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Adaptation to organic rearing system of eight different chicken genotypes: behaviour, welfare and performance

Cesare Castellini[a], Cecilia Mugnai[b], Livia Moscati[c], Simona Mattioli[a], Monica Guarino Amato[d], Alice Cartoni Mancinelli[a] and Alessandro Dal Bosco[a]

[a]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, University of Perugia, Perugia, Italy; [b]Dipartimento di Scienze degli Alimenti, University of Teramo, Teramo, Italy; [c]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy; [d]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Ministero delle Politiche Agricole Alimentari e Forestali, Roma, Italy

ABSTRACT

The aim of the study was to define poultry adaptability to organic system, through the assessment of several endpoints. Eight hundred male birds of slow-growing birds (Ancona: A, Leghorn: L, crossbreed Cornish × Leghorn: CL), medium-growing (Gaina: G, Robusta Maculata: RM, Kabir: K, Naked Neck: NN) and fast-growing strains (Ross: R) were organically reared. A and L genotypes displayed a quicker reaction time when submitted to tonic immobility test, and a great variety of behaviour and exploiting all the pasture area. Concerning feather conditions L, A, CL G and RM showed the best values for all considered body regions, as well as the absolute absence of foot pad and breast blister lesions. Static behaviour of R and G chickens did not produce a significant oxidative burst whereas, the active behaviour of A, slow-growing birds, increased the oxygen demand. Plasma α-tocopherol followed the trend of kinetic and foraging activity being higher in slow-, intermediate in medium- and lower in fast-growing birds. The adaptability index showed the best result of slow-growing strains with intermediate results in medium-growing and the worst in fast-growing ones. There is a negative linear correlation between adaptation and daily weight gain. However, within the same sub-group (slow, medium and fast), there is no correlation between daily weight gain and adaptation to an organic system. Even if R chickens had the highest productive performance, they appeared no adapted to the organic system. Daily weight gain (<50 g/d) is a prerequisite for chicken adaptation, but even birds with similar weight gains showed wide variations in the adaptation.

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Behaviour; chicken genotype; organic rearing system; performance; welfare

Introduction

The Article 12 of Regulation (EC) n. 889/2008 provides that each Member State has to define the criteria for the definition of slow-growing poultry genotypes and to evaluate their adaptability to the organic system. This regulation establishes that '.in organic livestock production, the choice of breeds should take into account of their capacity to adapt to local conditions, their vitality and their resistance to disease and a wide biological diversity should be encouraged.' Therefore, the best equilibrium between animal welfare, adaptability to environment, biodiversity and productive performance should be found. Within the European Union, the choice of genotypes is based only on the daily weight gain; others have opted for egg-type chickens, but the definition of slow-growth and the relationship between growing rate and adaptability to the organic system is still unclear.

There are cases where improvement of welfare determines a reduction of the production costs (e.g. decrease disease and mortality) and an increase of people's perceptions related to sustainable systems (Napolitano et al. 2013). On the other hand, some behavioural pattern (e.g. kinetic and foraging activity), positively related to the bird welfare and meat quality, negatively affect the weight gain (Branciari et al. 2009).

On the basis of these considerations, the aim of the present study was to evaluate the behavioural aspects and welfare indicators of eight different chicken genotypes through a multifunctional approach (behaviour, tonic immobility (TI), feathers condition, presence of body lesions, antioxidant and immune status) in order to assess their adaptability to organic system.

CONTACT Cesare Castellini cesare.castellini@unipg.it Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università di Perugia, Borgo XX Giugno 74, Perugia 06100, Italy

Materials and methods

Birds, diets and slaughtering

The trial was performed at the experimental section of the Department of Agricultural, Food and Environmental Science of Perugia (Italy) from September to November 2013 and chickens were reared according to EU Regulation 834/07, EU Regulation 889/2008 and Italian directives (Italian Regulation 1992) on animal welfare for experimental and other scientific purposes.

Eight hundred male birds of the following genotypes (100 animals/genotype) were compared: Ancona (A), Leghorn (L), crossbreed Cornish × Leghorn (CL), Kabir (K), Naked Neck (NN), Robusta Maculata (RM), Gaina (G) and Ross 308 (R). On the basis of our previous studies (Castellini et al. 2002a, 2002b; Dal Bosco et al. 2014a, 2014b) and commercial information, the genotypes were sub-categorised with regard to their growth rate: slow (GR $<$ 24 g/d; A, L, CL), medium (25 $<$ GR $\leq$ 40 g/d; G, RM, K, NN) or fast-growing (GR $>$ 41 g/d; R).

The L, A and RM genotypes originated from conservation flocks maintained at the Department of Agricultural, Food and Environmental Science of Perugia since the 1960s. CL chicks were produced by crossing L hens with Cornish fowl, whereas G, K, NN and Ross 308 were furnished by a commercial poultry farm (Avicola Berlanda, Italy).

Chickens were kept after hatching until 20 d of age in a poultry house in separate pens, with temperatures ranging from 20 to 32 °C and with relative humidity ranging from 65 to 75%. Incandescent light (30 lux) placed at bird level was used for heating and illumination. Chicks were vaccinated against Marek and Newcastle diseases.

At 21 d of age, chicks were transferred to straw-bedded indoor pens (0.10 m^2/bird), each equipped with feeders and drinkers and with free access to forage paddock (4 m^2/bird). Each genetic strain was replicated in four pens containing 25 chicks each. Birds were confined to indoor pens during the night.

The pasture was not treated with pesticides or herbicides during the 3 years prior to organic production. The pasture also contained mature trees, bushes and hedges.

Birds were raised until the *minimum* slaughter age (81 d).

Chickens were fed *ad libitum* the same experimental starter (1–21 d) and grower-finisher (22 d to slaughter) diets containing organic ingredients, with the exception of 5% of GM-free soybean meal and vitamin-mineral premix (Table 1).

Table 1. Ingredients and calculated analysis of poultry diets.

	Starter	Finisher
Ingredients (%)		
Maize	52	46
Full fat soybean	30.5	12.5
Wheat	–	20.0
Soybean meal[a]	9.00	14.0
Alfalfa meal	2.80	2.80
Gluten feed	3.00	2.00
Vitamin-mineral premix[b]	1.00	1.00
Dicalcium phosphate	1.00	1.00
Sodium bicarbonate	0.50	0.50
NaCl	0.20	0.20
Chemical composition		
Dry matter (%)	90.9	90.8
Crude protein (%)	22.3	18.0
Ether extract (%)	7.95	4.98
Crude fibre (%)	4.67	4.01
Ash (%)	5.76	5.59
NDF – neutral detergent fibre (%)	10.7	10.1
ADF – acid detergent fibre (%)	5.58	5.06
Cellulose (%)	4.22	3.56
ADL – acid detergent lignin (%)	1.03	1.11
Hemicellulose (%)	5.16	5.05
Metabolisable energy[c], MJ/kg	12.5	12.9

[a]5% from conventional crops.
[b]Amount per kg: Vit. A 11 000 U; Vit. D_3 2000 U; Vit. B_1 2.5 mg; Vit. B_2 4 mg; Vit. B_6 1.25 mg; Vit. B_{12} 0.01 mg; α-tocopherol acetate 30 mg; Biotin 0.06 mg; Vit. K 2.5 mg; Niacin 15 mg; Folic acid 0.30 mg; Pantothenic acid 10 mg; Choline chloride 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg and Co 0.5 mg.
[c]Estimated according to Carrè and Rozo (1990).

Access to feed and water was freely available, and the two diets were formulated to contain adequate nutrient levels as defined by the NRC (1994). Individual body weights were recorded weekly, as well as the collective feed intake of each pen. The average feed consumption of the group was used to calculate the feed:gain ratio. The number of culled and dead birds was recorded. At 81 d, a sample of five birds per genotype per pen, each weighing between ± 10% of the group mean, were slaughtered in the processing plant of the Department of Agricultural, Food and Environmental Science of Perugia, 12 h after feed withdrawal. Just before slaughtering, birds of the six groups were subjected to plumage scoring and blood samples were collected.

Behavioural observation

Behavioural observations were recorded at 11 weeks of age in a period of 5 d each. Five animals per pen were randomly selected and marked with different colours on the tip of the tail. Behavioural observations were recorded during 3-h periods in the morning (9:00–12:00) and afternoon (15:00–18:00) using the focal animal scan sampling method (Martin & Bateson 1986). Before each observation session, 5 min were allowed for

the animals to adapt to the presence of observers; in this period, the interest shown by the birds towards the observer and the time spent outdoors or indoors were registered according to Lewis et al. (1997).

The behavioural observations included: feeding (feed from feeders and water from drinkers), moving (walking, running and foraging), resting (standing idle, lying on the sternum), comfort (dust bathing, self-preening, wing flapping, scratching and starching), and others (allopreening as gentle and severe pecking others, as described by Kjaer and Sørensen (1997)). Birds' behaviours were recorded on a custom-designed table, and their respective frequencies were calculated as a percentage of the total behaviour. Since no differences were found between days and hours, all data were pooled to obtain a mean value.

The maximum distance from the house was calculated as the distance reached by chickens during the observation. Time spent outdoor was expressed as the percentage of outdoor birds with respect to the total birds.

At the end of behavioural observation, birds were caught and submitted to the TI test that was induced by restraining the birds on their backs in a U-shaped wooden cradle for 10 s (Gallup 1979). 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.

At slaughter, in all birds the plumage condition and footpad lesions (FPD) were assessed. The plumage condition evaluation was assessed following a 4-point scale for each trait, where a score of 4 implied the best condition and a score of 1 the worst one (Tauson et al. 2005). The six parameters (neck, breast, cloacae/vent, back, wings and tail) for feather condition score were summarised, implying a total score ranging from 6 to 24 points. The FPD was recorded by assigning different classes to 1 of 3:0 = no mark (no lesion), 1 = mild lesions (superficial lesions, erosions, papillae and discolouration of the footpad), or 2 = severe lesions (deep lesions, ulcers and scabs) (Berg 1998). The presence of breast blisters (BBs) on the carcass was also recorded.

Oxidative status, native immunity and blood parameters

Immediately before slaughter, 5-ml blood samples were collected within 2 min of removing the birds from its pen. 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 haematocrit were collected in heparinised capillary tubes and centrifuged in a micro haematocrit centrifuge for 7 min.

Reactive oxygen species (ROS) and the antioxidant power of plasma (AP) of the samples were evaluated by a commercial kit (Diacron, Grosseto, Italy). The α-tocopherol level of plasma was assessed according to Schuep and Rettenmeier (1994).

The haemolytic complement assay (HCA) was carried out in microtitre plates (Seyfarth 1976). The complement titre is the reciprocal of the serum dilution causing 50% lyses of red blood cells (RBC) of ram (CH50). The volumes of the reagents were modified to perform the test in microtitre plates at a final volume of 125 µl/well (100 µl of serum dilutions + 25 µl of 3% rabbit erythrocytes). The 0 and 100% haemolysis controls were set up in each plate at the same volume in Veronal buffer (pH 7.3) and distilled water, respectively. Titres were expressed as 50% haemolytic units per 100 microlitres (the test volume of sera).

The serum bactericidal activity (SBA) was performed according to a previous method validated for cattle (Amadori et al. 1997). The test is based on the growth of non-pathogenic *Escherichia coli* until long phase in 20 ml of brain heart infusion broth; for each test, one aliquot was incubated at 37 °C until an optical density of 590 nm. Then, bacteria were diluted 1:100 in sterile saline solution. Test reagents were distributed into wells of sterile, U-bottomed microtitre plates according to the following scheme: 50 µl of test serum (in duplicate) added to 50 µl of Veronal buffer, 100 µl of BHI broth, and 10 µl of 1:100-diluted bacterial suspension. Controls of sterility were set up without bacteria (negative control). Controls of bacterial growth (positive control) were set up without serum. The missing components were replaced by Veronal buffer at the same volumes. Plates were incubated in a humidified box at 37 °C for 18 h. They were then read spectrophotometrically in an ELISA reader at 690 nm, with a blank set on the sterility control. Its concentration was expressed in %.

Serum lysozyme was measured by a lyso-plate assay (Osserman & Lawlor 1966), carried out at 37 °C for 18 min, in a humidified incubator. Briefly, serum samples were reacted with a suspension of *Micrococcus lysodeikticus* inside an agar gel in 10 cm Petri dishes and then distributed in duplicate in 3 mm holes, 2 cm apart, at a regular distance of 1.5 cm from the dish edge. The reaction was carried out in a humidified incubator for 18 h at 37 °C. The diameter of the lysed areas around serum samples and lysozyme standards of known concentration in phosphate buffer (0.066 M, pH 6.3) was assessed by callipers or rules. Under these

Table 2. Ethogram (%) of different poultry genotypes.

		L	A	CL	G	RM	K	NN	R	χ^2
Initial interest[1]	%	65^{c}	62^{c}	60^{c}	45^{ab}	50^{b}	45^{ab}	55^{b}	30^{a}	0.25
Time spent outdoor	% of total time	60^{d}	62^{d}	56^{c}	46^{bc}	49^{bc}	42^{b}	55^{c}	19^{a}	15
Distance from house	m	18.1^{c}	17.5^{c}	15.3^{bc}	11.6^{b}	15.3^{b}	11.2^{b}	14.5^{bc}	4.9^{a}	12.4
Eating	%	3.6^{a}	2.1^{a}	3.6^{a}	33.2^{c}	4.5^{a}	18.4^{b}	19.4^{b}	37.0^{c}	13.2
Moving	"	71.5^{d}	51.2^{c}	50.6^{c}	25.4^{b}	40.6^{bc}	20.3^{b}	35.0^{b}	7.0^{a}	34.1
Resting	"	21.6^{a}	34.3^{ab}	34.9^{ab}	40.0^{ab}	37.3^{ab}	60.2^{c}	45.0^{ab}	55.5^{bc}	25.1
Comfort	"	2.0^{ab}	3.0^{b}	3.2^{b}	0.2^{a}	3.1^{b}	1.0^{ab}	0.2^{a}	0.5^{a}	2.1
Other behaviour	"	1.2^{b}	9.3^{c}	7.4^{c}	1.0^{b}	14.2^{d}	0.0^{a}	0.0^{a}	0.0^{a}	4.2

N: five birds/four replications per genotype.
L, Leghorn; A, Ancona; CL, crossbreed Cornish × Leghorn; G, Gaina; RM, Robusta Maculata; K, Kabir; NN, Nacked Neck; R, Ross.
[a...d]Values within a row with different superscripts differ significantly at $p < 0.05$.
[1]Interest shown by the birds towards the observer on the first 5 min of the presence in pen.

conditions, lysozyme concentration (μg/ml) was proportional to the diameter of lysed areas and was determined from a standard curve created with reference preparations of egg white lysozyme (Sigma-Aldrich, St. Louis, MO).

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 (H), lymphocytes (L), monocytes, eosinophils, basophils, RBC, haemoglobin (HGB), haematocrit and platelets (PLT) were counted on each slide. The H:L ratio was also calculated.

Statistical analyses

The data were analysed with a linear model (STATA 2005) to evaluate the effect of the genotype; the significance of differences ($p < 0.05$) was evaluated by multiple *t*-tests. Linear regression analysis was also performed to verify the adaptability score in relation to the daily weight gain.

Adaptability index

On the 49 different traits recorded for each bird, a rank of adaptability (from 0 low to 7 high; STATA; proc ROWRANK) has been calculated. The sum of the different scores was calculated and the final ranking of adaptability was then calculated and normalised (mean = 1; proc STATA). Differences in ethogram, culled birds and mortality rates were evaluated by the X^2 (proc. FREQ).

Results and discussion

The ethogram of the eight genotypes is summarised in Table 2. The R genotype showed the lower initial interest, like G and K ones; other medium-growing strains (RM and NN) reached intermediate values. On the other hand, L, A and CL had the highest percentages; the L and A chickens spent about 60% of their budget time outdoor and performed much of their behaviour patterns far (18.1 and 17.5 m, respectively) from the shelter exploiting all the available space. Together with CL, these birds showed a significantly higher percentage of kinetic behaviour, while only the 7.0% of this behaviour was observed in R chickens.

On the contrary, the eating and the resting behaviours were less frequents in the slow-growing chickens and much more relevant in R and G chickens whereas K and NN birds showed for eating intermediate values. Regarding resting behaviour, K and R chickens had the highest value.

Behavioural observation confirmed that the R genotype had the worst results in term of initial interest and spent more time indoor than outdoor. In contrast, the slow-growing birds displayed a great variety of behaviour patterns and exploiting all the available pasture area. Medium-growing birds had intermediate results in term of initial interest, time spent outdoor and complexity of behaviours. Lewis et al. (1997), comparing chickens with different growth rate, observed marked differences in the behaviour of birds; in particular, slow-growing genotype were much more active and more interested to the observer, made greater use of the perches and fewer are at rest. Slow-growing genotypes showed a better ability to exploit the pasture, with many positive metabolic and qualitative outcomes (Dal Bosco et al. 2012). Indeed, in previous studies (Castellini et al. 2002a; Fanatico et al. 2005), it is stated that the level of activity was correlated with the foraging behaviour which is fundamental for fully exploiting the natural resources available.

R chickens are more inactive confirming the findings of Gordon and Charles (2002). Weeks et al. (1994) compared the behaviour of Ross broilers, reared under free-range or kept inside, showed that free-range birds tended to stay indoors and/or near the house, rather than forage in the pasture. The authors attributed this behaviour mainly to leg weakness, which prevented

Table 3. Effect of poultry genotype on tonic immobility (TI), feather condition and body lesions.

		L	A	CL	G	RM	K	NN	R	Pooled SE
TI	Sec.	38^{a}	25^{a}	62^{b}	111^{c}	48^{ab}	97^{c}	62^{b}	126^{c}	24.0
Breast	–	4.0^{c}	4.0^{c}	4.0^{c}	4.0^{c}	4.0^{c}	1.7^{a}	2.6^{b}	1.0^{a}	1.6
Wings	–	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	3.5^{a}	3.5^{a}	2.0^{a}	0.2
Back	–	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	3.8^{a}	3.8^{a}	2.0^{a}	0.1
Tail	–	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	3.7^{a}	3.7^{a}	2.0^{a}	0.2
Vent/cloaca	–	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	3.7^{a}	3.7^{a}	2.0^{a}	0.1
Neck	–	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	4.0^{b}	3.7^{a}	4.0^{b}	2.0^{a}	0.1
Total score	–	24.0^{c}	24.0^{c}	24.0^{c}	24.0^{c}	24.0^{c}	20.1^{b}	21.3^{b}	11.0^{a}	5.9
FPD	%	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	30.0^{c}	20.0^{b}	60.0^{c}	20.6
BB	%	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	25.0^{b}	20.0^{b}	40.0^{c}	16.7

N: five birds/four replications per genotype.
L, Leghorn; A, Ancona; CL, crossbreed Cornish × Leghorn; G, Gaina; RM, Robusta Maculata; K, Kabir; NN, Nacked Neck, R: Ross.
TI, tonic immobility; FPD, footpad lesions; BB: breast blister.
$^{a,\ldots c}$Values within a row with different superscripts differ significantly at $p < 0.05$.

the birds from pasturing and behaving naturally. Fast-growing chickens showed severe difficulties in movement caused by the high body and breast weights, which obliged the animals to bed resting, especially in the last days of rearing. The reduction of kinetic activity is a major cause of leg weakness and the long resting in litter produces skin lesions (Elfadil et al. 1996; Bokkers & Koene 2004).

In our trial, the feeding behaviour (eating from feeders) was less present in the slow-growing strains whereas, fast-growing birds rested in a group around the feeders in accordance with Fanatico et al. (2005). The animal selection has focused on maximising the productive traits inducing animals to allocate their resources to body growth, reducing their ability to respond to other physiological and ethological demands (e.g. response to environmental stimuli, immunity, scavenging activity). Accordingly, such selection pressure reduced the level of bird activity (Dunnington 1990). Our results confirm such assumption: R birds had a higher feed intake, which covers the energy expenditure of high meat production. On the contrary, slow- and somewhat medium-growing birds showed the lowest feeding efficiency and feed intake and performed less resting in favour of kinetic (walking, running, foraging and exploring) activities.

The TI test, feather conditions, FPD and BB lesions of the eight poultry strains are presented in Table 3. The A, L and RM birds showed a quicker reaction time (<50 s); CL and NN showed intermediate times (about 60 s), whereas, K, G and R ones the highest values (97, 111 and 126 s, respectively).

Concerning feather conditions the L, A, CL, G and RM chickens showed the best values for all considered body regions, as well as the absolute absence of FPD and BB lesions. A different situation was for K, NN and R birds that had a worst feather condition, whereas, concerning body lesions, NN and K showed intermediate results and R the worst. Hence, the frequencies of FPD and BB resulted dramatically higher in fast-growing birds when compared with slow-growing chickens. Indeed, the 60% of fast-growing birds had severe FPD score. The L, A, CL, K, RM and NN birds showed the higher ROS and TBARs values while R and G the lower ones (Table 4).

The higher AP was observed in K and NN birds. Blood α-tocopherol was higher in L, CL and RM subject, followed by A and G chickens; intermediate values was observed in NN and K and lower in R chickens.

Regarding native immunity, the lower HCA content was detected in RM birds, followed by G and R ones whereas, for lysozyme, K, NN and R had the higher values. SBA did not show any significant difference in the different groups.

The heterophils/lymphocytes ratio was higher in R birds and the lowest values were observed in L, RM and NN birds. Monocytes and eosinophils were higher in L, A, CL and G chicks, while concerning HGB, haematocrit and PLT, R birds always showed the lowest values. RBC did not show significant differences between groups.

As expected, this distinct kinetic activity associated with the intake of grass affected the oxidative metabolism of the body. The reasons for such trend are probably related to the activity of birds: on one side the low activity of fast-growing strain did not produce a significant oxidative burst, whereas active birds increased the oxygen demand due to kinetic activity. Accordingly, plasma ROS and TBARS and AP, that is the body response to oxidative drive, try to counterbalance such situation by activating a comparable antioxidant response.

Plasma α-tocopherol followed the trend of kinetic and foraging activity: higher in slow-, intermediate in medium- and lower in fast-growing birds. Since all the birds ate the same feed this trend was mostly due to grass ingestion, which is very rich in vitamin E (Sossidou et al. 2010). However, in our case, the

Table 4. Effect of poultry genotype on oxidative status, native immunity and blood parameters.

		L	A	CL	G	RM	K	NN	R	Pooled SE
AP	μm HClO ml^{-1}	70.1^{a}	70.7^{a}	92.0^{a}	64.6^{a}	75.3^{a}	160.6^{b}	150.6^{b}	75.3^{a}	37.5
ROS	mm H_2O_2	0.24^{b}	0.19^{ab}	0.19^{ab}	0.07^{a}	0.18^{ab}	0.26^{b}	0.23^{b}	0.12^{a}	0.05
TBARS	μg/ml	1.58^{c}	1.49^{bc}	1.59^{c}	1.38^{b}	1.56^{c}	1.46^{bc}	1.45^{bc}	1.24^{a}	0.18
α-tocopherol	"	5.03^{cd}	4.84^{c}	5.54^{d}	4.38^{c}	5.90^{d}	2.69^{b}	2.85^{b}	0.74^{a}	0.36
HCA[1]	CH_{50}	81.86^{c}	84.64^{c}	78.45^{c}	68.88^{b}	59.35^{a}	78.08^{c}	81.47^{c}	69.17^{b}	6.54
SBA[2]	%	70.82	68.89	71.97	66.93	72.02	73.05	68.35	65.29	7.21
Lysozyme	μg/ml	1.50^{a}	1.47^{a}	2.00^{a}	1.83^{a}	1.42^{a}	6.80^{b}	6.07^{b}	6.89^{b}	1.59
Heterophils (H)	%	44.75^{a}	47.07^{ab}	50.80^{b}	50.57^{b}	40.34^{a}	$48.94a^{bc}$	45.71^{ab}	56.61^{c}	3.24
Lymphocytes (L)	"	50.50^{b}	46.89^{ab}	44.67^{a}	44.98^{a}	56.11^{c}	48.14^{ab}	51.33^{b}	40.60^{a}	5.14
H/L	–	0.89^{a}	1.00^{b}	1.14^{bc}	1.13^{bc}	0.73^{a}	1.01^{b}	0.91^{a}	1.39^{c}	0.36
Monocytes	%	2.43^{c}	2.22^{bc}	1.80^{b}	1.75^{b}	1.00^{a}	1.06^{a}	1.64^{b}	1.40^{a}	0.29
Eosinophils	"	2.38^{b}	2.80^{b}	2.50^{b}	2.05^{b}	1.95^{b}	0.86^{a}	0.89^{a}	0.80^{a}	1.24
Basophils	"	0.38	0.19	0.48	0.65	0.60	0.86	0.32	0.38	0.22
RBC[3]	$10^{6}/ml^{-1}$	2.94	3.53	3.25	2.49	2.90	2.61	2.57	2.89	0.82
Hb[4]	g/dl^{-1}	21.48^{b}	22.14^{b}	19.70^{b}	17.58^{a}	19.68^{b}	18.17^{a}	17.64^{a}	17.48^{a}	2.16
Ht[5]	%	37.49^{bc}	40.93^{c}	35.83^{b}	32.14^{b}	35.87^{b}	34.16^{b}	32.37^{a}	28.72^{a}	2.73
PLT[6]	"	5.50^{ab}	7.78^{c}	5.63^{ab}	4.53^{a}	5.27^{ab}	6.35^{b}	5.57^{ab}	4.13^{a}	1.92

N: five birds/four replications per genotype.
L, Leghorn; A, Ancona; CL, crossbreed Cornish × Leghorn; G, Gaina; RM, Robusta Maculata; K, Kabir; NN, Nacked Neck, R, Ross.
[1]Haemolytic complement assay; [2]serum bactericidal activity; [3]red blood cells; [4]haemoglobin; [5]haematocrit; [6]platelets.
[a,...d]Values within a row with different superscripts differ significantly at $p < 0.05$.

increase in plasma tocopherol was not able to completely counteract the production of ROS and the oxidative by-products (TBARs) in very active birds (Clarkson & Thompson 2000).

The relationship among welfare, immunity and health has been considered by many authors (Padgett & Glaser 2003; Broom 2006; Mugnai et al. 2011). The general question arises whether and how much the selection for productivity affects the ability of an animal to respond to environmental stressors. Provided that the activation of the immune system is energetically expensive, animals would make a trade-off between production level and immune response. Fast-growing birds, being genetically programmed for high productivity, might have an impaired ability to make this trade-off, meaning that they are less capable of coping with environmental stress. The immune system reflects the capability to react against external stress (Broom 2006). It is difficult to establish the best immune profile but we could hypothesise that fast-growing animals, genetically selected for high production, have difficulty in adapting to the organic system (Bayyari et al. 1997; Franciosini et al. 2011). Our results showed that HCA were higher in slow-growing birds, intermediate in medium- and lower fast-growing birds and in CL. Bayyari et al. (1997) in turkeys selected for body weight obtained a similar trend. Further, high HCA levels indicate that the birds have not consumed the complement for specific immune reactions against various pathogens (Ricklin et al. 2010).

Medium and fast-growing birds also showed the higher lysozyme level whereas slow-growing strains had the lower one indicating a lower presence of acute and chronic inflammation (Carroll & Martinez 1979). Such trend agrees with Franciosini et al. (2011) which found a much lower lysozyme value in backyard turkey.

Concerning blood-related traits, it is widely known that the stress increases heterophils and reduce lymphocytes, so the H/L ratio is an index of response to a stressor (Maxwell 1990). Slow- and medium-growing birds showed the lower H/L ratios probably indicating a higher adaptation to the free-range system. Avian heterophils act in acute inflammatory response with highly phagocytic specify and accumulate in inflamed tissue (Campbell 1995).

Avian leukocytes are only transiently present in the blood and after this period, they leave the circulation and migrate into the tissues, where to perform their specific immune functions (Davison et al. 2008).

Lymphocytes play a key role in protection against infection and in tumour rejection. Monocytes, heterophils, basophils and eosinophils are categorised as inflammatory leukocytes (Davison et al. 2008). Moreover, eosinophils play a major phagocytes role in the defence against parasites (Glick et al. 1964). Retention of normal levels of circulating eosinophils is associated with resistance to stress (Woolaston et al. 1996; Hohenhaus et al. 1998), and changes in blood eosinophils appear as a genotypic or phenotypic hallmark of stress reactions (Malyshev et al. 1993; Hohenhaus et al. 1998). Their hypothesis is consistent with the present findings that eosinophilia was exhibited in the L, A and CL birds, and not in K, NN and R birds. Bush (1991) pointed out that low percentage of basophils in chickens could also cause poor immunity against disease and the low percentage may indicate poor health condition. Even if this situation seems to be in opposite

Table 5. Adaptability indexes of different poultry genotype.

	L	A	CL	G	RM	K	NN	R	Pooled SE
Adaptability	0.49[d]	0.50[d]	0.58[d]	−0.41[b]	0.94[c]	−0.56[b]	0.18[c]	−1.77[a]	0.50
	Slow-growing				Medium-growing			Fast-growing	
Mean and SD	0.53 ± 0.41				0.05 ± 0.90			−1.77 ± 0.48	

N: five birds/four replications per genotype.
L, Leghorn; A, Ancona; CL, crossbreed Cornish × Leghorn; G, Gaina; RM, Robusta Maculata; K, Kabir; NN, Nacked Neck, R, Ross.
(slow – 0, GR < 24 g/d; medium – 1 (25 < GR <40 g/d and fast – 2 growing, GR > 40 g/d).
[a...d]Values within a row with different superscripts differ significantly at $p < 0.05$.

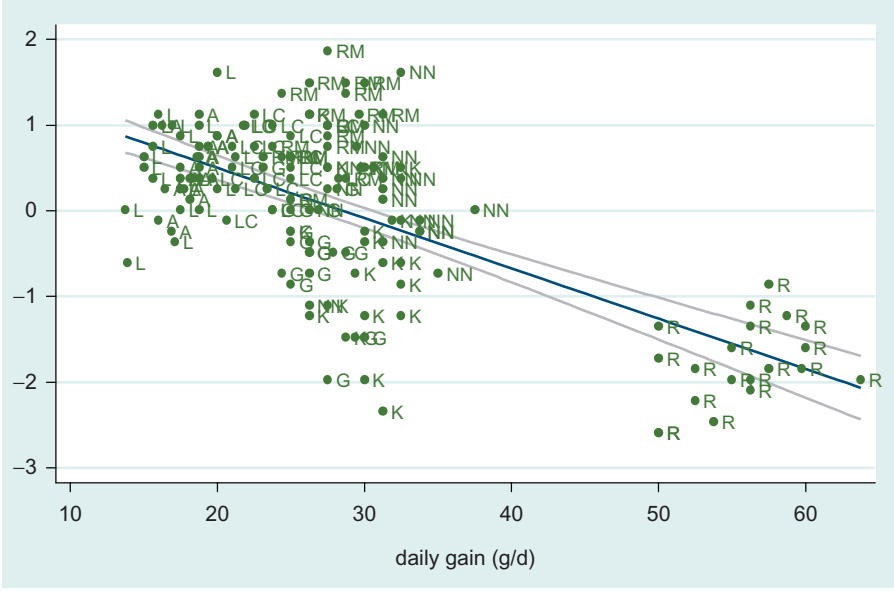

Figure 1. Fitted values of adaptability score versus daily gain (95% upper and lower limits). L, Leghorn; A, Ancona; CL, crossbreed Cornish × Leghorn; G, Gaina; RM, Robusta Maculata; K, Kabir; NN, Nacked Neck, R, Ross.

with all till now affirmed, a possible explanation could be the strong age-related haematologic profile observed in broiler strains (Talebi et al. 2005), in different breeds of chickens (Islam et al. 2004), in young cocks (Kral & Suchy 2000) and in pigeons (Seiser et al. 2000).

Monocytes constitute approximately 5–10% of peripheral blood lymphocyte, but this number varies in different chicken lines (Gordon & Taylor 2005). Besides modulating the immune response to pathogenic infections by producing proinflammatory cytokines, another important function of monocytes is their ability to give rise to tissue macrophages (Auffray et al. 2009). Heat stress (Altan et al. 2000) or transport (Ajakaiye et al. 2010) reduced monocyte.

The changes here observed in haemocrome, HGB, haematocrit are probably linked to the previously described distinctive level of activity done by birds. It is widely known that exercise enhances [Hb] and VO_2 max that is also proportional to the increase in the oxygen carrying capacity of blood (Calbet et al. 2006). The higher level of Ht in slow-growing birds may enhance oxygen delivery to the tissue. Also, this increment is supposed to be a factor for enhanced RBC as a reaction to increase body oxygen requirement. Julian and Mirsalimi (1992) also found that the oxygen saturation was higher in slow-growing chickens (91.6%) than in fast-growing chickens (86.0%). According to this, besides an obvious genotype effect on the behaviour and native immune parameters, less productive birds, if requested to enhance their natural defence (L, A and CL) seem to be better adapted, probably due to higher physiological homeostasis.

The standardised score of adaptability index is shown in Table 5. The worst adaptability index was shown by R chicks followed by K and G chickens. The L, A, CL and RM chicks showed the best adaptability to an organic system. A strict negative relationship between adaptability and daily weight gain is shown in Figure 1; however, within the same subclass (fast-, medium- and slow-growing) such strict relation disappeared (Figure 2).

As expected, R birds reached the highest slaughter weight (4400 g), K, NN and RM the intermediate (2380, 2500 and 2161 g, respectively), followed by CL and G (1865 and 2018 g, respectively), and slow-growing birds

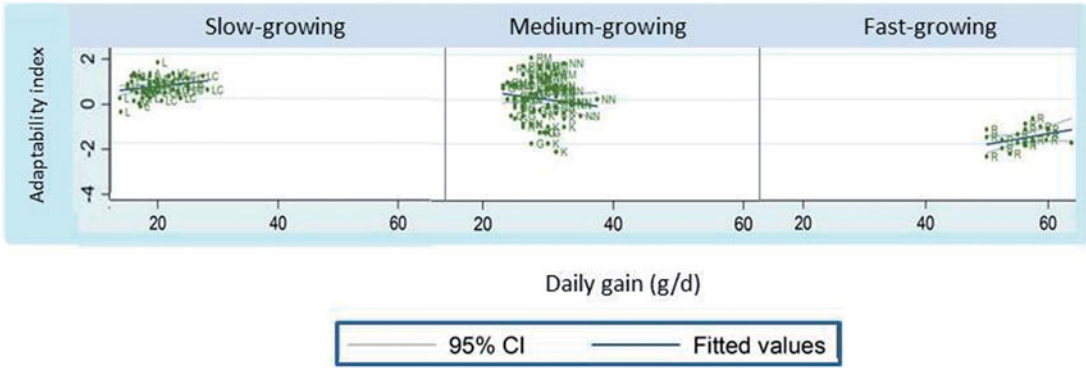

Figure 2. Fitted values of adaptability score versus daily gain within sub-groups. L, Leghorn; A, Ancona; CL, crossbreed Cornish × Leghorn; G, Gaina; RM, Robusta Maculata; K, Kabir; NN, Nacked Neck; R, Ross.

Table 6. Productive performance of different poultry genotypes.

		L	A	CL	G	RM	K	NN	R	Pooled SE/$^{(*)}\chi^2$
Live weight	g	1320^{a}	1370^{a}	1865^{ab}	2018^{b}	2161^{bc}	2380^{c}	2500^{c}	4400^{c}	120
Feed intake	g/d	78.3^{a}	80.6^{a}	89.7^{a}	87.7^{a}	97.7^{ab}	103.3^{b}	101.8^{b}	162.3^{c}	19.2
Daily weight gain	"	16.1^{a}	16.9^{a}	22.8^{b}	25.0^{bc}	26.7^{bc}	29.2^{c}	30.7^{c}	54.5^{d}	0.09
Feed:gain ratio		4.8^{b}	4.7^{b}	3.9^{b}	3.4^{ab}	3.9^{b}	3.4^{ab}	3.2^{ab}	3.0^{a}	0.28
Culled birds	%	0^{a}	0^{a}	2^{b}	2^{b}	2^{b}	3^{b}	2^{b}	5^{c}	24$^{(*)}$
Mortality	"	4^{a}	3^{a}	4^{a}	3^{a}	4^{a}	6^{a}	5^{a}	14^{b}	32$^{(*)}$

N: 25 birds/four replications per genotype.
L, Leghorn; A, Ancona; CL, crossbreed Cornish × Leghorn; G, Gaina; RM, Robusta Maculata; K, Kabir; NN, Nacked Neck, R, Ross.
$^{a,\ldots d}$Values within a row with different superscripts differ significantly at $p < 0.05$. *: X^2 $P < 0.05$.

(A 1370 and L 1320 g) (Table 6). Consequently, all the other productive traits (feed intake, daily weight gain) followed this trend, whereas feed to gain ratio increased in slow-growing birds.

Concerning mortality and culling rate, R chickens showed the highest values, whereas the A and L ones showed the lower value of culling rate. To the best of our knowledge, this is the first paper which analyses the adaptation of different poultry strains to the organic rearing system using a wide panel of physiological and behavioural traits. The relevance of such paper is related to the disposal of specific range for the poultry strains herein analysed but mainly for the definition of a synthetic index which assesses the adaptability to the organic free-range system. Such index is strictly connected with the daily gain of the birds: daily weight gain higher than 50 g/d resulted in very low values and these strains should be avoided in an organic system. Medium- and mainly slow-growing birds showed the highest adaptation. However, within the same sub-category (slow, medium and fast), the index showed a wide variability and the adaptation seems mostly independent from daily weight gains (e.g. NN has a higher daily gain than K while having a higher adaptation index). Thus, the daily weight gain is essential for the exclusion of extremely productive strains but it is not adequate for defining the adaptability of birds to an organic system.

Naturally, other data are involved in the choosing of a strain mainly related to the economic sustainability (e.g. feed to gain index). For example, the body weight of L and A chickens at 81 d was less than 2 kg, which is the under the minimum weight for the market (Saveur 1997), whereas the medium-growth chickens ranged from 2 to 2.5 kg.

Conclusions

This study confirms that the slow-growing chickens show better welfare status and adaptability to the organic system followed by medium-growing ones. Fast-growing chickens, although have the best productive performance, appeared no adapted to the outdoor environment as demonstrated by the high number of culled birds and mortality.

However, the definition of strains adapted to the organic system requires the measure of a wide panel of physiological and behavioural traits and not only daily weight gain. A multi-criteria analysis should be developed considering the economic, ecological, social and qualitative performance of different poultry genotypes

for identifying which of them better fit with the organic system requirements.

Only small national projects study these aspects of adaptability particularly in heat stress conditions typical of the Mediterranean area and commercial breeding companies do not seem interested in selecting suitable strains for these production systems. At the end, the problem remains unsolved with negative repercussion on the chicken organic production system as well as on the consumer perceptions towards its products quality traits.

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