

Bird density, stress markers and growth performance in the Italian chicken breed Milanino

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Primary Audience: Live Production Personnel, Organic Farmers, Researchers

SUMMARY

The Milanino is an Italian chicken breed included in a conservation project run by the University of Milan. It is characterized by good fertility, heavy body weights, high adaptation ability to adverse climate conditions, and disease resistance. Because of these characteristics, the Milanino could represent an important genetic resource for alternative production systems. This research was aimed at studying the effect of bird density on growth and slaughter performance, as well as stress response in Milanino chickens kept in outdoor pens. One hundred and sixty Milanino chickens were randomly assigned to 2 experimental groups kept at different densities (2 m²/bird and 8 m²/bird) and were slaughtered at 185 days of age. Growth and slaughter performance and stress condition were recorded. The interaction bird density * sex * age significantly affected body weight and an opposite trend was found between females and males: heavier females were found in the high-density group, while heavier males were found in the low-density group. Bird density did not affect carcass weight data. The stress marker (H/L ratio) was significantly higher in birds kept at the higher density (2 m²/bird). In conclusion, the Milanino provided satisfactory growth performance with different rearing density but the lower density, 8 m²/bird, should be preferred to minimize welfare problems for male birds.

Key words: body weight, chickens, breed, stress markers, bird density, slaughter, carcass yield

2015 J. Appl. Poult. Res. 24:529–535
<http://dx.doi.org/10.3382/japr/pfv044>

DESCRIPTION OF THE PROBLEM

According to the State of the World's Animal Genetic Resources for Food and Agriculture, 20% of documented livestock breeds are at risk of extinction: 1,500 of the 7,600 breeds around the globe may be lost forever in the near future [1]. Such a situation is greatly emphasized in the poultry sector where only selected strains are reared for meat and egg production while local breeds have been excluded from the intensive productive system [2]. In Italy, 90 lo-

cal avian breeds were described; the majority (61%) were classified as extinct and only 8.9% as still widely spread [3]. The need for conservation of avian genetic resources is well recognized and actions are requested from both public and private institutions [2]. In Italy, efforts to include local avian breeds within conservation programs have been increasing with the support of local institutions, mainly in the regions that have an important poultry tradition. Literature reports provide information on genetic variability, breeding performance, meat and egg quality and behavioral features of some Italian breeds involved in conservation projects in Veneto

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[4,5], Emilia Romagna [6], and Lombardia regions [7,8].

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 [9]. The breed is currently included in a conservation research project on poultry autochthonous breeds present in the Lombardia region (CoVAL project n. 1723, funded by Regione Lombardia). The morphological traits characteristic of the breed include white plumage, white skin, white shanks, and a large single comb. The mean egg production recorded in a small population during the breeding season, from January to June, was 58% with a peak of oviposition of 82%. High fertility values were recorded, range 85 to 91%, during the same whole reproductive season [9]. Because of these characteristics, the Milanino represents an important genetic resource for alternative production systems, especially where the demand for organic and free-range meat is increasing. Studies on biological, productive, and behavioral characteristics of the breed are required to develop a special management guide for extensive rearing systems and support the potential use of Milanino birds in sustainable productions, wishing to reach niche markets and marginal areas.

The purpose of this trial was to study the effect of bird density on growth and slaughter performance, and on stress response in Milanino chickens reared in outdoor pens.

MATERIAL AND METHODS

Birds, Rearing System, and Growth Performance

The study was carried out during the 2013 reproductive season, from May to November. One hundred and sixty straight-run Milanino chickens (79M:81F) were hatched at the Poultry Unit, Animal Production Centre, University of Milan (Lodi). The chicks were reared in a controlled environment from 1 to 35 days of age following standard management guidelines for chickens. At hatch, birds were labeled with a metal wing tag, weighed, and vaccinated for Marek's and Newcastle diseases. On day 35 of age, birds were

transferred to a local private farm and reared until 185 days of age in outdoor pens. The pens were equipped with feeders, drinkers, and a suitable shelter to confine chickens at night or during bad weather. Birds were fed ad libitum with a starter feed (12.13 MJ/kg of ME, 20% CP) from hatch to 21 day of age and a finisher feed (12.58 MJ/kg of ME, 18% CP) from 22 to 185 day of age. The total amount of feed given to each group was recorded. At transfer on 35 days of age, birds were randomly assigned to two experimental groups (80 birds/group) and kept at different densities in outdoor pens, corresponding to 2 m²/bird and 8 m²/bird. Birds were reared straight-run and each experimental group was kept in one pen according to the rural farming system used on the farm. Birds were individually weighed on day 35, 65, 95, 150, and 185 of age. 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].

Slaughter and Carcass Weight

At 185 days of age, 20 birds per treatment, 10 males + 10 females, were randomly chosen and slaughtered after 12 h feed withdrawal. Chickens were stunned by electrocution (110 V; 350 Hz) before killing. After killing, carcasses were plucked and weighed. Then, non-edible viscera (intestines, proventriculus, gall bladder, spleen, esophagus, and full crop) were removed and the weight of the partial eviscerated carcass (**PEC**) was recorded. Spleen weight was recorded and its proportion to the live body weight was calculated. Then the head, neck, legs, edible viscera (heart, liver, and gizzard) and fat (perivisceral, perineal, and abdominal) were removed in order to obtain the ready-to-cook carcass (**RCC**) [11] and its weight. The proportion of the PEC and RCC on the live body weight was calculated.

Stress Markers

Stress condition was assessed by the measurement of the heterophil/lymphocyte (H/L) ratio and of the spleen weight. In birds, H/L ratio is one of the most accepted indicators of stress

[12,13], and also the relative spleen weight is often used as a stress parameter [14].

On the day before slaughter, blood samples were randomly taken from the cutaneous ulnar vein of 40 birds per treatment (20 males + 20 females) to assess the leukocyte formula and then to calculate the H/L ratio. On the day of slaughtering, the spleen was dissected from the 20 birds slaughtered as previously described to record its weight and to calculate its proportion on the live body weight.

Statistical Analysis

Analysis of variance on the live body weight data was performed using the MIXED procedure of SAS [15]. The statistical model included fixed (sex, age, and 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.

Analysis of variance on the carcass weight data and on H/L ratio was performed using the GLM procedure of SAS [15]. The statistical model included sex and bird density as sources of variation, and the interaction sex * bird density. A *T* test was used to compare LS Means.

RESULTS AND DISCUSSION

Growth Performance

The fixed effects (sex, age, and bird density) and some of the interactions (sex * age, bird density * age and sex * bird density * age) considered in the analysis of variance significantly affected body weight during the growing period, ($P < 0.05$). Body weights were significantly different between the two sexes (males = 1,551.27 g \pm 14.07, females = 1,280.08 g \pm 14.36, $P < 0.001$) but not before day 65, and the difference increased with the age of the birds (Table 1). Males were significantly heavier than females from 65 to 185 days of age. The present result shows a clear sexual dimorphism in the Milanino breed. Mean body weight was different between the two sexes already on hatch, and this sexual dimorphism became markedly and constantly ev-

Table 1. Body weight (g) of males and females recorded at different ages during the growing period in the Italian chicken breed Milanino.

Age (days)	Males	S.E.	Females	S.E.
1	34.04	21.83	39.99	21.83
35	540.04	21.34	473.62	21.34
65	1,344.96 ^A	21.16	1,079.82 ^B	21.87
95	2,100.55 ^A	21.15	1,554.53 ^B	21.68
150	2,423.86 ^A	21.96	2,128.51 ^B	22.19
185	2,864.16 ^A	22.27	2,404.03 ^B	22.97

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

Table 2. Body weight (g) recorded at different ages in Milanino chickens reared in outdoor pens at different bird densities.

Age (days)	Density 2 (m ² /bird)	S.E.	Density 8 (m ² /bird)	S.E.
1	33.19	22.13	40.84	22.13
35	495.06	21.94	518.60	21.94
65	1,199.95	21.94	1,224.83	20.92
95	1,823.96	22.09	1,831.22	20.56
150	2,195.93 ^A	22.59	2,356.44 ^B	21.37
185	2,621.32	22.84	2,646.87	22.23

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

ident from 65 days of age onwards. According to their growth performance, both males and females could be slaughtered at different ages to produce different chicken meat products characteristic of the Italian poultry market [16]. Ready-to-cook carcasses could be produced with males and females slaughtered on 80 and 110 days of age, respectively, and meat cuts with males and females slaughtered on 150 and 185 days of age, respectively.

The body weight of Milanino chickens on 185 days of age ranged between 2,886 g and 2,381 g and these values are higher than those reported for other Italian chicken breeds, such as the Padovana breed reared outdoors and slaughtered at a similar age [17] and the Modenese and Romagnola breeds slaughtered at 210 days of age [6].

Bird density significantly affected body weight (2 m²/bird = 1,394.88 g \pm 14.44; 8 m²/bird = 1,436.47 g \pm 13.72, $P < 0.05$); however, the effect was age dependent and present only on 150 days of age (Table 2). At that age, the chickens kept at the lower density were

Table 3. Body weight (g) recorded at different ages in male and female Milanino chickens reared in outdoor pens at different bird densities.

Age (days)	Sex ¹	Density 2 (m ² /bird)	S.E.	Density 8 (m ² /bird)	S.E.
1	F	33.68	31.63	46.31	31.63
	M	32.71	31.63	35.37	31.63
35	F	460.11	31.63	487.13	31.63
	M	530.01	31.63	550.06	31.63
65	F	1,056.58	31.63	1,103.05	29.73
	M	1,343.31	30.42	1,346.61	29.43
95	F	1,552.00	32.30	1,557.05	28.45
	M	2,095.71	30.14	2,105.39	29.69
150	F	2,094.77	32.30	2,162.25	29.94
	M	2,297.10 ^A	31.59	2,550.63 ^B	30.51
185	F	2,450.46 ^a	33.00	2,357.60 ^b	31.48
	M	2,792.17 ^A	31.59	2,936.15 ^B	31.40

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

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.

¹M = males; F = females.

significantly heavier ($P < 0.001$). Moreover, also the interaction among age, sex, and bird density significantly affected body weight (Table 3). In females, bird density showed a significant effect on body weight only at 185 days of age ($P < 0.05$), and heavier females were found in the group kept at the higher density (2 m²/bird). In males, bird density showed a significant effect on body weight at 150 and 185 days of age ($P < 0.001$), and heavier males were found in the group kept at the lower density (8 m²/bird). Therefore, bird density significantly affected the growth, and the effect was age dependent and different between males and females. In males, the best growth performance was recorded in birds kept at the lower density of 8 m²/bird, and such positive effect was present from 150 days of age onwards. In contrast, in females, the highest mean body weight was recorded in birds kept at the higher density, corresponding to 2 m²/bird, and such an effect was present only in adult birds at 185 days of age. The effect of bird density on male growth performance may be related to male sexual behavior. Fights among males are frequent at the onset of sexual maturity in concomitance with the increase of testosterone blood level and the frequency of fights is positively related to rearing densities [18,19]. A higher frequency of fights can occur among males kept at the higher density of 2 m²/birds,

and, as a consequence, male growth performance could have been negatively affected. The same situation may also explain the highest weight of adult females reared at the same high density: if males spend more time to fight than to mate, females can feed undisturbed longer and gain more weight. In domestic fowls, the hierarchical system is rigorously established by aggressive behaviors, but once the hierarchy has been organized, the aggressiveness decreases and is substituted by demonstrations of dominance and submission [20].

According to the present results, a critical rearing period is from 95 to 150 days of age in male birds, in concomitance with the onset of sexual maturity, and from 150 to 185 days of age in females, in concomitance with the onset of mating behavior. The separate rearing of males from females after 10 weeks of age may be suggested and studied in order to better control sexual behavior. A way to overcome the aggressiveness of males due to the development of the reproductive function during the long rearing period of Milanino birds could be to use males to produce capons, a traditional niche market product during the Christmas period in Italy [21]. It is known that castration is followed by a deficiency of testosterone and males soon show changes in appearance and behavior, becoming more docile and unwilling to mate [22]. Caponization is usually performed from 4 weeks to 8 weeks of age [23]; therefore, this practice will prevent the hormonal and behavioral changes associated with the onset of the reproductive function.

The overall mean feed consumption measured in the growing period, from 35 to 185 days of age, was very similar in both treatments and corresponding to 94 g/bird/day. Daily feed consumption progressively increased from 53 to 129 g/bird in the same period. The cumulative feed consumption recorded was 14.1 kg/bird/150 days rearing period. According to the present results on growth performance, further studies are suggested to collect more detailed data on variations in feed consumption according to the age and the sex of birds.

Mortality recorded during the brooding period (1 to 35 days of age) was 2.4%. Mortality recorded during the growing period (36 to 185 days of age) was 1.2 and 2.4% in the low and high density group, respectively.

Table 4. Carcass weight data recorded in male and female Milanino chickens slaughtered at 185 days of age.

Carcass weight data ¹	Females	Males	S.E.
LW (g)	2,398.80 ^A	2,661.75 ^B	59.42
CW (g)	2,191.00 ^a	2,369.95 ^b	52.50
PEC (g)	1,893.75 ^A	2,264.50 ^B	59.25
RCC (g)	1,583.00 ^A	1,785.90 ^B	38.50
PEC (% LW)	79.20 ^A	84.93 ^B	1.36
RCC (% LW)	66.16	67.13	0.77

¹LW = live weight; CW = carcass weight; PEC = partial eviscerated carcass; RCC = ready-to-cook carcass.

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

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.

Slaughter Performance

The sex was the only source of variation significantly affecting the carcass weight data. Bird density and the interaction between density and sex did not affect carcass weight data ($P > 0.05$). Significant results related to the slaughter performance are reported in Table 4. The live body weight (BW) measured before slaughter was higher in males ($P < 0.05$), compared to the BW of females, and carcass weight showed the same difference between sexes ($P < 0.05$). The weight of the PEC and RCC was heavier in males ($P < 0.001$) compared to the same weight recorded in females (Table 4). The PEC expressed as percentage of the BW had higher values in males ($P < 0.05$), while the RCC expressed as percentage of the BW presented no significant difference between males and females ($P > 0.05$).

The Milanino, like other local breeds reared in free-range systems, should be able to provide satisfactory productive performance. The recommendations of the Network for Animal Health and Welfare in Organic Agriculture [24] suggest that the use of commercial strains should be avoided to reduce welfare problems and, in contrast, chickens with a slow growing rate should be preferred for extensive production systems because they show more “natural” behavioral patterns. On the other hand, strains or breeds with a slow growth rate require a very long rearing time [25,26], resulting in high production costs. With this background, the position of local breeds as an organic and free-range product could be strengthened if their true ability

Table 5. Stress parameters recorded in male and female Milanino chickens raised in outdoor pens.

	Females	Males	S.E.
H/L ratio ¹	0.75 ^a	0.41 ^b	0.09
Relative spleen (%)	0.11	0.13	0.007

¹H/L ratio = circulating heterophil/lymphocyte ratio.

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.

to have good performance in very different environmental conditions will be confirmed. In the present study, bird density and the interaction between density and sex did not affect carcass weight data. Therefore, the Milanino can be considered a high adaptable breed, able to have good productive performance under both organic and free-range systems. The slaughter performance of the Milanino breed of chicken was good in both sexes and a high proportion of carcass yield was measured, corresponding to 66% and 67% RCC in female and male birds, respectively. Lower proportions of RCC were reported in other Italian chicken breeds, 63% and 62% in the Modenese and Romagnola breeds, respectively, both slaughtered at 210 days of age [6]. A lower RCC proportion (64%) has been also reported in the Delaware breed (64%), slaughtered at 105 days of age, and today promoted for small-scale poultry production in the United States. [27]. The proportion of semi-eviscerated and fully eviscerated carcasses measured in the male Milanino chickens are in agreement with the results reported in the Baicheng-You indigenous breed, one of the native breeds commonly raised for high-quality meat production in China [28]. However, it should be considered that the comparison of results from different reports is difficult due to differences in bird management, age, and weight at slaughter.

Stress Parameters

The most important parameter involved in stress response, the H/L ratio, was significantly affected by sex ($P < 0.05$) and bird density ($P < 0.05$). The circulating H/L ratio was higher in female (0.75) than in male (0.41) chickens (Table 5), and in birds kept at the higher density (0.74 vs 0.42) (Table 6). In contrast, the

Table 6. Stress parameters recorded in Milanino chickens reared in outdoor pens at different bird densities.

	Density 2 (m ² /bird)	Density 8 (m ² /bird)	S.E.
H/L ratio ¹	0.74 ^a	0.42 ^b	0.09
Relative spleen (%)	0.13	0.12	0.007

¹H/L ratio = circulating heterophil/lymphocyte ratio.

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.

proportion of the weight of the spleen on the body weight was not different ($P > 0.05$) between the two sexes and the two bird densities. The interaction between sex and bird density considered in the statistical model did not show a significant effect ($P > 0.05$) on stress parameters.

A number of environmental stressors affects H/L ratio [12,14], which makes this measurement one of the most accepted indicators of the chronic stress condition in birds. The rearing system and management might have great influence on stress conditions in poultry. There are many stress-inducing factors in domestic bird rearing, such as feed restriction, high stocking densities or inability to perform specific behaviors like dust or sand bathing [19]. An increased circulating H/L ratio indicates a stress state [12,29] and a high H/L ratio is negatively correlated with body weight [14,30]. To evaluate the best density at which to rear Milanino chickens during the growing period, it is important to understand if bird density affects the stress of the birds and, finally, growth performance. In the present study, the birds kept at the higher density presented a higher H/L ratio, and it affected male, not female, body weight at the end of the rearing period. In fact, as reported by Queiroz and Cromberg [19], environmental stress is a possible triggering factor of aggressive behavior, and this could contribute to a higher frequency of fights among males kept at 2 m²/bird close to puberty. The H/L ratio was higher in females suggesting different stress effects between males and females, in the rearing conditions utilized for this study. Aggressiveness and subordination are complex behavioral expressions that involve genetic differences between breeds, strains, and individuals [31]. Milman and Duncan [20] reported mating problems related to an increased aggressiveness

in broiler breeders during the reproductive phase. Males showed extreme aggressiveness during mating, causing serious injuries to females, or even causing death. It would be of interest to investigate the mating behavior in the Milanino breed to understand if male aggressiveness may be related to the higher stress condition found in females.

Overall results provided in this report on the free-range rearing system for the Milanino chicken breed are the first available. The present trial was performed in field conditions, therefore each experimental group was reared in one pen. Even in this situation, which could have created highly variable results, significant effects of bird density on growth performance and stress parameters were found. The present results suggest further studies are required to improve the management of the birds during the rearing period in free-range system.

CONCLUSIONS AND APPLICATIONS

1. The Milanino is a heavy breed with sexual dimorphism in relation to adult body weight: 2,864 g in males and 2,404 g in females at 185 days of age.
2. Flock density affects body weight for this breed. The major effect was recorded in males, who gained more weight at the low density.
3. Slaughter performance was recorded at 66 to 67% carcass yield for the breed. Carcass yield was not affected by flock density.
4. Flock density did affect bird stress levels with high bird density associated with an increase in H/L measurements. A rearing density of 8 m²/bird is recommended in outdoor pens to prevent welfare problems in both male and female chickens.

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Acknowledgments

This study was funded by Regione Lombardia (project n. 1723 - CoVAL). The authors would like to thank Merial Italy S.p.A. for the provision of Marek vaccine and the farm Il Roncone (Figino Serenza, Como, Italy) for housing experimental birds.