid because of their genetic origin directed toward body growth rather than to high egg production. Hybrid birds have a trend of lower lipid content because they direct energy to egg production rather than to muscular tissue and thus low-fat depots in muscle may be observed.

Precocity may be evaluated by carcass composition (Castellini, 2005) and by the mean age at sexual maturity. The R genotype was less precocious than the E breed given that at 168 d of age, the former reached 68% and the latter 80% of adult BW (Veneto Agricoltura, 2002; Rizzi et al., 2008). In the present trial, the R hens exhibited poorer precocity compared with hybrids and E because the R onset of lay started later (they started laying at 25 wk of age) and they showed the highest BW increment.

Breast meat had higher protein and lower lipid in comparison to thigh muscles. It should be noted that breast meat involved only one muscle (superficialis major), whereas thigh meat included many muscles with intra- and intermuscular fiber fat depots. Lipid of breast meat is similar or lower than those reported by Cherian et al. (1995) on other strains of laying hens that had been intensively reared, whereas the thigh lipid is higher. Several factors such as diet composition, genetic origin, walking or exercising, environmental conditions, and physiological state may affect meat

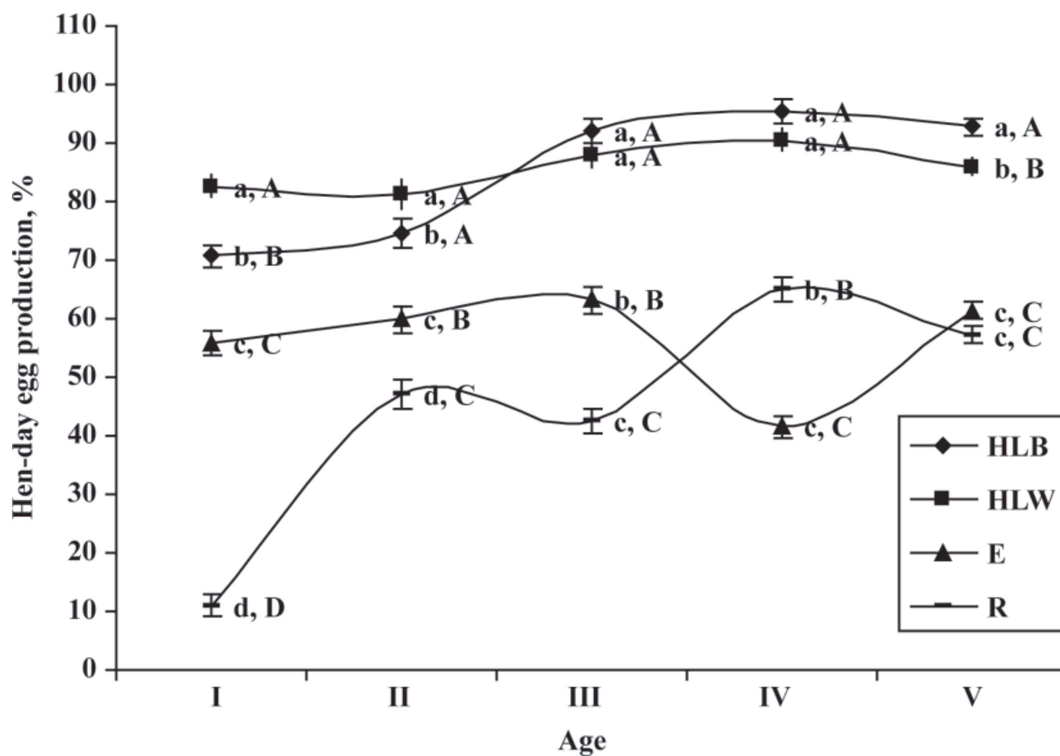

Figure 1. Hen-day egg production of 4 genotypes of laying hens throughout the trial. Age included the following: I (from 25 to 28 wk), II (from 29 to 32 wk), III (from 33 to 36 wk), IV (from 37 to 40 wk), and V (from 41 to 44 wk). Hybrid genotypes were Hy-Line Brown (HLB) and Hy-Line White (HLW), and Italian dual-purpose genotypes were Ermellinata di Rovigo (E) and Robusta Maculata (R). Per each given age (n = 28 observations per genotype), values (least squares means ± SEM) designated by different letters differ significantly: $^{a-d}$($P < 0.05$) and $^{A-D}$($P < 0.01$).

lipid, given that the hens tested showed a breast lipid content similar to that of younger commercial broilers (Castellini et al., 2002b).

A relationship between breast and thigh meat lipid and the age of the birds has been documented (Castellini et al., 2002b), but the lowering environmental temperature, occurring in the last period of the trial, may also have been significant in modifying corticosteroids and thyroid hormones (Tempel and Leibowitz, 1994; Chiericato et al., 1995). Hypothetically, the response of the adult hens to environmental temperature might have been different according to strains, and interactions between genotype and environmental conditions may have occurred.

In this experiment, the 19-wk period analyzed occurred during the summer and autumn when the out-

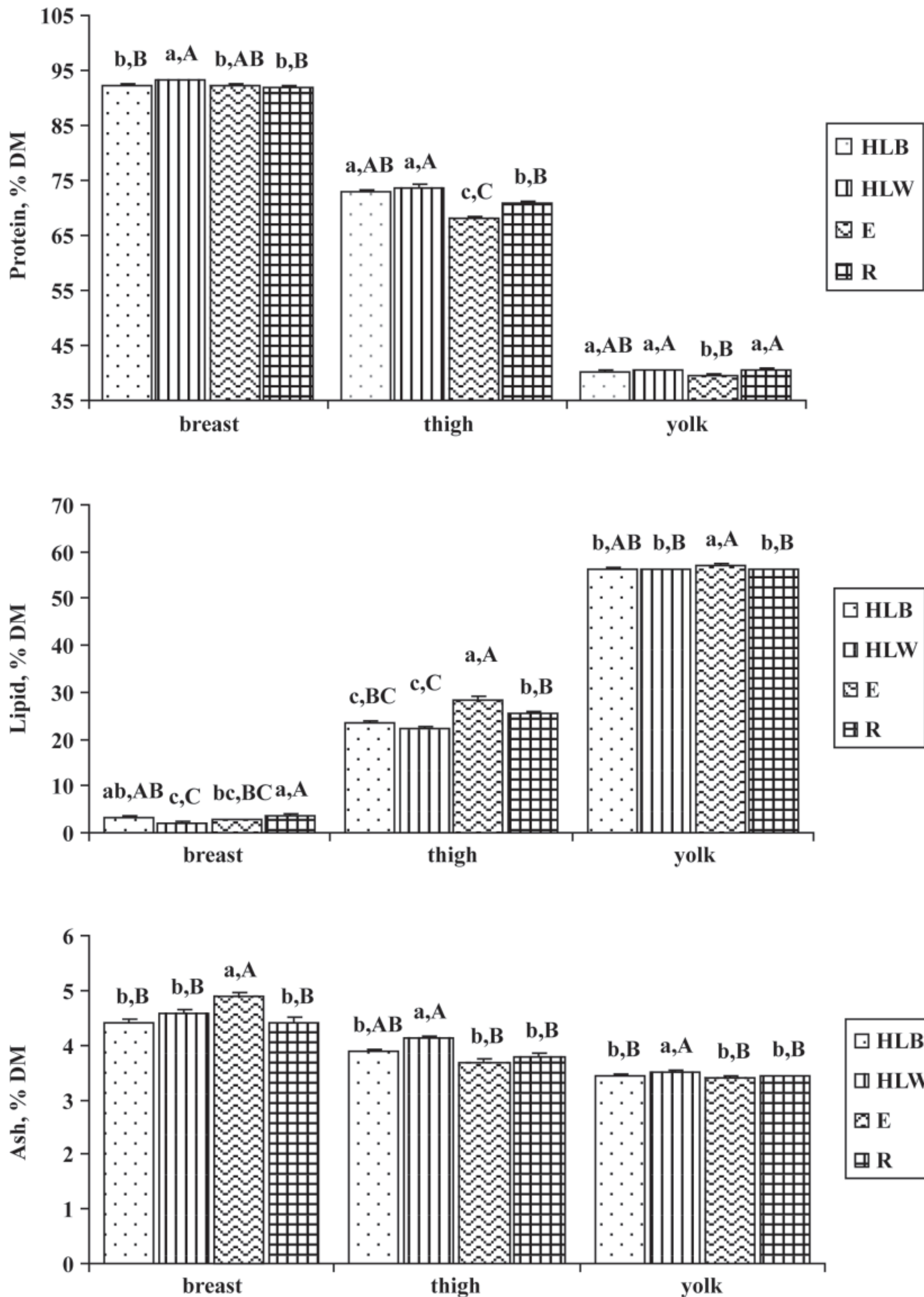

Figure 2. Chemical composition of breast, thigh, and yolk of 4 genotypes of laying hens at 44 wk of age. Hybrid genotypes were Hy-Line Brown (HLB) and Hy-Line White (HLW), and Italian dual-purpose genotypes were Ermellinata di Rovigo (E) and Robusta Maculata (R). Values (least squares means ± SEM) for each parameter designated by different letters differ significantly: $^{a–c}$($P < 0.05$) and $^{A–C}$($P < 0.01$).

Table 2. Productive performance of the laying hens[1]

Item	Genotype[2]				SEM
	HLB	HLW	E	R	
BW at 25 wk,[3] kg	$1.65^{b,B}$	$1.47^{c,C}$	$2.21^{a,A}$	$2.25^{a,A}$	1.46
Carcass weight,[4] kg	$1.50^{c,C}$	$1.21^{d,D}$	$2.02^{b,B}$	$2.49^{a,A}$	0.127
Daily growth/IBW,[5] %	$25.4^{b,B}$	$13.8^{d,D}$	$20.0^{c,C}$	$35.1^{a,A}$	8.72
Feed intake,[6] g/d	124	109	114	123	9.06
Feed intake/MBW,[7] g/g					
I	$6.50^{a,A}$	$6.54^{a,A}$	$6.00^{b,B}$	$4.92^{c,C}$	0.432
II	$8.04^{b,B}$	$8.45^{a,A}$	$6.44^{c,C}$	$5.79^{d,D}$	0.436
DEM/MBW,[8] g/g					
I	$2.51^{b,B}$	$3.34^{a,A}$	$1.46^{c,C}$	$0.19^{d,D}$	0.121
II	$3.59^{b,B}$	$3.71^{a,A}$	$1.65^{c,C}$	$1.43^{d,D}$	0.151

$^{a–d}$Means within the same row followed by a different superscript are significantly different ($P < 0.05$).

$^{A–D}$Means within the same row followed by a different superscript are significantly different ($P < 0.01$).

[1]From 25 to 44 wk of age; I = data collected from 25 to 28 wk of age (summer phase); II = data collected from 41 to 44 wk of age (autumn phase).

[2]HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta Maculata.

[3]HLB: n = 70; HLW: n = 67; E: n = 69; R: n = 68.

[4]Weight of eviscerated carcass, kept at 4°C; observations per each genotype = 14.

[5]Calculated as [daily growth/initial BW (IBW)] × 100; IBW = at 25 wk of age; HLB: n = 70; HLW: n = 67; E: n = 69; R: n = 68.

[6]Each genotype group consisted of 3 groups of birds; it refers to a 25 to 44-wk-of-age period.

[7]Calculated as [feed intake/metabolic BW (MBW)] × 100; HLB: n = 37; HLW: n = 46; E: n = 47; R: n = 41.

[8]Daily egg mass (DEM)/MBW; the daily egg mass was calculated as hen-day egg production × daily egg weight; HLB: n = 37; HLW: n = 46; E: n = 47; R: n = 41.

door temperatures initially reached about 30°C and then decreased to 5°C. Such variation of thermal conditions does not occur in intensive production houses where hybrid hens are reared at controlled temperatures. It should be pointed out that in many wild birds, the seasonal photoperiod variations induce the birds to modify their metabolism so as to better manage the seasonal temperature variations. Throughout the summer months, the decreasing photoperiod and, thereafter, the decreasing temperature generally allow outdoor animals to modify their hormonal profile and then their metabolism, and thus they gradually adapt to the low temperatures. In this trial, the natural photoperiod was supplemented with artificial light, as established by the European Union (1999), to maintain a constant light:dark ratio, thus providing for a higher hen-day egg production (Morris, 2004).

It may be hypothesized that under the present conditions, the genotypes demonstrated different physiological responses to the concurrent increasing egg production, yolk size and egg size, and body thermal regulation. Indications of the effect of light on physiological responses according to genotype (Morris, 2004) are scarce because previous works (Renema and Robinson, 2001) mainly refer to the light intensity effect with laying hens.

It is to be noted that HLW hens showed lower lipid values in the thigh and yolk if compared with the other genotypes. It is possible that HLW hens, which had the most favorable biological efficiency (daily egg mass:metabolic BW ratio), used nutrients to obtain the highest quantity of eggs, and thus less lipid deposition occurred in tissues and yolk. It is not known how much energy for production and body requirements the birds obtained from fat and from feed in relation to genotype.

In Table 3, cholesterol and some mineral content of muscles and yolks are reported. The HLW breast showed the highest ($P < 0.05$) cholesterol content and E the lowest. Thigh cholesterol content was higher ($P < 0.05$) in HLW than in the other groups. Yolk cholesterol was higher ($P < 0.01$) in purebreds than in hybrids. When considering the cholesterol content of the yolk of a table egg, because the 4 genotypes showed differences in yolk weight (data not shown), the HLB eggs had a lower ($P < 0.05$) content than HLW.

Lipid and cholesterol of meat and yolk varied among genotypes. For thigh and breast lipid, variations were as much as 22 to 40%, whereas in yolks, only slight differences (2%) were detected. The small lipid variations observed in eggs point out the role of the yolk in sustaining the life and growth of the embryo and therefore wide variations do not occur. Cholesterol showed similar variations among genotypes for breast and thigh (9%) and 7% for yolk. Variations of yolk cholesterol are higher than yolk lipid that, similar to protein, is deposited in rather constant quantity to guarantee embryo development. Embryo livability and growth do not depend on high cholesterol levels (Elkin, 2006).

The cholesterol content of breast and thigh meat showed limited differences among genotypes because it may depend on the number and dimensions of fibers and fat depots (Hoelscher et al., 1988; Kompdra et al., 2002); yolk cholesterol was higher in local breeds probably because they direct more cholesterol toward the yolk given their lower egg-day productions (Elkin,

2006). Breast cholesterol of the hens studied was lower than that of commercial broilers, whereas the thigh content was not so different (Crespo and Esteve-Garcia, 2001; Rule et al., 2002).

As far as meat mineral content is concerned (Table 3), breast sodium differed ($P < 0.05$) between HLW and HLB and it was higher ($P < 0.01$) than the Italian breast. The HLW breast showed a potassium level higher ($P < 0.01$) than the other groups. Breast iron did not differ among the groups. The R thigh showed the highest sodium content ($P < 0.01$) and E hens showed the lowest. A higher ($P < 0.01$) potassium level was found in HLW thigh meat when compared with the other groups. Significant variations were seen with iron content, which was higher ($P < 0.01$) in HLW and lower in R thigh meat. Hybrids had a higher ($P < 0.01$) iron content than the Italian hens.

Meat quality may be evaluated by quantifying some minerals because they have a role in human health. Although significant differences were observed among the groups, the data on meat mineral composition did not result in a strain with negative effects on human health because of a higher sodium content. The different content of lipid and iron between the breast and thigh muscles mainly depends on the type of fibers and on their metabolism (Hulot and Ouhayoun, 1999) according to the age of the birds. Thigh iron was higher in hybrid genotypes with lower muscle growth than in Italian breeds; this result agrees with that found by Berri et al. (2001).

Fatty Acid Composition of Breast and Thigh Meat and Egg Yolk

The fatty acid profiles of lipids of breast meat, thigh meat, and yolk are shown in Tables 4, 5, and 6. As far as breast saturated fatty acids (**SFA**) are concerned (Table 4), the E group showed the highest ($P < 0.05$) percentage and the HLW showed the lowest. Thigh SFA (Table 5) were significantly different ($P < 0.01$) between hybrids. In HLW and R eggs, SFA (Table 6) were higher than HLB ($P < 0.01$) and E ($P < 0.05$).

Fatty acid composition of the diet is very important both for physiological and production conditions of the birds and for the quality of their products. In this trial, HLW meat and HLB yolks had the lowest percentage of SFA. Previously, Cherian et al. (1995) and Ayerza and Coates (2000) found no differences in SFA yolks between brown and white hens. Saturated fatty acids showed little variation among strains for breast (3%) and yolk (5%), but it was higher in thigh (18%). Lopez-Ferrer et al. (1999) stated that, in relation to the tissues, the modification of fatty acid composition of i.m. fat is more limited than the separable fat deposits such as abdominal and s.c. fat.

Breast monounsaturated fatty acids (**MUFA**; Table 4) were higher ($P < 0.01$) in E than in HLW and R. Thigh MUFA (Table 5) proved similar between hybrids and different ($P < 0.01$) between Italian breeds. In yolk (Table 6), MUFA were higher ($P < 0.01$) in HLB and E than in the other genotypes. Monounsaturated fatty acids showed variations among genotypes of 7, 11, and 9%, respectively, for breast, thigh, and yolk. In intensively reared broilers, the MUFA of meat mainly depend on the dietary fatty acid composition rather than on hepatic synthesis (Ayerza et al., 2002): under the present conditions, this observation appears to be valid for the breast, whereas in the thigh, de novo synthesis was observed.

Significant variations were observed for polyunsaturated fatty acids (**PUFA**): in breast (Table 4), they were lower ($P < 0.01$) in E than in the other groups. Thigh PUFA (Table 5) differed significantly among the

Table 3. Cholesterol and some mineral content of breast, thigh, and yolk

	Genotype[1]				
Item	HLB	HLW	E	R	SEM[2]
Breast[3]					
Cholesterol, mg/100 g	164^{ab}	166^{a}	151^{b}	161^{ab}	14
Na, mg/g	$2.43^{b,AB}$	$2.63^{a,A}$	$2.37^{b,B}$	$2.38^{b,B}$	0.187
K, mg/g	$12.1^{b,B}$	$13.0^{a,A}$	$12.4^{b,B}$	$12.3^{b,B}$	0.443
Fe, mg/kg	13.5	12.6	13.9	11.6	4.08
Thigh[3]					
Cholesterol, mg/100 g	256^{b}	281^{a}	257^{b}	257^{b}	19
Na, mg/g	$3.86^{ab,AB}$	$4.02^{a,AB}$	$3.57^{b,B}$	$4.20^{a,A}$	0.372
K, mg/g	$10.8^{b,B}$	$11.8^{a,A}$	$10.9^{b,B}$	$10.9^{b,B}$	0.569
Fe, mg/kg	$46.5^{ab,AB}$	$48.9^{a,A}$	$43.3^{bc,BC}$	$41.1^{c,C}$	3.94
Yolk[3]					
Cholesterol, mg/g	$25.4^{b,B}$	$25.2^{b,B}$	$27.0^{a,A}$	$27.3^{a,A}$	0.077
Cholesterol, mg/egg	$215^{c,B}$	$222^{b,B}$	$256^{a,A}$	$252^{a,A}$	6.95

[a–c]Means within the same row followed by a different superscript are significantly different ($P < 0.05$).

[A–C]Means within the same row followed by a different superscript are significantly different ($P < 0.01$).

[1]HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta Maculata.

[2]Breast and thigh: n = 10 per genotype; yolk: n = 10 (HLB and E) and n = 9 (HLW and R).

[3]Dry matter basis.

Table 4. Major fatty acid composition of breast meat lipids[1]

Item	Genotype[2]				SEM[3]
	HLB	HLW	E	R	
	% of total fatty acids				
SFA	34.0^{ab}	33.7^{b}	34.7^{a}	34.5^{ab}	1.04
C14:0	0.487^{a,AB}	0.394^{b,B}	0.557^{a,A}	0.559^{a,A}	0.077
C16:0	23.7^{ab,AB}	23.0^{b,B}	24.0^{a,A}	24.1^{a,A}	0.779
C18:0	9.17	9.45	9.43	9.26	0.523
MUFA	28.2^{ab,AB}	27.2^{b,B}	29.3^{a,A}	27.2^{b,B}	1.27
C16:1	1.32^{ab,AB}	1.14^{b,B}	1.43^{a,A}	1.21^{b,AB}	0.213
C18:1	26.3^{ab,AB}	25.4^{b,B}	27.2^{a,A}	25.6^{b,B}	1.21
PUFA	37.4^{a,A}	38.5^{a,A}	35.5^{b,B}	37.8^{a,A}	1.13
n-6 PUFA	35.0^{b,A}	36.2^{a,A}	32.8^{c,B}	34.9^{b,A}	1.08
C18:2n-6	28.3^{a,A}	29.1^{a,A}	25.4^{b,B}	28.1^{a,A}	1.35
C20:4n-6	6.06	6.36	6.69	6.23	1.04
n-3 PUFA	2.46^{bc,BC}	2.25^{c,C}	2.65^{ab,AB}	2.91^{a,A}	1.05
C18:3n-3	1.08^{b,AB}	0.96^{b,B}	1.05^{b,AB}	1.32^{a,A}	0.242
C20:5n-3	0.036^{ab}	0.039^{ab}	0.030^{b}	0.045^{a}	0.013
C22:5n-3	0.370^{ab}	0.337^{b}	0.365^{ab}	0.418^{a}	0.068
C22:6n-3	0.966^{ab}	0.911^{b}	1.21^{a}	1.12^{ab}	0.280

$^{a–c}$Means within the same row followed by a different superscript are significantly different ($P < 0.05$).
$^{A–C}$Means within the same row followed by a different superscript are significantly different ($P < 0.01$).
[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
[2]HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta Maculata.
[3]n = 10 per genotype.

groups: HLW showed higher ($P < 0.01$) values than HLB and E. Yolk PUFA (Table 6) showed little variations among the genotypes, being higher ($P < 0.01$) in R than in the other groups.

Total PUFA exhibited low variations among genetic groups in breast (6%) and yolk (7%) and was higher in thigh (23%). The present results on hens agree with those reported by another author, who observed that broilers have a low capacity to modify the breast lipid fraction (Barroeta, 2007).

In breast, n-6 PUFA (Table 4) were higher in HLW than HLB and R ($P < 0.05$) and E ($P < 0.01$). Thigh n-6 PUFA (Table 5) differed ($P < 0.01$) between hybrids, being higher ($P < 0.01$) in HLW. The n-6 PUFA were higher ($P < 0.01$) in R yolk (Table 6) when compared with the other groups. The n-6 PUFA showed significant ($P < 0.01$) variations among genotypes in breast (9%, HLW vs. E) and yolk (7%, R vs. HLB, HLW, and E) and these variations were lower than in thigh (24%, HLW vs. HLB and E).

Table 5. Major fatty acid composition of thigh meat lipids[1]

Item	Genotype[2]				SEM[3]
	HLB	HLW	E	R	
	% of total fatty acids				
SFA	33.1^{a,A}	27.0^{b,B}	32.8^{a,AB}	30.4^{ab,AB}	4.57
C14:0	0.665^{b,A}	0.510^{c,B}	0.778^{a,A}	0.666^{b,A}	0.112
C16:0	24.0^{a,A}	19.0^{b,B}	23.5^{a,A}	21.7^{ab,AB}	3.29
C18:0	7.72	6.91	7.64	7.39	1.11
MUFA	35.4^{b,AB}	33.6^{b,B}	37.8^{a,A}	33.9^{b,B}	2.23
C16:1	2.40^{b,B}	2.30^{b,B}	2.95^{a,A}	2.10^{b,B}	0.388
C18:1	32.4^{b,AB}	30.9^{b,B}	34.3^{a,A}	31.3^{b,B}	1.85
PUFA	31.0^{bc,B}	39.2^{a,A}	29.6^{c,B}	35.5^{ab,AB}	6.23
n-6 PUFA	28.9^{b,B}	37.2^{a,A}	27.9^{b,B}	33.7^{ab,AB}	6.12
C18:2n-6	28.1^{bc,AB}	35.6^{a,A}	27.0^{c,B}	32.8^{ab,AB}	5.92
C20:4n-6	0.528^{b,B}	1.13^{a,A}	0.631^{b,B}	0.627^{b,B}	0.315
n-3 PUFA	2.08^{a,A}	1.92^{b,AB}	1.69^{c,C}	1.87^{b,B}	0.139
C18:3n-3	1.81	1.81	1.61	1.74	0.240
C20:5n-3	—	—	—	0.017	0.010
C22:5n-3	0.221^{a,A}	0.022^{b,B}	0.044^{b,AB}	0.048^{b,AB}	0.152
C22:6n-3	0.038^{ab}	0.093^{a}	0.031^{b}	0.072^{ab}	0.060

$^{a–c}$Means within the same row followed by a different superscript are significantly different ($P < 0.05$).
$^{A–C}$Means within the same row followed by a different superscript are significantly different ($P < 0.01$).
[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
[2]HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta Maculata.
[3]n = 10 per genotype.

Regarding breast n-3 PUFA (Table 4), they were higher ($P < 0.01$) in the Italian breeds as opposed to hybrids. In thigh, n-3 PUFA (Table 5) differed both between hybrids ($P < 0.05$) and between Italian breeds ($P < 0.01$). The fatty acid C20:5n-3 was not detected, except in the R group. As regards yolk n-3PUFA (Table 6), R showed the highest ($P < 0.01$) values. Relevant strain effects on n-3 PUFA may be observed because breast, thigh, and yolk exhibited significant variations of 23, 19, and 16%, respectively, between extreme values for the 4 genotypes. Previously, Cherian et al. (1995) had detected no significant differences among strains for n-3 PUFA in white and dark meat of laying hens.

Generally, yolk lipid and fatty acids did not show wide variations among the 4 strains, but for n-3 PUFA, breed differences were more relevant. Higher incorporation of total n-3 PUFA in yolks was observed for HLB in comparison to HLW. Bean and Leeson (2003) and other authors (Cherian et al., 1995) did not find any strain effect on yolk n-3PUFA; C22:6n-3 was higher in brown hens in comparison to white hens (Ayerza and Coates, 2000; Bean and Leeson, 2003). The interpretation of the results of the different trials is difficult because different ages and diets were used; as far as genotype effects are concerned, liver size should affect docosahexaenoic acid (**DHA**) synthesis because Scheideler et al. (1998) reported higher DHA levels in egg yolks of European strains when compared with American strains because the former have larger livers and may therefore have a higher capacity to elongate α-linolenic acid and change it to DHA.

In the present trial, the eggs, particularly Italian breed eggs, had a higher DHA content than conventional eggs (Payet et al., 2004), and therefore they seem to be an interesting vehicle to supply DHA in the form of phospholipids compared with triacylglycerols (Payet et al., 2004).

Meat and yolk n-3 PUFA were not high because the diet did not contain a high quantity of n-3 PUFA. However, an increase of long-chain PUFA was observed, but with a low rate of transformation from linolenic acid (Barroeta, 2007). The hens demonstrated the ability to elongate and desaturate linolenic acid to form DHA, but only traces of eicosapentaenoic acids were observed.

Other authors have reported that high values of n-3 PUFA of meat and yolk lipid found in free-range-fed hens were caused by consumption of green leafy vegetables, fresh and dried fruits, insects, and worms (Simopoulos and Salem, 1992; Ayerza and Coates, 2000, Castellini et al., 2006). These conditions may only be achieved with particular grass varieties and if a great quantity of vegetables, insects, or worms is available rather than feed. In the present trial, factors such as age, genotype, and environmental conditions may have been involved.

In Figure 3, some ratios between fatty acid groups are shown. The n-6:n-3 ratio was lower ($P < 0.01$) in Italian breasts than in HLW ones. In thigh, it was lower ($P < 0.01$) in HLB than in HLW and in yolk, it was significantly lower ($P < 0.01$) in R. The SFA:PUFA ratio was higher ($P < 0.01$) in E breast than in the other groups; in thigh, it was significantly ($P < 0.05$) lower in HLW than in HLB and in yolk, the lowest ($P < 0.05$) value was seen in R. The SFA:n-3 ratio showed the lowest value in R breast ($P < 0.05$), in HLW thigh ($P < 0.01$), and HLB and R yolks ($P < 0.01$) when compared with the other groups. Products with adequate dietary n-6:n-3, SFA:PUFA, and SFA:n-3 ratios must

Table 6. Major fatty acid composition of yolk lipids[1]

Item	Genotype[2] HLB	HLW	E	R	SEM[3]
	% of total fatty acids				
SFA	33.0^{b,B}	34.4^{a,A}	33.9^{b,AB}	34.7^{a,A}	0.692
C14:0	0.247^{c,B}	0.235^{c,B}	0.295^{a,A}	0.279^{b,A}	0.017
C16:0	24.0	24.4	23.9	24.4	0.584
C18:0	8.60^{b,B}	9.38^{a,A}	9.43^{a,A}	9.71^{a,A}	0.415
MUFA	38.7^{a,A}	37.4^{b,B}	38.7^{a,A}	35.2^{c,C}	1.01
C16:1	1.80^{a,A}	1.40^{bc,B}	1.47^{b,B}	1.31^{c,B}	0.149
C18:1	36.7^{a,AB}	35.7^{b,B}	36.9^{a,A}	33.7^{c,C}	0.933
PUFA	28.2^{b,B}	28.3^{b,B}	27.4^{b,B}	30.1^{a,A}	1.17
n-6 PUFA	26.0^{b,B}	26.3^{b,B}	25.3^{b,B}	27.7^{a,A}	1.07
C18:2n-6	23.5^{b,AB}	23.6^{b,AB}	22.7^{b,B}	24.8^{a,A}	1.07
C20:4n-6	1.88^{b,B}	1.97^{b,B}	1.89^{b,B}	2.23^{a,A}	0.141
n-3 PUFA	2.11^{b,B}	1.97^{c,B}	2.06^{bc,B}	2.34^{a,A}	0.112
C18:3n-3	1.03^{b,AB}	1.00^{bc,B}	0.95^{c,B}	1.10^{a,A}	0.072
C20:5n-3	0.007^{ab}	0.007^{ab}	0.006^{b}	0.010^{a}	0.003
C22:5n-3	0.121^{b,B}	0.083^{c,C}	0.113^{b,B}	0.151^{a,A}	0.020
C22:6n-3	0.940^{bc,B}	0.873^{c,B}	0.973^{b,AB}	1.063^{a,A}	0.091

[a–c]Means within the same row followed by a different superscript are significantly different ($P < 0.05$).

[A–C]Means within the same row followed by a different superscript are significantly different ($P < 0.01$).

[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[2]HLB = Hy-Line Brown; HLW = Hy-Line White; E = Ermellinata di Rovigo; R = Robusta Maculata.

[3]n = 10 (HLB and E) and n = 9 (HLW and R).

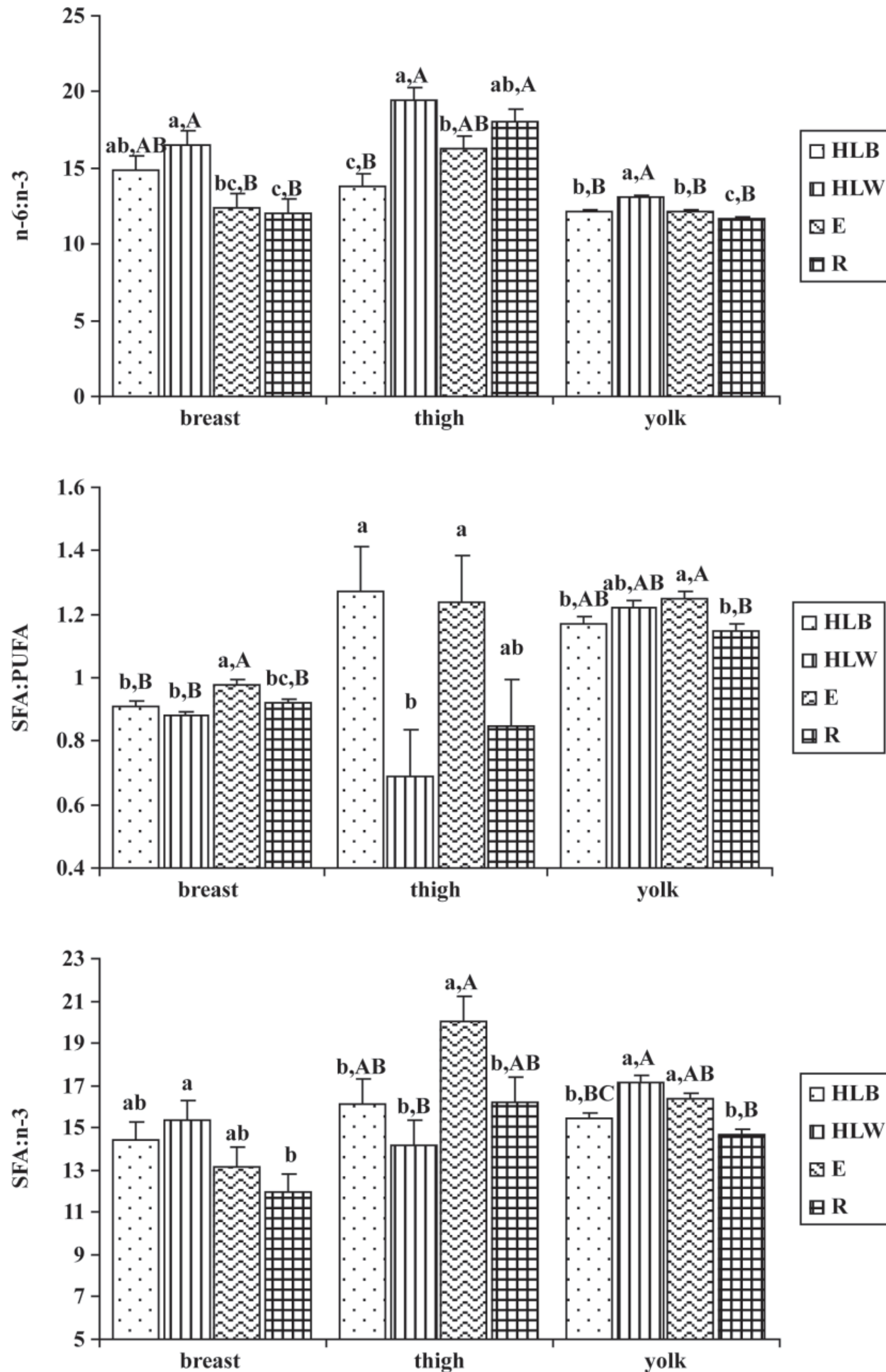

Figure 3. n-6:n-3, saturated fatty acid (SFA):polyunsaturated fatty acid (PUFA), and SFA:n-3 ratio of breast, thigh, and yolk of 4 genotypes of laying hens at 44 wk of age. Hybrid genotypes were Hy-Line Brown (HLB) and Hy-Line White (HLW), and Italian dual-purpose genotypes were Ermellinata di Rovigo (E) and Robusta Maculata (R). Values (least squares means ± SEM) for each parameter designated by different letters differ significantly: $^{a\text{–}c}$($P < 0.05$) and $^{A\text{–}C}$($P < 0.01$).

be provided because they are considered good indices for positively affecting human health (Kinsella et al., 1990; Broughton et al., 1991).

Under the experimental conditions, the n-6:n-3 ratio was higher than the suggested ratio of 5:1 or less (Canada Health and Welfare, 1990; Simopoulos and Robinson, 1998). Strain affected the n-6:n-3 ratio because variations of 27 and 29% were observed in breast and thigh meat, respectively, and only 11% variation was observed in yolk. In some strains, n-6:n-3 values of 12 were observed and this was 45% lower than the ratio (20) found in conventional eggs (Cherian et al., 1995; Ayerza and Coates, 2000).

The SFA:PUFA ratio showed wide variations (45%) among strains in thigh meat, whereas in breast (8%) and yolk (8%), small variations were observed. The SFA:PUFA ratio in meat and eggs of 4 genotypes is in line or even better than the 1:1 recommendation made by some health organizations (LaRosa, 1990; Canada Health and Welfare, 1990). The SFA:n-3 ratio was higher than suggested values but lower than values reported by other authors (Ayerza and Coates, 2000) on yolks.

Conclusions

The comparison between hybrid and Italian breed hens reared using an organic production system demonstrated the quality of their products after the first half of the laying cycle. Genotypes differed for egg and meat yields (Rizzi et al., 2007) but also for egg and meat quality. The results indicate that the genotypes used showed different abilities to incorporate fatty acids; a possible interaction between strain and environment may exist on absorption and utilization of dietary components as well as production yields.

Strain differences for lipid content and fatty acid composition changed according to body product. As well as cholesterol and lipid content and its fatty acid composition, breast and thigh meat and yolk showed an acceptable composition from a health point of view, especially in hybrids and R hens reared under organic conditions.

Given the high variability of environmental conditions in organic farming systems, further research is needed to determine the effect of environmental temperatures on the hen's responses in terms of lipid synthesis and energy utilization and to determine the relationship to other phases of the productive cycle and to different dietary compositions. The capability of certain genotypes either to synthesize or to transfer to tissue a high quantity of some fatty acids, minerals, or other molecules considered functional factors for human health could be an advantageous factor for rearing these genotypes under organic farming or free-range system conditions.

In conclusion, the Italian breeds showed a meat production higher than hybrids but a lower egg production; at 44 wk of age, under the experimental conditions, the dual-purpose breed R and the hybrids seemed to be suitable for healthy products.

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