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In most developed countries about 90% of hens are kept in cages (Tauson 1998). From 2012 the Council Directive 1999/74/EC will prohibit the use of conventional cages within the European Union but will permit the use of modified cages along with non-cage systems. Therefore, it is crucial to understand the effects of these housing conditions on the current laying hens, which have been selected mainly for production traits. Several studies have reported that heterophil to lymphocyte (H/L) ratio is affected by stressors and can be used as a sensitive haematological indicator of stress response among chicken populations (Gross and Siegel 1983). Acute phase proteins (APPs) are a major group of serum proteins whose concentration is altered during pathophysiological conditions in order to promote a return to homeostasis. Recently it has been suggested that 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 (Murata 2007). Alpha-1 acid glycoprotein (AGP) is a positive APP (increases in response to a challenge), while albumin behaves as a negative APP (falls in response to a challenge) both in mammals and chickens (Adler and others 2001; Murata and others 2004). The aim of this study was to investigate whether the exposure of laying hens to different housing systems was associated with H/L ratio, serum AGP and albumin changes over time.

Isa Brown layers used in this experiment came from the same flock. From the day of hatch birds addressed to cage systems were reared in decker breeder cages (200 cm^2 per bird until the age of 4 wk and 400 cm^2 per bird until the age of 17 wk), while birds assigned to free range system were housed on all-litter house (10 birds/m^2 until the age of 8 wk and 6 birds/m^2 until the age of 17 wk). At the age of 18 wk 24 birds were assigned to one of the three different housing system as follows. Birds housed in cages were moved in 6 conventional cages (CC, 550 bird/cm^2, 4 hens per cage, one cup drinker, 10 cm feed trough per hen); 3 modified cages (MC, 750 bird/cm^2, 8 hens per cage, two cup drinkers, 12 cm feed trough and 15 cm perch per hen, a nest box, a dust bath). The lighting regimen was 16L:8D, the temperature was 20-25 °C and the relative humidity was 60-80%. Birds

reared in all-litter house were moved to the free range (FR, one pen, 1.2 bird/m^2, one fountain drinker, one feeder, perches, 4 birds/nest) when the natural photoperiod (end of april 2008) was 15L:9D. The average monthly temperature (min and max) and relative humidity were respectively: 12.1-20.8 °C and 71.5% (April); 12.1-20.8 °C and 71.5% (May); 16.3-24.8 °C and 75.5% (June); 17.1-27.1 °C and 70.1% (July); 17.0-26.7 and 69.9% (August). Feed and water were provided *ad libitum*. All the animals were fed the same commercial diet (11.5 MJ metabolizable energy/kg feed; 16.6% crude protein) and were allowed to acclimate to their environment before the experimentation began. Individual body weight was recorded at the age of 18, 22, 30 and 40 wk. Feed consumption and egg production were recorded during the periods 20-22, 28-30 and 36-38 wks of age. Fourteen birds, individually identified using a shank ring, were bled by venipuncture (needle size 21 G) 3 times within each group. Blood samples were obtained via brachial vein after 15 days (T1), 2 months (T2) and 4 months (T3) upon arrival at the new accommodation. A total of 5 ml of blood was collected for each bird using one tube containing EDTA and one tube without anticoagulant. A blood smear was prepared from a drop without anticoagulant. Capillary tubes were used in order to control the quantity of blood deposited on the slide. The counts of total red and white cells were determined in improved Neubauer's haemocytometer. Differential count was evaluated according to Campbell (1995) in order to determine the H/L ratio. To obtain serum we left tubes to clot in a standing position at room temperature for about 2 hours. Serum was separated by centrifugation (700 *g*, 15 minutes) and frozen at -80°C until use. Serum AGP concentration (mg/ml) was assayed using a commercially available radial immunodiffusion tray (Cardiotech Services, Inc.). Serum albumin (g/dl) was measured using an automatic chemistry analyzer (Instrumentation Laboratory 300 plus). After checking normality and homogeneity of variances data were processed using SPSS software package (2003). Data were analysed by ANOVA (repeated measures for blood parameters and body weight) followed by Tukey's post-hoc test. The main factors were the housing system between groups and the time of sampling within group. Results are presented as mean ± standard deviation (s.d.).

The mean individual body weight resulted 1680±78 g, 1810±85 g and 1875±101 g and 1920±73 g at the age of 18 wk, 22 wk, 30 wk and 40 wk respectively, no statistical differences were found between groups. Egg-laying rate resulted 55.7% (CC), 54.2% (MC), 55.2% (FR) for the period 20-22 wks; 91.7% (CC), 92.1% (MC) , 85.1% (FR) for the period 28-30 wks; 89.5% (CC), 86.1% (MC), 80.2% (FR) for the period 36-38 wks. The egg weight range was 48.3-50.2, 54.7-57.2 and 55.1-58.7 for the periods 20-22 wks, 28-30 wks and 36-38 wks of age respectively, non statistical differences were found between groups.

Total count of red and white cells and the H/L ratio did not show statistical differences between groups. These data are presented for each group as mean value of T1, T2 and T3 because no effect of time of sampling was found within groups (Table 1).

In spite of differences in the magnitude of the response between individuals, a clear time course of changes in the concentration of AGP could be defined. On sampling T1, CC and MC groups showed similar high mean values of AGP (1.21 and 1.16 mg/ml) while on T2 (0.32 and 0.37 mg/ml respectively) and on T3 (0.28 and 0.38 mg/ml respectively) showed lower mean values ($P<0.01$) than T1 (Table 2). An opposite trend occurred in FR group: AGP concentration on T1 and T2 was similar (0.34 and 0.28 mg/ml respectively), whereas on T3 (0.70 mg/ml) was significantly higher ($P<0.01$). Albumin trend tended to decrease over time for all groups and the lowest mean value was recorded in FR group (1.62 g/dl). The magnitude of this decrease was significant ($P<0.01$) only for ISA Brown kept in MC.

In this study the H/L ratio was not affected neither by the type of housing system, nor by the time of sampling (Table 1). Data reported in the literature about the interaction between the housing system and H/L ratio are controversial. Campo and others (2008) found that the effect of housing system (litter *vs* free range) on H/L ratio varied from breed to breed and was not significant in three of the five breeds studied. Shini (2003) reported that in Brown layers kept in conventional cages, H/L ratio was significantly higher than in layers housed in modified cages and intensive free range system,

indicating a linkage between the different housing system and an increase in the non-specific immune reactive cells, such as heterophils.

In general, avian species react comparably to mammals on inflammation, infectious diseases or other changes of the homeostasis (Chamanza and others 1999). Recently, some studies regarding mammals 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, Piñeiro and others 2007, Salamano and others 2008).

The hens in the present study were clinically normal and apparently healthy. Their haematology profiles (Table 1) were within the normal range (Campbell 1995) and this together with the absence of clinical signs could support that changes in APP concentrations were not related to infection or disease. A possible explanation for the high AGP values (Table 2) measured at early sampling times (T1) in CC and MC could be that hens kept in cage, being in restricted space, probably were obliged to have intensive stressful interactions between cage mates. The level of overt aggression in a small stable flock decreases in time as each bird learns its relationship with all other individuals, and a hierarchy or peck order is formed (Grigor, 1995). The relevant AGP decrease on T2, and the stabilization recorded on T3, could be due to the dominance-subordinance relationship establishment. However, to confirm this hypothesis behavioural assessments should have been employed. ISA Browns kept under FR conditions recorded an opposite AGP time trend and the lowest albumin mean value (Table 2 and 3). Commercial laying hens, such as ISA Brown, are selected cage-adapted hens for high productive traits, and they could have lost the flexibility to adapt to other types of environments. These genotypes might not be robust enough to face outdoor conditions and this could explain why AGP values in hens reared in FR increased over time, while AGP values in CC and MC reached steady values. Furthermore we observed that a rural Italian breed (Bianca di Saluzzo, particularly adapted to free range systems) showed constant AGP and

albumin concentrations over time (Schiavone and others 2009), even though this breed was reared simultaneously to the FR group of the present study, in the same conditions and in close pens.

In general furnished cages retain many of the advantages of conventional cages without the drawback of severe behavioural restriction (LayWel 2006). For instance, access to a nest site is a high-ranking priority for laying hens, preferred over food as oviposition approaches (Weeks and Nicol 2006) and this behavioural priority can be satisfied by modified cages. Nevertheless in this study AGP values in CC and MC behaved in the same way and MC albumin mean value was significantly lower than value recorded in CC (Table 2 and 3). Anyway the number of sampled animals should be increased along with the number of replications for each housing system.

Further research is needed to define the usefulness of APPs as potential markers of poultry welfare and to evaluate other major APPs in chicken, such as ovotransferrin and ceruloplasmin.

Data of this study suggest that AGP may be a useful tools to evaluate and compare the response of laying hens reared in different housing systems.

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Table 1**:** Erythrocyte and leukocyte count, and heterophil to lymphocyte (H/L) ratio by housing system (n=14; T1, T2, T3* mean ± s.d.)

	Conventional cage	Modified cage	Free range
Erythrocyte (10^6/µl)	2.25 ± 0.66	2.11 ± 0.77	2.18 ± 0.81
Leucocyte (10^3/µl)	17.13 ± 10.30	17.04 ± 8.07	18.08 ± 8.89
H/L ratio	0.74 ± 0.59	0.84 ± 0.50	0.82 ± 0.40

*T1 = 15 days, T2 = 2 months and T3 = 4 months upon arrival at the new accommodation.

Table 2: Serum alpha-1 acid glycoprotein (mg/ml) by housing system and time of sampling* (n=14; mean ± s.d.)

	Conventional cage	Modified cage	Free range
T1*	$1.21^{X} \pm 0.51$	$1.16^{X} \pm 0.69$	$0.34^{Y} \pm 0.13$
T2*	$0.32^{Y} \pm 0.21$	$0.37^{Y} \pm 0.23$	$0.28^{Y} \pm 0.06$
T3*	$0.28^{Y} \pm 0.08$	$0.38^{Y} \pm 0.14$	$0.70^{X} \pm 0.49$
Mean (T1, T2, T3)*	0.61 ± 0.55	0.64 ± 0.53	0.45 ± 0.34

X, Y: $P \leq 0.01$ on the same column for T1, T2 and T3.
*T1 = 15 days, T2 = 2 months and T3 = 4 months upon arrival at the new accommodation.

Table 3: Serum albumin (g/dl) by housing system and time of sampling* (n=14; mean ± s.d.)

	Conventional cage	Modified cage	Free range
T1*	2.00 ± 0.27	1.99^{X} ± 0.16	1.71 ± 0.20
T2*	1.96 ± 0.28	1.82^{X} ± 0.35	1.60 ± 0.19
T3*	1.79 ± 0.19	1.48^{Y} ± 0.19	1.55 ± 0.30
Mean (T1, T2, T3)*	1.91^{A} ± 0.26	1.74^{B} ± 0.31	1.62^{B} ± 0.24

X, Y: P < 0,01 on the same column for T1, T2 and T3
A, B: P < 0.01 on the same line.
*T1 = 15 days, T2 = 2 months and T3 = 4 months upon arrival at the new accommodation.