

Free-Range Rearing Density for Male and Female Milanino Chickens: Growth Performance and Stress Markers

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Primary Audience: Live Production Personnel, Free-range and Organic Farmers, Researchers

SUMMARY

The Milanino is a heavy Italian chicken breed and represents an important genetic resource for alternative production systems. Due to the absence of standard management guidelines for local chicken breeds, this study aims to determine the space requirement to achieve a good equilibrium between growth performance and stress response in Milanino chickens reared according to a separate-sex free-range program. A total of 140 birds (70M:70F) were reared in outdoor pens from 75 to 235 d of life according to the following experimental groups (35 birds/group): (M2) males in 2 m²/bird; (F2) females in 2 m²/bird; (M10) males in 10 m²/bird; (F10) females in 10 m²/bird. Growth performance and stress condition were recorded. Bird density affected male body weight, that was higher at the lower density of 10 m²/bird. In contrast, females showed a consistent growth rate irrespective of the bird density suggesting lower space requirements. An overall good adaptability of the breed to the separate-sex free-range system was found, irrespective of both sex and bird density. Moreover, the stress markers values assessed at the end of the growing period suggest the ability of Milanino birds to respond well to environmental stressors. The results provided in this report will be implemented into free-range management guidelines for meat production in the Milanino breed: the separate-sex rearing is recommended during the growing period in outdoor pens and the bird density has to be planned according to the sex.

Key words: autochthonous chicken breed, free-range, rearing density, body weight, stress markers

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DESCRIPTION OF THE PROBLEM

The Milanino is a composite chicken breed (Valdarnese × Orpington) selected mainly for meat production at the beginning of the 20th

century in the rural area close to Milan in the North of Italy [1]. Milanino is a heavy breed with a characteristic sexual dimorphism in relation to adult body weight providing a high carcass yield in both sexes [2]. The meat is characterized by high-protein and low-fat contents compared to the standard broiler meat and it shows interesting

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quality traits, such as color and tenderness [2, 3]. In addition, Milanino hens have laying ability, with a mean oviposition rate of 58% recorded during a breeding season from January to June, and a peak of oviposition of 82% recorded in March [1].

In Italy, over the past decade, the amplified demand for traditional local products in the agrifood sector has opened new prospects concerning the poultry production system and rural rearing using local breeds has increased. However, due to the absence of standard management guidelines for local chicken breeds, farmers have to rely on personal experience in determining the space requirement to achieve the best equilibrium between productive performance and animal welfare. In fact, welfare concerns affect consumers of poultry products, and stocking density is perceived as a priority for animal welfare [4]. Therefore, peculiar management guidelines for autochthonous birds need to be studied.

Most of the scientific reports on chicken density and stress condition focused on commercial strains and intensive farming, while few data are available on local breeds reared according to free-range system. Some experimental trials have been carried out concerning poultry stress response to several factors, such as the type of rearing system and transport in Italian [5–7] and Spanish chicken breeds [8, 9].

Milanino birds are not competitive for intensive farming, whereas are suitable for extensive rearing, such as in free-range and organic systems. Therefore, they represent an important genetic resource for the development of a local meat production system for niche markets of potential interest for the diversified farms within the agricultural sector in Italy.

Previous studies have been performed to outline specific management guidelines for Milanino chickens reared in free-range system. If reared in common pens, a sex-dependent effect of bird density on growth performance was shown; furthermore, female birds showed higher level of the heterophil/lymphocyte ratio stress marker compared to males [6]. The protein dietary level was also investigated in both sexes during the growing period [2].

In order to improve both welfare condition and growth performance during the slow growing period of Milanino birds, the purpose of the

present trial was to study the effect of bird density on growth performance and stress response in male and female birds reared in separate-sex free-range system.

MATERIALS AND METHODS

Birds, Rearing System, and Growth Performance

The study was carried out during the 2017 reproductive season, from March to November. The trial was approved by the Committee for Animal Welfare of the University of Milan (OPBA_24.2017) according to the EU Directive 2010/63/EU.

One hundred and sixty Milanino chickens (82M:78F) were hatched at the Poultry Unit, Animal Production Centre, University of Milan (Lodi). The chicks were reared straight-run in indoor floor pens in a controlled environment from 1 to 75 d of age following standard management guidelines for chickens. At hatch, birds were labeled with a metal wing tag, individually weighed and vaccinated for Marek's and Newcastle diseases. On day 21, birds were provided with a second vaccination for Newcastle disease. Birds were fed ad libitum with a commercial starter diet (12.13 MJ/ME/kg, 22% crude protein, 3% lipids, 3% fiber, 5% ash) from hatch to 35 d of age, and with a commercial grower diet (12.58 MJ/ME/Kg, 19% crude protein, 4% lipids, 3% fiber, 5% ash, 30 mg vitamin E/kg) from 35 to 75 d of age. The total amount of feed given to the birds was recorded to calculate the overall mean feed consumption in the brooding period. On day 75 of age, birds were transferred to a local private farm and reared in outdoor free-range pens equipped with feeders, drinkers and a suitable shelter to confine chickens at night or during bad weather. At transfer, birds were sexed and randomly assigned to four experimental groups, according to the following sex and bird density combinations: group (M2) males in 2 m²/bird; group (F2) females in 2 m²/bird; group (M10) males in 10 m²/bird; group (F10) females in 10 m²/bird. In total, 140 birds were used (35 birds/group). Birds were fed ad libitum the same commercial grower diet used from 35 d of age until the end of the trial. Birds had unlimited access to pasture during the day and,

according to the overall low rearing-density, grass cover and plant variety were consistent until the end of the rearing period. Birds were individually weighed on days 75, 120, 150, 180, 205, and 235 of age. The cumulative feed intake of each group was recorded from 76 to 120, 121 to 150, 151 to 180, 181 to 205, and 206 to 235 day of age to calculate the overall mean feed consumption in each specific phase of the growing period. Total feed intake and mean weight gained recorded in the same phase were used to estimate the feed conversion ratio (FCR) in each experimental group.

Bird mortality was recorded daily. Bird handling was in accordance with the principles presented in Guidelines for the Care and Use of Agricultural Animals in Research and Teaching [10].

Stress Markers

Bird welfare was assessed by the measurement of different stress markers: heterophil/lymphocyte (H/L) ratio, serum lysozyme (LYS), and total plasma antioxidant activity (OXY). Stress markers have been assessed on 90 d of age, 15 d after the outdoor transfer, and on 215 d of age corresponding to the end of the growing period. The same birds have been sampled at both ages. Blood samples (3 mL) were taken from the ulnar vein of 10 birds per treatment and split into 2 aliquots: 1.5 mL placed into ethylene diamine tetra acetic acid tubes for H/L ratio and OXY assessment, and 1.5 mL into tubes without anticoagulant for LYS evaluation. Blood aliquots were immediately cooled to 5°C after sampling and transferred to the laboratory. Blood was collected soon after the removal of the bird from its pen. To assess the leukocyte formula and then to calculate the H/L ratio, the procedure described by Castellini et al. [7] was used. LYS ($\mu\text{g}/\text{mL}$) was assessed by the lyso-plate assay according to Osserman and Lawlor [11]. OXY was measured using commercially available kits and quantified in $\mu\text{M HClO}/\text{mL}$ (OXY-Adsorbent Test, Diacron Labs S.r.l., Grosseto, Italy).

Statistical Analysis

Analysis of variance for repeated measures was performed using the MIXED procedure of SAS [12] on the variables: body weight, H/L

ratio, LYS, OXY. The statistical model included fixed (sex, age, bird density) and random (the chicken) effects and the relative interactions (sex * age, sex * bird density, age * bird density, sex * age * bird density). A *t*-test was used to compare LS Means.

The present trial was performed in field condition, and each experimental group was reared in 1 pen according to the facilities available on farm. Therefore, in order to assess the variability within each treatment, birds were individually identified and repeated measures of all parameters were recorded on the same bird.

RESULTS AND DISCUSSION

Growth Performance

According to the results of the analysis of variance, the fixed effects sex, age and bird density, and only the interactions sex * age and sex * bird density significantly affected body weight during the growing period ($P < 0.001$).

The overall mean body weight was significantly different between the 2 sexes, corresponding to $2,602.35 \pm 16.25$ g in males and $1,898.75 \pm 14.63$ g in females ($P < 0.001$), and the significant difference between sexes increased with the age of the bird according to the progressive increase in body weight from 75 d of age onwards (Table 1). The clear sexual dimorphism in relation to body weight observed in Milanino chickens is in agreement with previous studies [2, 6]. The present results on adult body weight in both males and females also confirm the Milanino to be a heavy Italian breed compared to several Italian chicken breeds, such

Table 1. Body Weight in Males and Females Recorded at Different Ages During the Growing Period in Milanino Chickens Reared in Free-Range System.

Age (days)	Females ¹	s.e.	Males ¹	s.e.
75	1065.33 ^A	35.30	1541.56 ^B	38.47
120	1592.38 ^A	35.31	2268.36 ^B	41.23
150	1929.87 ^A	35.82	2641.74 ^B	42.63
180	2173.76 ^A	36.09	2853.11 ^B	37.53
205	2291.79 ^A	36.12	3097.78 ^B	39.99
235	2339.35 ^A	36.42	3211.52 ^B	38.81

¹LS Means of body weight (g) are shown.

^{A,B}Values within a row with different superscripts differ significantly at $P < 0.001$.

as the Padovana [13, 14], the Modenese and Romagnola [15], and the Bionda Piemontese and Bianca di Saluzzo [16]. In contrast, similar body weights have been reported in Spanish (Mos, Menorca) and Portuguese (Amarela, Preta Lusitanica, Pedres Portuguesa) chicken breeds [17]. Furthermore, in all these European breeds also, sexual dimorphism on adult body weight was a very clear trait [17].

In free-range and organic poultry production, the choice of chicken genotypes should consider the relationship between slow-growing rate and adaptability to local conditions [18]. However, the definition of slow-growing poultry genotypes and the evaluation of the true adaptability to alternative rearing systems is still unclear. Castellini et al. [7], on the basis of personal studies and commercial information, proposed a classification of poultry genotypes in slow-, medium- and fast-growing according to their growth rate: slow < 24 g/d, 25 < medium < 40 g/d, and fast-growing > 41 g/d. The study of a panel of physiological and behavioral traits in 8 chicken genotypes revealed a negative linear correlation between adaptation and daily weight gain, and a daily weight gain lower than 50 g/d was identified as a prerequisite for chicken adaptation [7]. However, according to the only daily weight gain, it is not possible to identify free-range adaptable chicken genotypes, but only to exclude extremely productive strains. According to the growth rate performed in the present trial, the overall daily weight gain of Milanino chickens was 10 g/d; therefore, the Milanino breed can be classified as slow-growing genotype potentially suitable for free-range and organic farming.

Bird density significantly affected body weight and the birds reared at 10 m²/bird were significantly heavier compared to the birds reared at 2 m²/bird: 2,303.16 ± 14.96 g vs. 2,197.93 ± 15.94 g ($P < 0.001$). However, the bird density effect was sex dependent and present only in male birds that showed the best growth performance at the lower density of 10 m²/bird (Table 2). In contrast, the growth performance of females was independent by bird density and very similar in pens providing 2 and 10 m²/bird (Table 2). In a previous study on straight-run Milanino chickens, bird density affected growth performance and an opposite trend was found

Table 2. Body Weight Recorded in Male and Female Milanino Chickens Reared in Outdoor Pens at Different Bird Densities During the Growing Period.

Sex	Bird density (m ² /bird)			
	2	s.e.	10	s.e.
F ¹	1,886.91	20.95	1,910.58	20.43
M ¹	2,508.95 ^A	24.04	2,695.74 ^B	21.87

¹LS Means of body weight (g) recorded from 75 to 235 d of age are shown.

^{A,B}Values within a row with different superscripts differ significantly at $P < 0.001$.

according to the sex [6]. A critical period during the rearing of slow-growing male birds is the onset of sexual maturity and the effect of rearing density on male growth performance may be related to male sexual behavior [19]. Fights among males are frequent from 90 d of age onwards, in concomitance with the increase of testosterone blood level, and the frequency of fights is positively related to bird densities [19–21]. A higher frequency of fights can occur among males kept at the higher density of 2 m²/bird, and, consequently, male growth performance could have been negatively affected. The negative effect of rearing density on Milanino male growth performance was previously shown in straight-run chickens [6] and confirmed in the present study in separate-sex chickens. In contrast, the separate-sex rearing system after 10 wk of age prevented the negative effect of bird density on female growth performance previously found in the straight-run rearing system [6] confirming its relation to male mating behavior.

The cumulative feed consumption recorded during the whole rearing period was 22.5 kg/bird/235 d. The mean FCR in males and females reared at different bird densities during the growing period was very similar. The FCR calculated in each sex at 125, 150, 180, 205, and 235 d of age presented the following values: M = 5.03, 5.46, 6.49, 6.89, 7.75; F = 5.79, 6.23, 6.79, 7.87, 8.93. Few data on feed consumption and FCR of local chicken breeds are available. Tixier-Boichard et al. [22] reported that feed efficiency ranged from 4 to 6.5 in 7 French local chicken breeds and, in particular, feed efficiency in the well-known Bresse breed was 4.59. After 180 d of age, both male and female Milanino birds presented an FCR higher than 6. Compared

Table 3. Blood Stress Parameters Recorded at the Beginning and at the End of the Outdoor Growing Period in Milanino Chickens.

Stress parameters ¹	Age (days)		s.e.
	90	215	
H/L	0.08 ^A	0.33 ^B	0.04
OXY ($\mu\text{M HClO/mL}$)	192.18 ^A	228.49 ^B	7.6
LYS ($\mu\text{g/mL}$)	2.48 ^A	1.64 ^B	0.31

¹LS Means of blood stress parameters are shown. H/L = circulating heterophil/lymphocyte ratio, OXY = total plasma antioxidant activity, LYS = serum lysozyme

^{A,B}Values within a row with different superscripts differ significantly at $P < 0.05$ between the age.

with the commercial strains, the local chicken breeds are characterized by slow growth rates, requiring a very long rearing time [23], and low feed efficiency values, resulting in high production costs. In this context, further studies are required to customize the feeding program to the nutrient requirements of the Milanino breed in order to optimize the profitability of meat production.

Mortality recorded during the straight-run brooding period (1–75 d of age) was 1.25%. Mortality recorded during the growing period (75–235 d of age) was 8.6% in M2 group and 2.8% in F2, F10, and M10 groups.

Stress Parameters

According to the results of the analysis of variance, the age of birds, corresponding to the time of blood sampling, was the only fixed effect significantly affecting the stress markers ($P < 0.001$); all the interactions considered in the statistical model did not show a significant effect ($P > 0.05$). All significant results related to the stress parameters are reported in Table 3.

The stress condition assessed in Milanino chickens reared in separate-sex free-range system was not affected by either sex and bird density. The overall mean H/L, LYS, and OXY values were 0.20 ± 0.04 , $2.06 \pm 0.30 \mu\text{g/mL}$, and $210.33 \pm 7.66 \mu\text{M HClO/mL}$ respectively.

The H/L ratio and LYS values were consistent to those measured in several chicken genotypes reared under organic conditions typically related to many stress types (environmental, nutritional, physical, social, psychological, and pathological) [7]. It is widely known that stress increases heterophils and reduces lymphocytes, so the H/L

ratio is an index of response to a stressor: an increased circulating H/L ratio is indicative of a stress state [24] and a high H/L ratio is negatively correlated with body weight [25]. The LYS is an indicator of inflammatory response and a lower LYS level is indicative of a lower presence of acute or chronic inflammation [26]. The very low H/L ratio and LYS values assessed in Milanino birds suggest a true adaptability of the breed to the separate-sex free-range system. In contrast, a previous study reported higher H/L ratio in female compared to male Milanino chickens reared in straight-run free-range system, suggesting a stress condition in females probably related to mating behavior [6].

The high kinetic activity and foraging behavior observed in slow-growing genotypes [27, 28] has been associated to the increase in oxygen demand affecting the oxidative metabolism of the tissues. OXY values represent the ability of body response to oxidative stressors and high values have been recorded in Milanino chickens, irrespective of the sex and rearing conditions. The high antioxidant activity of Milanino birds suggests good adaptability to the potential stressors acting in free-range condition. In contrast, lower OXY values have been reported in 8 different genotypes, including local breeds, slow- and fast-growing birds [7].

H/L ratio and OXY mean values significantly increased from 90 to 215 d of age and, in contrast, an opposite trend was shown in the LYS mean value (Table 3). At the end of the growing period LYS and OXY showed the lowest and the highest value respectively, suggesting the ability of Milanino birds to respond well to environmental stressors. On the contrary, a higher value of the H/L ratio was found at the second sampling time, corresponding to an apparent increase of stress condition at the end of the rearing period. However, the change observed may be related to an age-related trend in the heterophil and lymphocyte profiles, in agreement with the H/L progressive increase observed in both sexes in relation mainly to the onset of sexual maturity [29].

CONCLUSIONS AND APPLICATIONS

1. The Milanino is a heavy breed with sexual dimorphism in relation to adult body weight:

- 3,211 g in males and 2,339 g in females at 235 d of age.
- Flock density affected body weight of male chickens reared in separate-sex free-range system, and a rearing density of 10 m²/bird is recommended to obtain higher growth rate; on the contrary, low space requirements, such as 2 m²/bird, are suitable to perform the full potential in female growth rate.
 - The Milanino breed showed an overall good adaptability to the separate-sex free-range system, irrespective of both sex and bird density, and the ability to respond well to environmental stressors; within the farming program, bird density has to be planned according to the sex.
 - Overall results provided in this report will be implemented into free-range management guidelines for meat production in the Milanino breed.

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