

# Effect of Genotype and Husbandry System on Blood Parameters, Oxidative and Native Immune Status: Welfare and Implications on Performance of Organic Laying Hens

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**Abstract:** The aim of the present work was to compare the effect of organic production vs standard cage system on welfare and performance of a pure breed (Ancona) vs a commercial strain (Brown Hy-Line) of laying hens through a multifunctional approach (behaviour, tonic immobility, feathers score, antioxidant status of the body, blood parameters, innate immunity and mortality). The trial was carried out in farm of the Dpt. of Applied Biology (University of Perugia), where 17-weeks-old females of Ancona breed (n=200) and Brown Hy-Line (n=200) were divided in 2 homogenous groups of 100 birds each and assigned to cage or organic rearing systems.

The genotype of the hens affected in a degree which depends on rearing system almost all the variables. The feeding and resting activities were higher in caged whereas moving activities were higher in organic hens and especially in Ancona birds. Social relationships showed genotype and rearing system effects being higher in Brown Hy-Line hens particularly when reared in cage. The TI duration was affected by genotype and rearing system being the immobility time longer in Brown Hy-Line. The plumage condition showed a great rearing system effect and genotype effects was observed only between organic groups where Ancona hens showed the better feathers condition. Regarding native immune status serum bactericidal activity showed the highest values in organic hens and mainly in Ancona ones. On the contrary, lysozyme showed greater value in caged hens. The haptoglobin value was lower in organic Ancona group, the same hens showed about 30% greater reactive oxygen substances, but accompanied by highest the antioxidant power. Organic rearing system, mainly in the Ancona hens, induced the highest red blood cells, haemoglobin and hematocrite values. Lymphocytes were higher in Organic birds thus reducing the H/L ratio in Organic hens. As expected, organic birds showed the worst feed efficiency and percentage of deposition, but the lowest mortality rate. In conclusion, on the basis of all these information together with the low mortality rate, Ancona hens showed the better welfare status, mainly when they were organically reared.

**Keywords:** Laying hen welfare, genotype, rearing system.

## INTRODUCTION

A correct assessment of animal welfare should involve multiple indicators such as behaviour, physiology, body injuries, disease and performance [1]. In recent years it has become evident that there is a strong correlation between animal behaviour, stress and the neuro-endocrine and immune systems [2-4]. Therefore, unfavourable environmental conditions could lowered homeostatic functions, such as the immune response and in particular the innate immune system [5] and the health status of animals [6].

Most of the intensive housing and management systems used in commercial poultry farms have been developed in order to reduce the production cost and are not the best with respect to the ethological needs of animals. The welfare of laying hens raised in commercial cages has been placed under intense scrutiny. The housing of egg-type chickens in cages is now widely considered to have a negative effect on the welfare of hens [7-11]. Throughout Europe, concern for

the welfare of laying hens in conventional cages has prompted changes in housing systems [12]. Cage-based systems are being phased out and those that are retained must meet high welfare standards [13]. The EC Directive 74/99 establishes the minimal requirements for hen protection and specifies new guidelines that provide for the progressive replacement of conventional cages that are to be completely eliminated by January 1, 2012 (European Commission, 1999). This directive stated that all existing cages must meet the 750 cm<sup>2</sup>/bird space requirements and that each cage must be enriched with facilities that will allow birds to express their normal behaviours.

Among the different alternative farming systems organic egg production has increased during the last 15 years. In 2006, the market share in Italy of organically-produced eggs was 7.6% of all the organic products in retail and over a two-year period the request for organic eggs increased by 4.6% [14].

To date, there has been no clear evidence that welfare is improved when hens are raised under the organic production system. Few studies have been published on this subject and the results are conflicting, due to the great variation in the

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breeds used, production methods, diets used, pasture availability. Some studies [15, 16] and review [10, 17] concerning the effect of conventional and *free-range* systems on productivity and product quality did not establish one system as being better than the other [18-23]. The overall main opinion is that successful organic farming will depend on the right combination of layout and management of the henhouse and free range on the one hand, and on the choice of the right breed of hen on the other, thus the balance between these aspects will probably be of major importance. Regarding the genotype of the hens to be used in order to assure a good welfare status, the EC Regulation 1804/99 and the final recommendation of Network for Animal Health and Welfare in Organic Agriculture (2003), suggest utilizing local breeds for their higher rusticity. Hybrid birds selected to produce under highly controlled conditions, seem to be quite unsuitable for extensive systems, such as the organic one because the environment is less controlled and the rations are less equilibrated. The use of less selected strains, which still conserve natural behaviours, could also be a valuable alternative, particularly if they are in danger of extinction [24]. Italy is the country of origin of some egg-type chicken strains that have seen a drastic decline in number; the Ancona breed which was widespread through Europe is an example. It has good productivity (about 280 eggs/year); the eggs are white and weigh 54-56 g [25]. As other pure breeds, Ancona has been progressively replaced by hybrids expressly selected for intensive egg production.

Being animal welfare a “state” [1] that encompasses many complex aspects of the animals it includes biological, psychological and ethical components. The biological components can be further divided into physical, physiological and behavioural. Most of the physical components of welfare are easy to determine, as it includes parameters traditionally used by the producers to evaluate performance and health. Behaviour is frequently used by experienced farmers to determine potential problems in animals [26] and the status of birds’ integument has recognised of considerable impact on the interpretation of bird health and welfare [27, 28]. Physiological parameters which include hormone levels such as cortisol or corticosterone, heart rate or immune status [29-30] are frequently used as reliable indicators of the welfare status as well [31-33]. Moreover the haematological stress indicator H/L is expected to increase if hens experience mild to moderate long-term stress [34]. Thus the aim of the present work was to compare the effect of organic production vs standard cage system on welfare (through a multifunctional approach as behaviour, tonic immobility, feathers condition, antioxidant status of the body, blood parameters and innate immunity) and performance of a pure breed (Ancona) vs a commercial strain (Brown Hy-Line) of laying hens.

## MATERIAL AND METHODS

### Animals, Housing and Feeding

Five hundred 1-day-old chicks of the Ancona and two hundred 1-day-old female chicks of the Brown Hy-Line genotypes were reared during the first four weeks under identical conditions in floored pens, covered with wood shavings. The floored pens were located indoors in an environmentally-controlled building at the farm of the Dpt.

of Applied Biology (University of Perugia). All animals were vaccinated against Marek and Newcastle diseases; the beaks were not trimmed and no other pharmacological treatments were given.

At 4-weeks-of-age, the Ancona females were divided in two homogenous groups of 100 birds each and the Brown Hy-Line were divided in two homogeneous groups, too. Two groups of them, the Control groups (Brown Hy-Line and Ancona; CB and CA, respectively), were housed in cages, under standard rearing conditions (single bird cages, 0.75m<sup>2</sup> of three tier batteries that were provided with a linear automatic feeder and drinker; at 17 weeks-of-age an artificial photoperiod of 16 h per day of light was applied. The building was under a controlled ventilation regime (10 m<sup>3</sup>/hen/h), the temperature ranged from 15 to 29 °C (mean/average daily temperatures, winter 13-17, and summer, 23-35, extremes) and the relative humidity ranged from 50 to 80%.

The lasts groups were transferred to a farm with building labeled for the requirements of the Council Directive 1999/74 and Directive 1804/99 regarding organic production. Both groups (Brown Hy-Line and Ancona; OB and OA, respectively) were reared in two covered, straw-bedded houses (6 bird/m<sup>2</sup>) with access to two pens with natural grazing (10 m<sup>2</sup>/bird). Feed and water were provided indoors with manual feeders and automatic drinkers respectively. Inside the paddocks, there was a small hut with nests (1 per 6 hens) and perches. Environmental temperature, humidity and photoperiod were the natural ones. A conveyor belt running along the back of the nests collected the eggs.

All the animals were reared according to Italian directives [35] on animal welfare for experimental and other scientific purposes along the year 2008.

A standard layer feed was given *ad libitum* to all groups; for organic groups more than 90% of the ingredients (maize, wheat and whole soybean) were organically grown. The nutritive characteristics of the feed were similar for all the groups: the main differences regarded the vitamins which, for organic groups, were in lower amount and coming from natural fonts; conventional diets had greater vitamin quantity of synthetic origin.

### Behavioural Observations

Behavioural observations, Tonic Immobility Test (TI) and plumage evaluation were performed during four seasons, in a period of 5 days each. Ten animals per group were randomly selected and marked with different colours on the tip of the tail. Behavioural observations were recorded during three-hour periods in the morning (9.00-12.00) and afternoon (15.00-18.00) using the focal animal scan sampling method [36]. Before each observation session, 5 minutes were allowed for the animals to adapt to the presence of observers. The behavioural observations included: moving (walking, running and foraging), resting (standing, lying), feeding (food and water), comfort (dust bathing, self preening, scratching and starching), social relationships (allo-preening) and gentle and severe pecking others [37]. Behaviours were recorded on a custom-designed table, and their respective frequencies were calculated as a percentage of the total observed behaviours. Since no differences were found between days and hours, all data

were pooled to obtain a mean value. At the end of behavioural observation birds were caught and submitted previous to the TI test [38] and then to plumage condition evaluation [28].

TI was induced by restraining the birds on their backs in a U-shaped wooden cradle for 10 s [38]. A bird was defined as being in a state of TI if it remained immobile for a minimum of 10 s after restraint had ended. A maximum of three inductions and a test ceiling of 3 min in TI were applied. The total duration of TI, i.e. until the bird righted itself, was recorded.

The plumage scoring system assigned values of 1 to 4 points for each trait [28], where a score of 4 implied the best and a score of 1 the worst condition. The 6 parameters (neck, breast, cloacae/vent, back, wings and tail) for plumage condition were summarized, implying a total score ranging from 6 to 24 points.

### Sample Collection and Analytical Determinations

Blood samples for analyses were collected during four seasons, in all the different phases of the productive activity from the same ten birds after feather evaluation in the last day of behavioural observation. After collection from the brachial vein, blood samples were immediately sent to the laboratory where they were centrifuged and frozen at -80 °C until analysis. Blood samples for hematocrite were collected in heparinized capillary tubes and centrifuged in a micro hematocrite centrifuge for 7 min. Serum lysozyme was measured by a lyso-plate assay [39], carried out at 37 °C for 18 minutes, in a humidified incubator. The method is based on the lyses of *Micrococcus lysodeikticus* in 1% agarose. The diameter of the lysed zones was measured with a ruler and was compared with the lysed zones of a standard lysozyme preparation (SIGMA, Milan, Italy, M 3770). The serum bactericidal activity (SBA) was performed according to a previous method validated for cattle [40]. The test is based on the challenge of sera with no pathogenic *E. coli* and its concentration was expressed in %.

The haemolytic complement assay (HCA) [41] was carried out in microtitre plates. The complement titre is the reciprocal of the serum dilution causing 50% lyses of red blood cells of ram (CH<sub>50</sub>).

Haptoglobin (Hp) was measured by a commercial kit (Phase Haptoglobin Colorimetric Assay, Tridelata Development Ltd, Kildare, Ireland) according to the manufacturer's directions. The test is based on the different peroxidase activity in acidic environment of haptoglobin-hemoglobin complexes and free hemoglobin, respectively. Briefly, a known amount of hemoglobin is added at low pH to the serum sample; the residual peroxidase activity of hemoglobin is directly proportional to serum haptoglobin concentration.

Reactive oxygen species (ROS) and the Antioxidant Power of plasma (AP) of the sample were evaluated by a commercial kit (Diacron, Grosseto, Italy).

The leukocyte counts have been done on two drops of blood, and blood smears were made on duplicate glass slides. Both the slides were counted and the means were calculated for each bird. These smears were stained with Wright stain in 15 min. One hundred leucocytes, including

heterophils, lymphocytes, monocytes and eosinophils were counted on each slide. The H/L ratio was also calculated.

### Productive Performance

Data for calculating the % of deposition, feed intake (FI) and bird mortality were recorded per cage/pen throughout the productive cycle by the farm workers. Feed consumption per cage/pen was recorded weekly. Mortality was recorded daily, while hen live weight (LW) was recorded at the end of the blood sampling.

### Statistical Analyses

A linear model [42] was used to assess the effects of genotype and of rearing system and their interactions. For simplicity of exposition the season/age effect was not showed. Significance of the differences was assessed by the multiple t-tests and X-square was used for the non-parametric variables.

## RESULTS

Generally, the genotype of the hens affected in a degree which depends on rearing system almost all the variables. Ancona breed was the responsible of such an interaction due the different response of the birds to the rearing system.

**Behaviour of birds** (Table 1). The feeding activity was higher in CB birds respect all other group, whereas resting activities were greater in CA birds, intermediate in CB and OB groups and lower in OA bird. Moving activities were higher in organic hens and especially in OA birds, while comfort activities were higher in OB and CA hens. Social relationships, in particular for allo-preening and gentle pecking behaviours, showed genotype and rearing system effects, showing Ancona hens the higher social relation ship percentage, whereas gentle/severe peaking were higher in Brown Hy-Line hens particularly when reared in cage; on the contrary OA showed the lowest value. It is important to underline that genotype did not affect the amount of severe pecking (0.5 vs 0.1 %, caged and organic hens, respectively) that occurs in close proximity of nests.

**TI test and plumage conditions** (Table 1). The TI time was affected by genotype and rearing system being the immobility time longer in Brown Hy-Line. Regarding Ancona hens the caged group showed the higher time of immobility. The plumage condition showed a great rearing system effect and genotype effect was observed only between organic groups where Ancona hens showed the better feathers condition.

**Innate immunity and oxidative parameters** (Table 2). SBA showed the highest values in organic hens and mainly in Ancona ones. On the contrary, lysozyme showed greater value in caged hens. The HP value was lower in OA group, whereas ROS were about 30% greater in organic hens. The highest AP value was shown by OA group.

**Hematic parameters** (Table 3). Organic rearing system, and within organic the Ancona hens, showed the highest red blood cells, haemoglobin and hematocrite values. Lymphocytes were higher in Organic birds thus reducing the H/L ratio in Organic hens. Monocytes and Eosinophils showed different trends: both groups of Ancona showed the

**Table 1. Effect of Genotype and Rearing System on Behavior, TI and Feather Condition in Laying Hens**

		Brown Hy-Line		Ancona		X <sup>2</sup>
		Control	Organic	Control	Organic	
Feeding	%	33.1 <sup>b</sup>	19.2 <sup>a</sup>	15.4 <sup>a</sup>	14.4 <sup>a</sup>	2.2
Resting	“	37.6 <sup>b</sup>	35.3 <sup>b</sup>	45.5 <sup>c</sup>	20.4 <sup>a</sup>	4.1
Moving	“	10.9 <sup>a</sup>	20.3 <sup>b</sup>	13.8 <sup>a</sup>	47.7 <sup>c</sup>	3.5
Comfort	“	8.7 <sup>a</sup>	17.4 <sup>bc</sup>	19.2 <sup>c</sup>	14.1 <sup>b</sup>	2.4
Social relationships	“	0.9 <sup>a</sup>	1.0 <sup>a</sup>	3.6 <sup>b</sup>	3.0 <sup>b</sup>	0.5
Gentle/severe peaking		8.9 <sup>d</sup>	6.8 <sup>c</sup>	2.5 <sup>b</sup>	0.4 <sup>a</sup>	0.9
						<i>Pooled SE</i>
TI	s.	127.5 <sup>c</sup>	156.3 <sup>d</sup>	93.8 <sup>b</sup>	32.5 <sup>a</sup>	21.2
Feather score	tot.	15.9 <sup>a</sup>	17.5 <sup>b</sup>	16.5 <sup>ab</sup>	24.0 <sup>c</sup>	4.1
Neck		3.0 <sup>a</sup>	4.0 <sup>b</sup>	3.0 <sup>a</sup>	4.0 <sup>b</sup>	0.5
Breast		3.0 <sup>a</sup>	4.0 <sup>b</sup>	3.0 <sup>a</sup>	4.0 <sup>b</sup>	0.4
Cloaca/Vent		2.5 <sup>a</sup>	2.0 <sup>a</sup>	2.5 <sup>a</sup>	4.0 <sup>b</sup>	1.1
Back		3.0 <sup>a</sup>	3.0 <sup>a</sup>	3.0 <sup>a</sup>	4.0 <sup>b</sup>	0.7
Wing		2.2 <sup>a</sup>	2.5 <sup>a</sup>	2.5 <sup>a</sup>	4.0 <sup>b</sup>	0.4
Tail		2.2 <sup>a</sup>	2.0 <sup>a</sup>	2.5 <sup>a</sup>	4.0 <sup>b</sup>	0.5

N = 40 per group (10 observed hens per 4 seasons); a..d: P<0,05.

**Table 2. Effect of Genotype and Rearing System on Innate Immunity and Oxidative Parameters in Laying Hens**

		Brown Hy-Line		Ancona		<i>Pooled SE</i>
		Control	Organic	Control	Organic	
Lysozyme	µg <sup>-1</sup> ml	7.4 <sup>c</sup>	3.2 <sup>b</sup>	8.7 <sup>c</sup>	1.4 <sup>a</sup>	3.2
SBA	%	46.9 <sup>a</sup>	48.3 <sup>ab</sup>	49.7 <sup>ab</sup>	51.9 <sup>b</sup>	4.8
Complement	CH <sub>50</sub>	103.3	109.8	106.8	102.7	30.2
Hp	mg/ml	0.54 <sup>b</sup>	0.49 <sup>b</sup>	0.48 <sup>b</sup>	0.24 <sup>a</sup>	0.21
ROS	mM H <sub>2</sub> O <sub>2</sub>	77.4 <sup>a</sup>	101.8 <sup>b</sup>	67.2 <sup>a</sup>	101.7 <sup>b</sup>	24.1
AP	µM HClO ml <sup>-1</sup>	245.5 <sup>a</sup>	216.1 <sup>a</sup>	215.5 <sup>a</sup>	301.2 <sup>b</sup>	39.8

n=40 per group (10 blood samples per 4 seasons); a..c: P<0,05.

highest values of Monocytes, while caged hens showed the highest values of Eosinophiles.

**Productive traits** (Table 4). As expected, OA hens showed the lower live weight and feed index. Organic birds showed the worst feed efficiency and percentage of deposition. Ancona birds showed a lower egg laid and lower number of marketable eggs (egg laid out nest and broken or cracked eggs). On the contrary, organic hens showed the lowest mortality rate.

## DISCUSSION

For decades, animal breeding has focused on maximizing production traits. Such a selection pressure induces farm animals to allocate a large portion of their resources to a particular production trait, reducing their ability to respond to other demands (response to environmental stimuli, immunity, etc). Accordingly, even the natural behaviour was affected. Indeed, selection for feed conversion efficiency

(low residual food intake) has been found to be correlated with lower levels of activity compared to less efficient animals [43]. Our results confirm such assumption: selected hens had a higher feed and ME intake, which should compensate birds for an enhanced energy expenditure of higher egg production. On the same time Hy-lyne had greater percentage of resting behaviour also when reared in organic condition. On the contrary, Ancona hens, showed the lowest feeding efficiency and feed intake and, when the possibility was given, performed less resting at favour of kinetic (walking, running, foraging and exploring) activities. However, even the more productive strain positively respond to free range system: organic hens of both genotypes showed lower feeding and higher moving due to the given possibility of explore the environment and to forage. According to previous works [44] cage avoids the occurrence of some natural behaviours and animals supply to boredom through increasing their feeding activity.

**Table 3. Effect of Genotype and Rearing System on Hematic Parameters in Laying Hens**

		Brown Hy-Line		Ancona		Pooled SE
		Control	Organic	Control	Organic	
Red cells	10 <sup>6</sup> /ml	2.1 <sup>a</sup>	2.4 <sup>b</sup>	2.4 <sup>b</sup>	2.7 <sup>c</sup>	0.79
Hemoglobin	g/dl	14.9 <sup>a</sup>	14.5 <sup>a</sup>	14.0 <sup>a</sup>	16.5 <sup>b</sup>	4.15
Hematocrite	%	30.1 <sup>a</sup>	31.7 <sup>a</sup>	28.8 <sup>a</sup>	34.2 <sup>b</sup>	7.24
Heterophils (H)	%	36.3 <sup>b</sup>	30.0 <sup>a</sup>	38.6 <sup>b</sup>	26.5 <sup>a</sup>	4.48
Lymphocytes (L)	“	56.0 <sup>a</sup>	66.2 <sup>b</sup>	51.5 <sup>a</sup>	68.5 <sup>b</sup>	8.28
H/L		0.65 <sup>b</sup>	0.45 <sup>a</sup>	0.75 <sup>c</sup>	0.39 <sup>a</sup>	3.15
Monocytes	%	3.6 <sup>a</sup>	6.0 <sup>b</sup>	6.4 <sup>b</sup>	5.8 <sup>b</sup>	3.15
Eosinophiles	“	4.0 <sup>b</sup>	2.0 <sup>a</sup>	3.6 <sup>b</sup>	1.5 <sup>a</sup>	0.98

n=40 per group (10 blood samples per 4 seasons); a..c: P<0,05.

**Table 4. Effect of Genotype and Rearing System on Some Productive Traits of Laying Hens**

		Brown Hy-Line		Ancona		Pooled SE
		Control	Organic	Control	Organic	
Live weight*	kg	2.07 <sup>d</sup>	1.96 <sup>c</sup>	1.89 <sup>b</sup>	1.69 <sup>a</sup>	0.09
Feed intake <sup>†</sup>	g d <sup>-1</sup>	121.4 <sup>b</sup>	120.6 <sup>b</sup>	110.6 <sup>a</sup>	114.0 <sup>a</sup>	6.0
ICA**		2.52 <sup>a</sup>	2.95 <sup>b</sup>	2.84 <sup>b</sup>	3.35 <sup>c</sup>	0.15
Egg deposition	%	74.1 <sup>b</sup>	63.8 <sup>a</sup>	70.9 <sup>b</sup>	68.1 <sup>b</sup>	9.8
Egg laid	g d <sup>-1</sup>	48.2 <sup>c</sup>	40.8 <sup>b</sup>	39.0 <sup>b</sup>	34.0 <sup>a</sup>	4.6
Egg laid out nest	%	-	2.4	-	1.0	0.2
Broken or cracked eggs	“	0.5 <sup>a</sup>	2.7 <sup>c</sup>	1.1 <sup>ab</sup>	1.6 <sup>ab</sup>	0.4
Mortality***	%	4.3 <sup>c</sup>	2.5 <sup>b</sup>	4.1 <sup>c</sup>	1.6 <sup>a</sup>	0.7

\*: N = 100 per group; \*\*: fed for egg laid<sup>-1</sup>; \*\*\*: X<sup>2</sup>; a..b.: P<0,05.

Tonic immobility evaluates fearful behaviour in the chicken [45] that represents a terminal defensive reaction and can be used as criterion for measuring the wellbeing and stress levels of the birds. Ancona hens organically reared gave the better response in term of tonic immobility and plumage condition. According to some Authors [46] that have reported that gentle pecking resembling a stereotypy, the higher rate of gentle pecking was found in caged hens. Regarding feathers conditions both the effects (genotype and housing condition) were significant; in fact it is largely documented that feather pecking is a multi-factorial problem affected by the genetic background of the birds [47-49] and group size and stocking density [50-52]. According to these Authors Brown Hy-Line hens performed the higher percentage of gentle pecking the other bird feathers that probably worsened their plumage condition. More over Brown Hy-Line hens are a typical commercial hybrid selected for egg production and Bestman and Wagenaar [53] described that some degree of feather pecking was seen in 71% of organic layer flocks of 50 weeks and older. They also described that the degree of feather pecking in flocks was less when more hens used the outdoor run as in the case of organic Ancona. These “behaviour” results can be interpreted as a greater welfare status in birds reared in more extensive conditions. Such hypothesis was confirmed by

their better innate immunity and oxidative status evaluation mainly in pure breed.

Regarding innate immunity, lysozyme is a strong antibacterial enzyme (against Gram<sup>+</sup>) that has a synergic action with immune humoral response and factors of the serum complement [54]. It is essential related with the function of the macrophage system and basically indicates the presence of inflammation. SBA is a major parameter of innate immunity. The capacity of the serum to inhibit bacteria growth is assessed by the presence of complement factors and modulates the concentrations of natural antibodies against some more ubiquitous environmental bacterial agents, mainly *Enterobacteriaceae* (Gram<sup>-</sup>). It gives same indications about the defence mechanisms of the animal that activates the complement system. The HCA is a test that shows the complement activity and is of great help in assessing the risk of infectious disease onset or the severity of already existing pathologies. Confirming behavioural results, AO hens showed better values for lysozyme and SBA. This hypothesis is confirmed by the lower value of HP; that is considered to bind free haemoglobin (Hb) derived from inflammation processes in a stoichiometric ratio *in vivo* and *in vitro*. The resultant HP-Hb complex is rapidly removed from the circulation by the reticuloendothelial system [55]. Moreover, these animals

showed the higher moving and foraging activities and the consequent higher ROS haematic concentration, but accompanied by an increased haematic AP value that permitted to animal to adapt to the higher oxidative metabolism [56]. Oxidative stress, resulting from an increased production of free-radicals and ROS and/or a decrease in AP, makes damage to biological macromolecules and disturbs normal metabolism and physiology [57]. Oxygen free-radicals generated during biological processes are involved in the pathogenesis of several diseases and various reports have indicated that oxidative stress alters immune competence [58]. High level of ROS significantly reduces the primary and secondary antibody responses; further, the presence of sub-inflammatory process, evidenced by ROS and haptoglobin, enhances the lysozyme release by neutrophils and macrophages and reduces the free complement which is mainly found in immuno-complexes.

Concerning haematic traits, heterophils increase and lymphocytes decrease when chickens are stressed, so that the ratio between them is a good index of response to a stressor. There is a genetic component to heterophils and lymphocytes responses to stressors [59]. Bayyari *et al.* [60] reported that lymphocyte numbers were lower in a line of turkeys selected for heavier BW than in a line selected for increased egg production. The differences for heterophils, lymphocytes, and the ratio between them observed in our experiment may be caused both by breed and housing. Gross and Siegel [61] suggested that reference values for the heterophil to lymphocyte ratio of about 0.2, 0.5, and 0.8 are characteristic of low, optimal, and high degrees of stress, respectively. On the basis of this affirmation organic hens showed H/L values comprised between a low and optimal degrees of stress, on the contrary the caged ones showed values near a high degree of stress and between those the Ancona hens, being the more active suffered more of special constraining.

As previously affirmed, the question arises whether the selection for higher productivity affects the ability of animal to respond to environmental stressors. Responding to stress (i.e., adapting to a changing situation to maintain homeostasis) could be theoretically an energy demanding process. Many stressors that animals encounter are immunological challenges. Provided that having and activating an immune system is energetically costly [62, 63], animals would have to make a trade off between immune response and maintaining production level. Being genetically programmed to efficient production, selected animals might have an impaired ability to make this trade-off, meaning that they are less capable to cope with an immune-related stress. According to this, beside an obvious genotype effect on the native immune and oxidative parameters analysed, less productive hens if requested to enhance their natural defence (OA) seem to be better adapted, probably linked to their lower productive performance that allowed to maintain their physiological homeostasis.

In conclusion, on the basis of all these information (greater AP response to ROS production, lower lysozyme, higher SBA, lower HP concentration, lower H/L) together with the low mortality rate it is possible to affirm that the Ancona hens showed the better welfare status, especially when they were organically reared.

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