Egg quality and blood parameters of "Bianca di Saluzzo" and Isa Brown hens kept under free range conditions

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ABSTRACT - Egg quality traits and some blood parameters of Bianca di Saluzzo hen (BSh) were compared to Isa Brown hen (IBh). Birds were reared in free range conditions. Weight of the whole egg was higher (P≤0.01) for IBh, due to its higher amount of albumen (P≤0.01) whereas BSh egg showed the highest weight and relative percentage of yolk (P≤0.01). IBh and BSh egg chemical composition was similar. Total red cells did not show statistical differences between groups, while total leucocytes were lower (P≤0.05) for BSh hens. Heterophyl/Lymphocyte (H/L) ratio of BSh was lower (P≤0.01) than IBh (0.33 *vs.* 0.82). IBh α-1 acid glycoprotein (AGP) mean concentrations were affected by time: the values registered after 15 d (T1) and 2 months (T2) of observation were similar (345±132 and 279±58 µg/ml respectively), while AGP value registered after 4 months (T3) were significantly higher (700±487 µg/ml); BSh did not display any AGP statistical variation over time. IBh albumin mean concentration (1.62 g/dL) was statistically lower than BSh values (1.89 g/dL); in both groups no effect of time of sampling was recorded. Acute phase protein (APP) values of BSh suggested a more adaptive attitude to free range conditions and appeared more constant over time. The present findings suggest genetic or adaptive differences affecting egg quality, physiological and immunological parameters.

Key words: Bianca di Saluzzo, Egg quality, Acute phase protein, Heterophil to lymphocyte ratio.

Introduction – In recent years the interest for laying hen alternative housing systems is increasing as within EU conventional battery cages will be banned from 2012 (1999/74/EC). Similarly saving biodiversity and local population is a matter of great importance also for poultry species (Weigend and Romanov, 2001). Free range system is one of the most appreciated rearing system by the consumer as it suggests the idea of high condition for animal well-being. Commercial laying hen have been selected over several years for productive purposes and to be adapted to live in battery cages. Studying rural population adapted to free range conditions could represents a valuable source for biodiversity. Few studies have been performed on "Bianca di Saluzzo" (or "Bianca di Cavour") hen, a dual-purpose breed originated in the countryside of Piemonte region (north-west Italy).

Several studies have reported that heterophil to lymphocyte ratio (H/L) is affected by stressors and can be used as sensitive haematological indicator of stress response among chicken populations (Gross and Siegel, 1983). α-1 acid glycoprotein (AGP) is a "positive" acute phase protein (APP) in chicken (Murata *et al*., 2004), while albumin behaves as a "negative" APP both in mammals (Mackiewicz, 1997) and chickens (Adler *et al*., 2001). Levels and response profiles of APPs differ among species and their concentrations may increase (positive APPs) or decrease (negative APPs) in response to a challenge. Recently it

has been suggested that acute APPs could be useful not only for monitoring the inflammatory process for diagnostic and prognostic purposes but also for analysing various non-inflammatory conditions, which it was previously thought did not affect APP values. In fact, some studies have highlighted a linkage between APP response and non-inflammatory psychophysical stress, suggesting that this response is inducible also by stressful events to which domestic animals are ubiquitously exposed during daily management (Murata, 2007; Salamano *et al*., 2008). Measurements of H/L ratio, serum AGP and albumin concentrations and their changes over time were employed in order to assess welfare and stress response in two different hen genotypes. Furthermore some egg quality traits were evaluated.

Material and methods - In this study the local breed Bianca di Saluzzo (BSh) was compared to the commercial strain Isa Brown (IBh). Both genotypes were reared in free range conditions at a density of 2 hens/m^2. IBh and BSh, at the age of 18 and 20 weeks respectively, were housed at "Centro Interdipartimentale Servizio Ricovero Animali (CISRA)", University of Torino (Italy). Each group of hens was composed by 24 birds individually identified by a shank ring. Animals received the same commercial feed and care management. Feed and water were provided *ad libitum*. Experimental observations were carried out after 15 days (T1), 2 months (T2) and 4 months (T3) upon arrival at CISRA. For each sampling time 30 eggs have been collected during two consecutive days. Whole egg weight as well as shell, albumen and yolk weights were measured. Albumen and yolk have been lyophilized and stored at -18°C until analysis. AOAC (1984) methods were used to evaluate the chemical composition (dry matter, crude protein, lipids, ash). Blood samples (n=14 for each sampling) were obtained from brachial vein. The counts of total red and white cells were determined in improved Neubauer's haemocytometer after mixing with Natt-Herrick solution. Blood smears were prepared from a droplet without anticoagulant, stained with May-Grünwald and Giemsa-Romanowski stains. Differential leucocytes count was evaluated in order to determine H/L ratio. Serum AGP concentration was assayed using a commercially available radial immunodiffusion tray (Cardiotech Services, Inc.). Serum albumin was measured using a chemistry analyzer (Instrumentation Laboratory 300 plus). Data were analyzed using two-way ANOVA with genotype and time of sampling as main factors.

Results and conclusions - Egg general traits and chemical composition of albumen and yolk are reported in Table 1. Weight of the whole egg was higher ($P \leq 0.01$) for IBh, due its higher amount of albumen ($P \leq 0.01$). BSh egg showed the highest weight and relative percentage of yolk ($P \leq 0.01$). IBh and BSh egg chemical composition was similar and results are consistent with general data reported for chicken egg (Cerolini *et al*., 2008).

Blood parameters are reported in Table 2. Total red cells did not show statistical differences among genotypes, while total leucocytes were lower ($P \leq 0.05$) for BSh. H/L ratio of BSh was lower ($P \leq 0.01$) than IBh (0.33 *vs.* 0.82). IBh albumin concentration (1.62 g/dL) was significantly lower than in BSh (1.89 g/dL); in both genotypes no effect of time of sampling was recorded. IBh AGP mean concentrations were affected by time: on sampling T1 and T2 were similar (345±132 and 279±58 µg/ml respectively), while AGP value on T3 was significantly higher (700±487 µg/ml); BSh did not display any AGP statistical variation over time. APP values of BSh suggested a more adaptive attitude to free range conditions and appeared more constant over time. A possible explanation could be that BSh is a rustic breed while IBh has been selected as a cage-adapted hen. Thus, laying hens should be genetically suited to the alternative housing systems to realize its full welfare advantages.

The present findings suggest genetic or adaptive differences affecting egg quality, physiological and immunological parameters. Data suggest an interesting question whether hens from a specific breed have higher or lower basal levels of a specific metabolite, or if some genotypes are more stressful/stressed in these specific experimental conditions.

Table 1. Egg traits and chemical composition of albumen and yolk according to the hen genotype (mean ±s.d.) (n=30).

Egg traits		Isa Brown	Bianca di Saluzzo
Whole egg	(g)	67.2^{A} ± 4.4	61.4^{B} ± 3.0
Shell	(g)	8.2 ± 1.2	7.6 ± 0.8
	(%)[1]	12.2 ± 1.8	12.4 ± 1.1
Albumen	(g)	41.9^{A} ± 4.2	34.9^{B} ± 2.9
	(%)[1]	62.2^{A} ± 3.4	56.8^{B} ± 2.5
Yolk	(g)	16.5^{B} ± 1.6	18.5^{A} ± 1.1
	(%)[1]	24.6^{B} ± 2.8	30.1^{A} ± 2.3

Chemical composition of albumen and yolk	Albumen		Yolk	
	Isa Brown	Bianca di Saluzzo	Isa Brown	Bianca di Saluzzo
Dry matter (%)[2]	12.4 ± 3.2	12.0 ± 2.6	48.3 ± 3.7	49.7 ± 3.6
Crude protein (%)[2]	10.1 ± 2.2	10.1 ± 2.2	15.1 ± 1.4	16.0 ± 1.1
Lipids (%)[2]	trace	trace	26.7 ± 2.2	27.1 ± 1.9
Ash (%)[2]	0.7 ± 0.1	0.7 ± 0.1	1.6 ± 0.2	1.7 ± 0.2

[1]% of whole egg. [2]% on fresh matter basis. A, B: P≤0.01

Table 2. Blood parameters value according to the hen genotype (mean ±s.d.) (n=42 for total mean; n=14 for T1,T2 and T3 respectively).

	Isa Brown	Bianca di Saluzzo
Erythrocytes (RBC/µl)	2183571 ± 124807	2499261 ± 128108
Leucocytes (WBC/µl)	18077^{a} ± 1372	12895^{b} ± 718
H/L ratio	0.82^{A} ± 0.09	0.33^{B} ± 0.03
albumin (g/dL)	1.62^{B} ± 0.24	1.89^{A} ± 0.15
AGP (µg/ml) (total mean)	446 ± 344	287 ± 58
T1	345^{AY} ± 132	286^{B} ± 63
T2	279^{Y} ± 58	271 ± 36
T3	700^{AX} ± 487	304^{B} ± 70

a, b on the row: P≤0.05; A, B on the row: P≤0.01; X, Y on the column: P≤0.01.

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