ENVIRONMENT, WELL-BEING, AND BEHAVIOR

The impact of phenotypic appearance on body weight and egg production in laying hens: A group-size- and experience-dependent phenomenon

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ABSTRACT Alterations of birds' phenotypic appearance (PA) may lead to unwanted behaviors, potentially impairing poultry welfare, health, and productive performance. Likewise, group size may play an important role modulating the expression of adaptive behaviors. This study evaluates whether changes in the PA of Hy-line Brown laying hens may affect their BW and egg production, and if so, whether these effects depend on group size. A total of 1,050 one-day-old chicks were randomly assigned to 1 of 45 pens. Groups were of 10, 20, or 40 individuals (8 hens/m^2). At arrival, the PA of 0, 30, 50, 70, or 100% of the birds within each group was artificially altered by marking the back of their heads black. The remaining birds within groups were unaltered. The 30% marked hens within groups of 10 individuals had a lower BW at 24 wk of age than their 70% unmarked counterparts, whereas the other groups showed similar BW. No differences were detected in egg laying performance during this phase. Next, within the initially homogeneous groups (0 and 100%), 30, 50, and 70% of the hens were either marked or unmarked (PA changed) sequentially at 34, 38, and 44 wk of age. Hens within the initially heterogeneous groups of 30, 50, and 70% marked birds remained unchanged and were used as controls. Groups of 40 individuals showed a reduction in BW gain and weekly hen-day-egg production after 30% PA changes, as compared with control counterparts. No differences were found in pens of 10 hens, and the groups of 20 showed intermediate results. A transient reduction in egg production was found after 50% PA changes. No further productive effects were observed after 70% changes. Our findings suggest that differences in hen appearance, which may occur due to variations in health status, injuries, and other natural causes, can be critical for production and welfare management practices depending both on the flock size and the birds' previous experience in exposure to group phenotypic heterogeneity.

Key words: phenotypic appearance, group size, social dynamics, body weight, egg production

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INTRODUCTION

The poultry industry faces new challenges and changes to adapt to new market interests, new regulations, and to continue improving the productivity as well as the welfare of birds. All these changes can have serious economic implications for the industry. In modern alternative production systems, birds have the advantage of wider behavioral opportunities and freedom of movement (Fröhlich et al., 2012). Nonetheless, these opportunities may also increase the appearance of unwanted behaviors, leading to negative consequences in terms of animal welfare, health, and productivity of laying hens (Sossidou and Elson, 2009). One of the major problems detected is the higher risk for injuries and mortality as a consequence of aggression and cannibalism that can occur even when beak trimming is applied (Elson, 2008). These behaviors, once they appear, can easily expand within the populations by learning (Cloutier et al., 2002), with dramatic consequences in terms of bird health, welfare, performance, and economic returns to the farmers. In battery cages, unwanted social interactions are likely to remain isolated to the surroundings of the small groups of birds housed in the cage, while in furnished cages and other alterative production systems, with larger numbers of birds per group, the problem can easily spread to the entire population, affecting

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many more individuals. Therefore, several management problems of alternative production systems arise as a result of the maintenance of larger groups in both enriched cages and aviaries. Furthermore, large group sizes (**GS**) have been associated with increased mortality and fear responses, damage to feathers and skin, and of particular relevance to the present study, to a reduced BW and egg production (Hughes and Duncan, 1972; Craig and Adams, 1984; Bilcik and Keeling, 1999; Nicol et al., 1999; Keeling et al., 2003; Rodenburg and Koene, 2007).

Social aggressive interactions within a group of domestic fowl are known to be directed toward subordinates (Guhl, 1968; McBride, 1969). These interactions do not appear to occur at random, and have been linked to specific phenotypic appearance (**PA**) of the aggressor and the receiver of the interaction, including aspects such as body mass, comb size, and even their previous social experience (Guhl and Ortman, 1953; Cloutier and Newberry, 2002). A winning experience in a previous encounter will enhance the likelihood of winning again in future interactions, whereas a prior losing experience will increase the chances of subsequent defeats (Hsu and Wolf, 1999; Cloutier and Newberry, 2000; Beacham, 2003; Hsu et al., 2006). Familiarity, or lack thereof, is also an important factor with demonstrated relevance in the group dynamics of domestic fowl (Lindberg and Nicol, 1996; Marin et al., 2001). However, D'Eath and Keeling (2003) proposed that individual recognition, although relevant in small groups, may be less so in large groups where aggressive interactions and other types of social relationships might be based in badges of status (Pagel and Dawkins, 1997). These authors indicated that in large groups, badges of status may take place over active dominance relationships, and suggested that identifiable marks, either positive or negative, might increase the likelihood of recognition of the birds' social status, but not of the individual itself. Interestingly, it has also been observed that birds that are phenotypically different from their conspecifics (for instance, due to natural variation in the feathering coloration) are at higher risk of being pecked and possibly cannibalized as has been noted experimentally (McAdie and Keeling, 2000; Estevez et al., 2003; Dennis et al., 2008), as well as in commercial facilities (I. Estevez, personal observation). Guhl and Ortman (1953) and Guhl (1968) discovered that chickens that were experimentally altered in their physical appearance (by dying feathers, adding feather extensions, and altering combs) received high levels of aggression from their prior subordinates when returned to their original flock. It has also been shown that the inclusion of marks on the birds can potentially alter not only behavioral responses but also the marked birds' stress-related hormonal responses (Dennis et al., 2008). All this taken together suggests that social dynamics in the domestic fowl can be much more complex than anticipated. Factors such as PA, previous experience, familiarity, and the GS where birds are housed might play a very important role on the relationships between laying hen groupmates, modulating their adaptation to new particular situations (Jones, 1996; Bilcik and Keeling, 1999; Estevez et al., 2003, 2007; Sossidou and Elson, 2009). The quality of these relationships can have important management, production, health, and welfare consequences.

The objective of this study was to evaluate whether differences or changes in the PA of Hy-line Brown laying hens may affect their BW and egg production, and if so, whether these effects are dependent on the GS and previous experience. In a first phase, we assessed the effects of GS in groups with either homogeneous or heterogeneous PA since 1 d of age. We aimed to determine whether variations in the GS and phenotypic composition since a very early age can affect the dynamic of the group, affecting main performance parameters (BW, puberty age, and egg production). In a second phase, when hens reached full adulthood and peaked egg production, different proportions of hens were subsequently PA altered within the initially homogeneous groups. The aim was to determine whether the sequential changes to the hens' phenotype can affect the group performance, affecting BW and egg production parameters. Similarly to the first phase, we also determined if that effect is dependent on the size of the group.

MATERIALS AND METHODS

Birds and Rearing Conditions

Twelve hundred 1-d-old Hy-line brown female chicks were obtained from Avigán Terralta (Tarragona, Spain) and transported to a new experimental poultry facility at the Neiker-Tecnalia research center (Vitoria-Gasteiz, Spain). Immediately upon arrival, 1,050 chicks were randomly assigned to 45 pens that housed groups of 10, 20, or 40 birds (GS of 10, 20, and 40, respectively; 15 pens per GS). The additional 150 birds were placed in supplementary enclosures to replace mortalities of experimental birds that occurred during the first 10 d of the growing period. All birds were kept at the same density (8 birds/m^2). Therefore, pen dimensions varied according to GS. Pens housing 10 birds were 0.75 × 1.78 m (1.25 m^2), 1.00 × 2.50 m (2.5 m^2) for 20 birds, and 2.00 × 2.50 m (5.00 m^2) for 40 birds. Each pen was provided with automatic drinkers and feeder space proportional to the number of birds housed (4 cm^2 feeder space per bird and 1 nipple every 5 birds). A black plastic sheet was attached to the sides of the pens to avoid potential visual contact among individuals from neighboring pens. Bedding was provided in the form of wood shavings (approximately 3 kg/m^2) and birds were fed ad libitum a commercial diet according to their rearing stage. A computerized system allowed light, ventilation, and temperature control, which followed commercial standard practices.

Table 1. Experimental design for phase I and II regarding phenotypic appearance (PA) assignment within groups from each group size (10, 20, or 40 individuals)[1]

		PA composition phase II (34 to 48 wk of age)		
Original marking treatment (%)	PA composition phase I (1 to 34 wk of age)	30% changed (34 wk of age)	50% changed (38 wk of age)	70% changed (44 wk of age)
0	100UM	30M and 70UM	50M and 50UM	70M and 30UM
30	30M and 70UM	30M and 70UM	30M and 70UM	30M and 70UM
50	50M and 50UM	50M and 50UM	50M and 50UM	50M and 50UM
70	70M and 30UM	70M and 30UM	70M and 30UM	70M and 30UM
100	100M	30UM and 70M	50UM and 50M	70UM and 30M

[1]UM = unmarked; M = marked.

At 2 d of age, all birds were labeled for individual recognition with 2 white laminated paper tags placed on each wing side (Cornetto and Estevez, 2001). Tags included the pen number and an individual bird number identification [2-digit black numbers printed on both sides, Dennis et al. (2008)] and were fixed with plastic filaments injected under the skin using the Swiftack system (Heartland Animal Health Inc., Fair Play, MO). Birds were remarked at 8 wk of age in both sides of the neck with larger tags (5 × 5 cm) according to bird size and feather growth. Before the laying period started, pens were provided with nests and perching space also proportional to GS.

The experiment was approved by the ethical committee at Neiker-Tecnalia in compliance with the Spanish legislation regarding the use of animals for experimental and other scientific purposes (Real Decreto 1201/2005). This study is part of a larger project that evaluates the effects of changes in PA and GS on behavioral and health-related variables.

Experimental Design Phase I: Same PA Through Time

Upon arrival, the birds' PA was either maintained unaltered (unmarked) or artificially altered (marked) by placing a black mark with a nontoxic dye on the back of the head, following experimental procedures detailed elsewhere (Dennis et al., 2008). Specifically, pens from each GS were assigned to one of the following PA treatments: 0, 30, 50, 70, or 100% of the birds marked, yielding 3 replicate groups for each PA option and within each GS (Table 1). All marks were as similar as possible and birds were remarked as needed through the growing stage. Thus, according to the birds' PA, the following treatments were studied: homogeneous groups with 100% individuals unmarked (**100UM**), homogeneous groups with 100% individuals marked (**100M**), heterogeneous groups with 30% individuals marked and 70% unmarked (**30M** and **70UM**), heterogeneous groups with 50% individuals marked and 50% unmarked (**50M** and **50UM**) and heterogeneous groups with 70% individuals marked and 30% unmarked (**70M** and **30UM**). All groups remained with the same assigned PA until 34 wk of age. The same birds used in phase I were also evaluated during phase II.

Experimental Design Phase II: Changing a Proportion of the Groups' PA Through Time

During phase II, groups with initially homogeneous PA (100UM and 100M for all GS: 10, 20, and 40) were altered by sequentially changing the PA of 30, 50, and 70% of individuals at 34, 38, and 44 wk of age (1st, 2nd, and 3rd PA change, respectively). The PA change was accomplished by either randomly marking the birds' head (for 100UM, using the same procedure described above), or unmarking them (for 100M, by applying an H_2O_2 solution during approximately 30 min to the dyed feathers; Table 1).

Specifically, the 30% of birds that were altered by either marking or unmarking them at 34 wk of age (1st PA change), remained with this assigned PA until the end of the study. At 38 wk of age, an additional 20% of the birds were altered (2nd PA change) within the same groups by either marking or unmarking to achieve a total of 50% of birds with altered PA. Similarly, at 44 wk of age, during the 3rd PA change, another set of 20% of the birds that still remained with their original phenotype, were either marked or unmarked to achieve a total of 70% of birds with altered PA. Remaining individuals within the group were unaltered, maintaining the original phenotype assigned at the onset of the study. The groups with initially assigned heterogeneous PA composition (30M and 70UM, 50M and 50UM, and 70M and 30UM) remained with the same phenotype composition for the whole study (no change of the original assigned PA during phase II) and served as controls for the sequential PA changes (Table 1).

Variables Measured

Individual BW were taken (PCE-PCS scale, PCE Instruments UK Ltd., Southhampton, Hampshire, UK) on 10 d, 12 wk, and 24 wk of age during phase I of the study, and again 4 wk after the 1st, 2nd, and 3rd PA changes. Daily egg production per pen was recorded for 33 wk beginning with the day on which the first bird laid an egg; this occurred at 15.5 wk of age and was considered d 1 of lay. During the laying period, mortalities or birds removed for health reasons were not replaced. To assess the onset of puberty, the average age (d) at first egg laid (**FEL**), at cumulative 25% egg production (**A25%EP**), and at cumulative 50% egg production (**A50%EP**) since d 1 of lay were calculated for each pen. A cumulative hen-day egg production (**HDEP**) was also determined per pen at the end of phase I, whereas for phase II a weekly HDEP was calculated. In addition, morphometric measures of the eggs (egg weight, length, and width) were also recorded 4 wk after the 1st, 2nd, and 3rd PA changes by randomly sampling 5 eggs from each pen.

Statistical Analysis

Data analyses were conducted following procedures described by Dennis et al. (2008). Mean BW per PA and pen was calculated for data collected on d 10 and wk 12 and 24 (phase I). Differences in mean BW were determined by a mixed-model ANOVA with PA treatments (100UM, 100M, 30M, 70UM, 50M, 50UM, 70M, and 30UM) and GS (10, 20, and 40) as fixed effects, and age at weighing as a repeated measure. Pen was included as a random effect. To correct for heterogeneity of variance, data were transformed to ranks.

During phase II, to avoid potential carrying over effects from phase I, we worked with BW gain that was also averaged according to PA types within pen. Separate mixed-model ANOVA were used to examine the main effects of PA, GS and their interaction on BW gain for each of the PA changes (1st, 2nd, or 3rd), as the control groups in each case were different. For the 1st PA change, there were 8 PA groups that were 30M, 70UM, 30UM, and 70M from groups with PA originated in phase I and unchanged during phase II, and 30M, 70UM, 30UM, and 70M from groups with PA that were changed at 34 wk of age. For the 2nd PA change, the 6 treatments were 50M and 50UM unchanged, original from phase I, and 50M, 50UM, 50UM, and 50M from groups with PA changed during phase II. Finally, for the 3rd PA change, the treatments included 70M, 30UM, 70UM, and 30M unchanged, original from phase I, and 70M, 30UM, 70UM, and 30M from groups with PA changed. Pen was also included as a random effect.

Additionally, to determine the evolution of BW gain through the sequential marking procedures, BW gain data during phase II were analyzed considering the order in which the PA of each bird was artificially changed (either unchanged or altered during the 1st, 2nd, or 3rd PA change). Thus, BW gain data from birds that were marked or unmarked during each of the PA changes were averaged per pen and analyzed using mixed-model ANOVA that examined the fixed effects of order of PA changing (PA that remain unchanged, PA altered during the 1st change, PA altered during the 2nd change, and PA altered during the 3rd change), GS (10, 20, and 40), time after the change was made (4 wk after 1st, 4 wk after 2nd, and 4 wk after 3rd change; as a repeated measure) and their interactions. Pen was included as a random effect.

Daily egg production data were evaluated per pen. Because it was not possible to distinguish eggs laid by marked or unmarked birds, PA treatments in this case were 100UM, 100M, 30M-70UM, 50M-50UM, and 70M-30UM. Data to assess differences in puberty parameters (FEL, A25% EP, and A50% EP) and cumulative HDEP at the end of phase I were analyzed by mixed-model ANOVA, which included PA treatment (as described) and GS as fixed effects. Differences in HDEP after 1st, 2nd, and 3rd PA change were evaluated by separated mixed-model ANOVA that incorporated PA treatment, GS and time after the PA change (before changes, and 1, 2, 3, and 4 wk after changes; as a repeated measure). There were 4 PA treatments for the 1st PA change (30M-70UM and 30UM-70M from groups with PA not changed, and 30M-70UM and 30UM-70M from groups with PA changed), 3 PA treatments for the 2nd PA change (50M-50UM from groups with unchanged PA, and 50M-50UM from groups with PA changed by marking them, and 50M-50UM from groups with PA changed by unmarking them), and 4 for the 3rd PA change (70M-30UM and 70UM-30M from groups with unchanged PA, and 70M-30UM and 70UM-30M from groups with PA changed). Morphometric egg data (egg weight, length, and width) collected during phase II were averaged by pen and evaluated by mixed-model ANOVA that considered the fixed effects of PA and GS.

Whenever significant effects were detected, least squares means were determined and contrasts were used for means comparisons.

RESULTS

Phase I: Same PA Through Time

The results of the study regarding BW revealed an interaction between initial PA, GS, and age ($F_{28, 96}$ = 2.11; $P = 0.004$; Figure 1). Body weights were similar in all groups at 10 d and 12 wk of age. However, at 24 wk, 30M hens within GS 10 showed lower BW compared with their 70UM groupmates ($P = 0.001$), and all other phenotype groups housed in groups of 10 individuals ($P < 0.05$, in all cases). Within groups of 20 or 40 individuals, no differences were detected for BW ($P > 0.05$; Figure 1).

Analysis on puberty parameters showed no effects of initial PA or GS ($P > 0.39$ in both cases) on FEL, A25%EP, or A50%EP (15.5 ± 0.3, 20.6 ± 0.2, and 23.8 ± 0.3 wk of age, respectively). Similarly, no PA or GS effects ($P > 0.60$ in both cases) were detected on the cumulative HDEP (0.96 ± 0.01) at the end of phase I (34 wk of age).

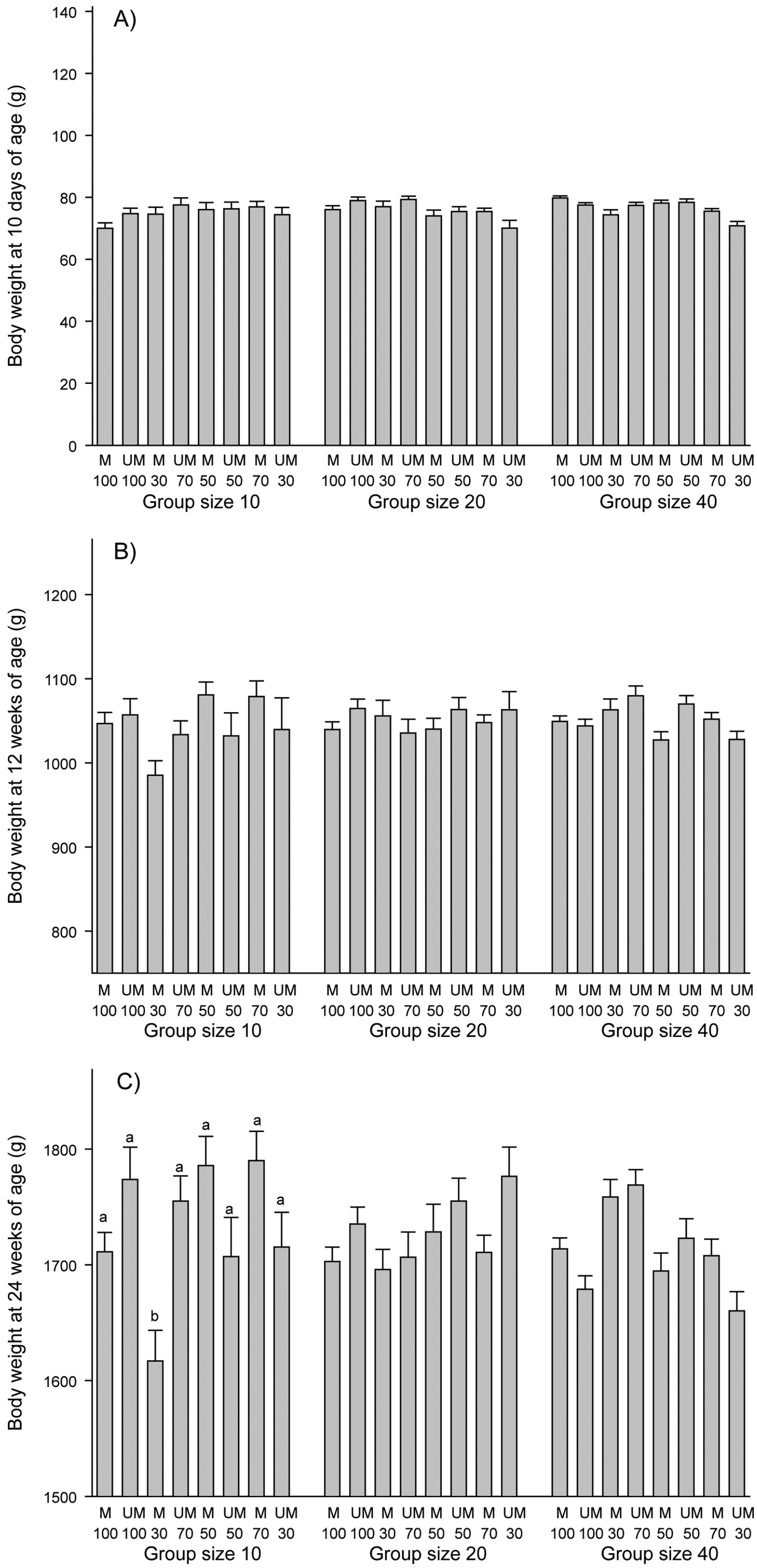

Figure 1. Body weight in birds with different artificial phenotypic appearance since 1 d of age. Bars represent the mean ± SE. M = marked; UM = unmarked; 100, 30, 50, and 70 = 100, 30, 50, and 70% of the birds within the group either marked or unmarked. Groups with no common letters (a,b) differ significantly ($P < 0.05$).

Table 2. Summary of BW gain statistical results (shown in Figure 2) after the phenotypic appearance (PA) changes of an increased proportion of the group[1]

	30% changed			50% changed			70% changed		
Effect	df	*F*-value	*P*-value	df	*F*-value	*P*-value	df	*F*-value	*P*-value
PA	7, 48	5.51	0.001	5, 36	0.83	0.533	7, 48	1.07	0.399
GS	2, 48	12.03	0.001	2, 36	14.24	0.001	2, 48	10.20	0.001
PA × GS	14, 48	3.29	0.002	10, 36	0.75	0.670	14, 48	0.71	0.748

[1]GS = group size.

Phase II: Changing a Proportion of the Group PA Through Time

Table 2 summarizes the results for the analysis of BW gain for phase II, after the 30, 50, and 70% PA changes occurring at 34, 38, and 44 wk of age, respectively. For the 30% PA change, PA, GS, and their interaction affected the birds' BW gain, whereas for 50 and 70% PA changes, only GS showed an effect (Figure 2).

After the first PA change (30%), no differences were detected among PA groups housed in groups of 10 individuals, whereas groups of 20 and 40 individuals showed declines in BW gain (Figure 2A). Specifically, within GS 20, the PA altered 30M group showed a lower BW gain compared with their 70UM (unaltered) groupmates and compared with their 30M control nonaltered counterparts (30M from the start of the study; $P < 0.01$ in both cases). The PA altered 30UM group showed intermediate values of BW gain that did not differ from the altered 30M group or their nonaltered 30UM controls. On the other hand, within GS 40, both the altered 30M and 30UM groups showed reductions in BW gain compared with their 70UM and 70M counterparts ($P < 0.01$ in both cases) and compared with their respective nonaltered 30M and 30UM controls ($P < 0.05$ in both cases). Subsequent PA changes, to complete a 50 or 70% PA changed, showed no further impact of PA or an interaction between PA and GS on BW gain. Only overall GS effects were detected where birds housed in groups of 10 showed higher BW gain values than birds housed in groups of 20 and 40 individuals (Table 2; Figures 2B and C).

Figure 3 depicts the mean BW gain (±SE) of hens according to the order of PA change. Table 3 summarizes the results of the statistical analyses. An interaction between the order of PA change, GS, and time after the change was found. Initially, 4 wk after the 1st PA change, the BW gain of the hens altered during this PA change showed a reduction ($P = 0.002$) compared with nonaltered hens. This reduction was observed within the groups of 20 and 40 hens but not within the groups of 10. However, BW gain after subsequent PA changes (2nd or 3rd) recovered to values equivalent to those of hens housed in groups that remain unchanged during phase II (other than on d 1 after arrival). On the other hand, regardless of GS, hens that were submitted to PA changes in 2nd or 3rd order showed no BW gain differences compared with their PA unchanged counterparts ($P > 0.42$ in all cases).

Table 4 summarizes the results of the statistical analyses for weekly HDEP. After changing the appearance of 30% of the hens (1st PA change), interactions between PA treatment and GS, PA treatment, and time after the changes, and GS and time after the changes were observed (Figure 4A, B, and C, respectively). No differences between PA treatments were detected among groups of 10 individuals. However, within GS 20, the changed 30M-70UM group showed a HDEP reduction compared with the other groups, and within GS 40, the changed 30M-70UM and 70M-30UM groups both showed reductions compared with their unchanged controls (Figure 4A). Compared with GS 10, the groups of 20 and 40 individuals showed transient reductions after the 1st PA change was applied (Figure 4B). Changed groups (30M-70UM and 70M-30UM) showed transient weekly HDEP reductions compared with their unchanged 30M-70UM and 70M-30UM controls. The lowest HDEP values were observed 2 wk after the PA change and the 30M-70UM group showed stronger reductions than the 70M-30UM group. Four weeks after the change, altered groups recovered similar egg production levels as their unchanged control groups (Figure 4C). Analysis of variance after completing the 2nd PA change (50% of the hens altered) showed that weekly HDEP was affected by an interaction between PA and the time after the application of the changes (Figure 5). A GS effect was also detected (Table 4). Hen-day-egg production was higher in birds housed in groups of 10 individuals (0.95 ± 0.01) than in birds housed in groups of 20 (0.92 ± 0.01) or 40 individuals (0.91 ± 0.02). After the 3rd PA change (70% of the hens altered), no effects of PA, GS, or interactions between factors were found ($P > 0.36$ in all cases). Only a time effect was found showing an overall decline in weekly HDEP at the last week of the study (0.90 ± 0.01) compared with the previous weeks (0.94 ± 0.01, 0.93 ± 0.01, 0.93 ± 0.01, and 0.93 ± 0.01).

The statistical analyses on the morphometric egg data collected during phase II showed no effects of PA, GS, and time after PA changes, nor interactions between them. Average egg weight, length, and width were 64.3 ± 0.3, 57.7 ± 0.1, and 0.61 ± 0.1, respectively.

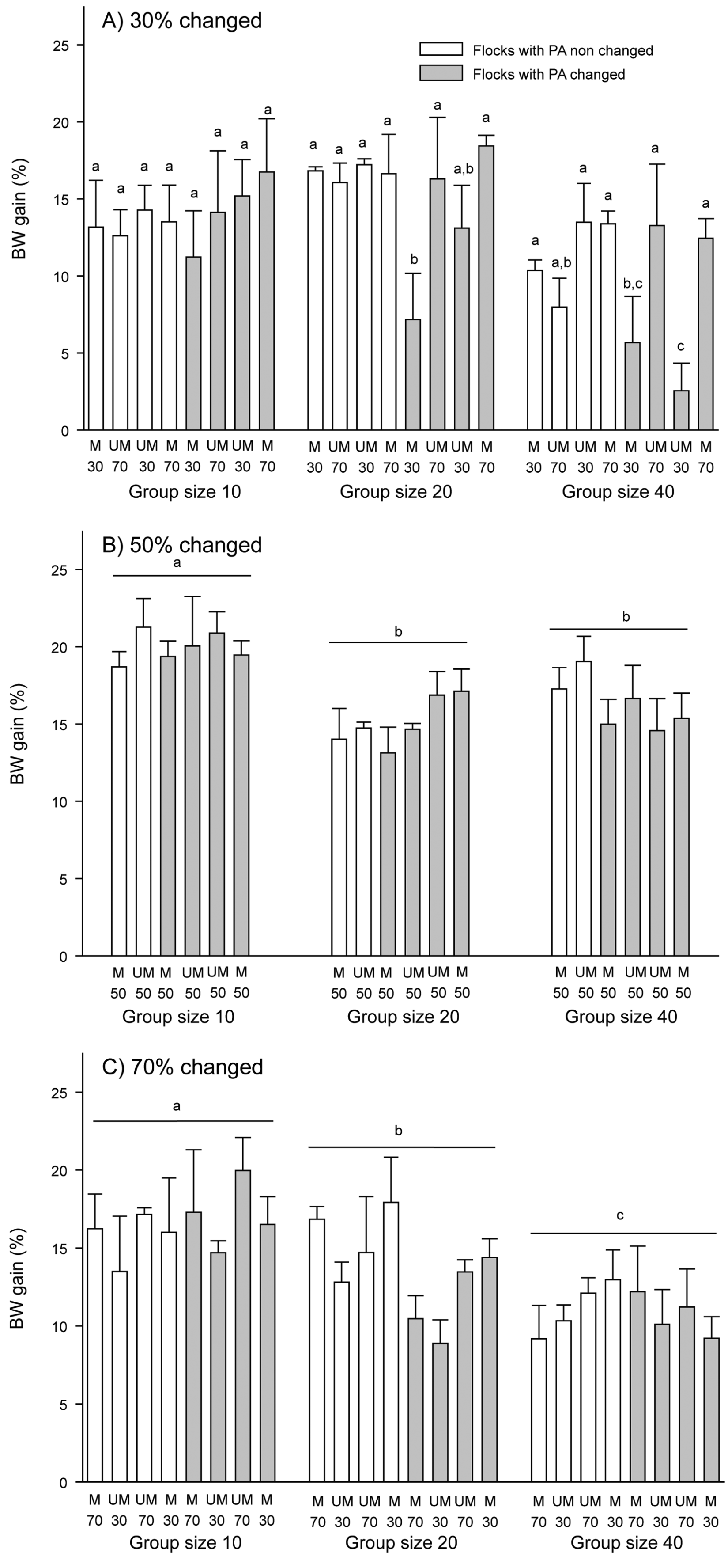

Figure 2. Body weight gain after the phenotypic appearance (PA) of an increased proportion of the group (30, 50, or 70%) was sequentially changed. Bars represent mean ± SE. M = marked; UM = unmarked; 30, 50, and 70 = 30, 50, and 70% of the birds within the group either marked or unmarked. A) Within each group size, phenotype groups with no common letters (a–c) differ significantly ($P < 0.05$); B) and C) group size pooled data with no common letters (a–c) differ significantly ($P < 0.05$).

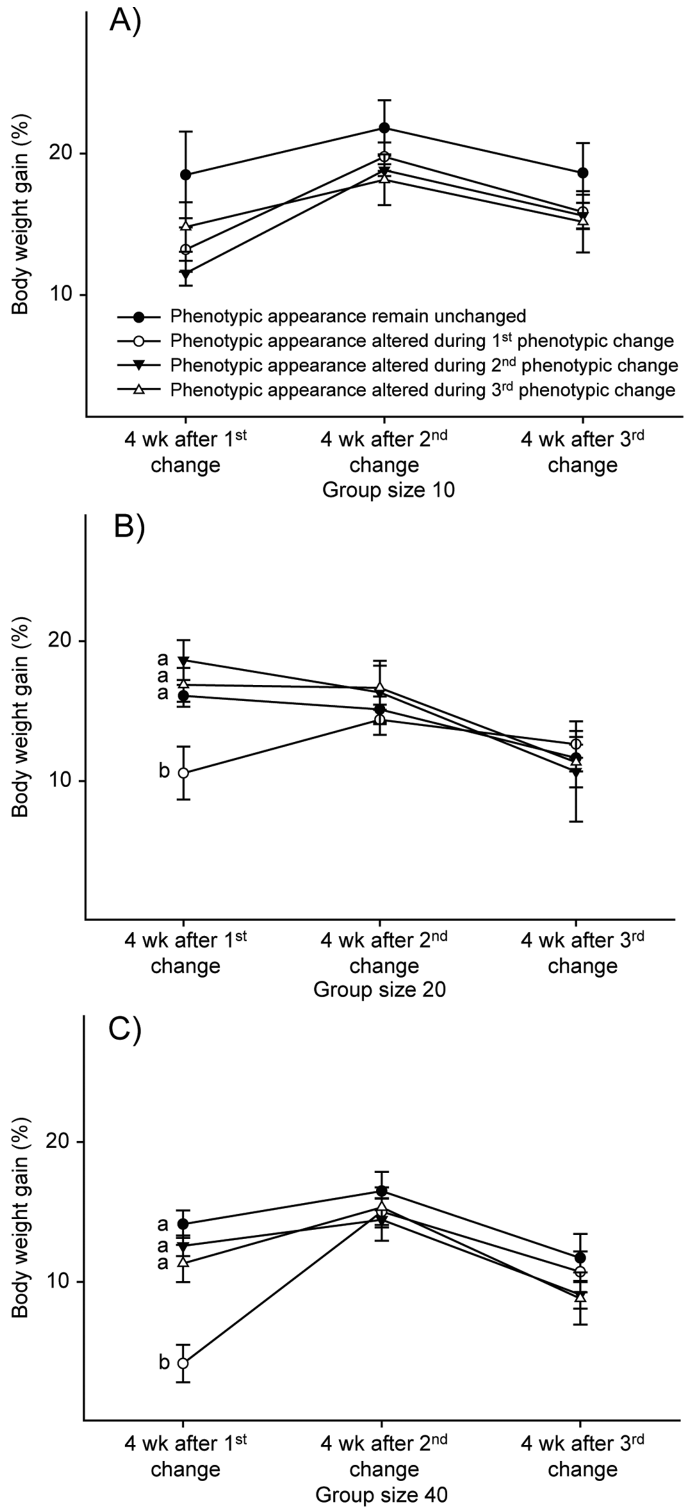

Figure 3. Body weight gain according to the time when the phenotype of the birds was artificially changed (either phenotypic appearance unchanged or altered during 1st, 2nd, or 3rd phenotypic changes). Data from altered birds (either marked or unmarked) during the same phenotypic change were pooled to facilitate visualization. Groups with no common letters (a,b) differ significantly ($P < 0.01$).

DISCUSSION

This is an experimental study designed to evaluate the potential productive effects derived from alterations of the PA in a layer strain (Hy-Line Brown). The experimental design was constructed to manipulate the degree of PA composition, varying from 30 to 70% heterogeneity, that were compared with homogeneous nonmarked (100UM) or all-marked (100M) groups. These treatments were combined with 3 GS because consequences of PA heterogeneity may differ depending on the social dynamics related to GS (Estevez et al., 1997, 2003). Although commercial poultry is normally reared in homogeneous groups (all sharing a similar plumage color and being of the same age) individuals may change their PA due to naturally occurring phenomena (e.g., diseases, injuries). Therefore, to simulate this effect the experiment included manipulations of the phenotypes in homogeneous groups during adulthood. Results could also be relevant from a practical standpoint considering that some European countries also raise mixed flocks of white and brown birds (Leenstra et al., 2012).

The results of phase I (PA changes applied on d 1) showed combined PA, GS, and age effects. No initial PA or GS effects were evident on BW at early and intermediate ages (10 d of age or 12 wk of age). The lack of differences across groups at these ages is not unexpected and seems to be consistent with the random assignation of birds to the PA and GS treatments at the beginning of the study, and with early filial learning processes (Bolhuis and Bateson, 1990; Bolhuis and Honey, 1998) that would facilitate groupmate recognition in despite of their PA and degree of group heterogeneity. Although BW in groups of 20 or 40 individuals continued to be similar regardless of PA at 24 wk, 30M birds within groups of 10 showed a lower BW compared with their 70UM groupmates, and to all other phenotype compositions housed in groups of 10 individuals. Egg laying started at 15.5 wk, reaching the 50% HDEP by 23.8 wk of age. Thus, BW differences observed after the onset of lay might relate to hormonal changes occurring at sexual maturation, a period where potentially dangerous social interactions are more frequently expected (Savory and Mann, 1997). The fact that the negative response was restricted to 30M birds, and not 30UM, and to small GS 10 exclusively, suggest, a priori, that group of hens can perceive as different the conspecifics with a conspicuous mark (black feathers on the head), but not to those that, although different, do not carry a conspicuous signal. Despite the clear effect in the small groups, the effects of a PA conspicuous change appear to be diluted in larger groups. The detrimental effects on BW of conspicuous marking are consistent with the results obtained by Dennis et al. (2008) for groups of broilers with a 20% of PA modified.

The restriction of effects to small groups appears to be consistent with the idea of hierarchy formation in small groups (Guhl, 1968) and of tolerant social dynamics in larger groups that require no individual recognition per se (Estevez et al., 1997, 2003). In this study, we manipulated the PA to constant proportions of birds along the different GS treatments. Therefore, in a small group, the 30% consisted of 3 birds, whereas

Table 3. Summary of BW gain statistical results (shown in Figure 3) according to the order in which the phenotypic appearance (PA) of the birds was artificially changed (PA unchanged or altered during the 1st, 2nd, or 3rd phenotypic changes)[1]

Effect	df	*F*-value	*P*-value
Order of PA changing	3, 60	2.51	0.068
GS	2, 60	12.18	0.000
Time after PA change	2, 120	61.25	0.000
PA × GS	6, 60	0.84	0.547
Order of PA changing × time after PA change	6, 120	8.58	0.000
GS × time after PA change	4, 120	12.02	0.000
Order of PA changing × GS × time after PA change	12, 120	2.37	0.009

[1]GS = group size.

for GS 20 and 40 the 30% consisted of 6 and 12 birds, respectively. It seems to some extent logical that to be one of the few in a small group might not be equivalent to be one of the few in a larger group, and the effect may be diluted by the larger number of birds that share a common PA. Plumage coloration is known to have a major role as a signal of status in birds (Krebs and Davies, 1987; Whitfield, 1987), especially conspicuously pigmented feather patches that may convey fighting ability (Senar and Camerino, 1998; Senar, 1999). However, it is also speculated that for a signal to be honest (and evolutionary stable) it must have an associated cost (Senar, 1999), potentially in the way of increased social challenge (Tibbetts and Dale, 2004). Feather pigmentation has also been shown to affect the risk of being targeted by feather peckers (Keeling et al., 2004). Thus, the pigmentation does not necessarily link to a signal with positive connotations. It is then possible that the reduced number of birds with changed PA in the way of a conspicuous black mark, within GS 10, may have received a greater number of social challenges from their group mates, and over time may have succumbed to the social cost of carrying the badges of status. These effects appear to start, in terms of BW, at wk 12 and become highly evident at 24 wk. On the contrary, if in larger groups the level of social pressure was lower, as speculated by a tolerant type of social organization rather than a hierarchical structure (Estevez et al., 1997, 2003, 2007), and if the social challenges were more dispersed among a greater number of phenotypically altered birds, then the effects on the PA changed individuals may have been undetectable in larger groups. This hypothesis seems to be supported by preliminary results of the aggressive interactions observed in these groups (I. Campderrich, M. G. Liste, and I. Estevez, unpublished data).

The results in egg production showed no effects of initial PA or GS on FEL, A25%EP, or A50%EP, and no effects on the cumulative HDEP were detected at the end of the first phase of the study (34 wk of age). These findings suggest that, even though behavioral and BW changes can be induced by the particular social conditions used herein (GS and PA combination), when these conditions are imposed from a very early age, they do not appear to significantly affect the group egg production. This can be explained considering processes of social structure modulation, learning, tolerant strategies, or a combination of these (McBride et al., 1969; Bessei, 1985; Keeling and Duncan, 1991; Jones, 1996; Estevez et al., 1997; Leone and Estevez, 2008; Leone et al., 2010) that would allow to minimize socially induced stress responses that otherwise could compromise the sexual maturation (Marin et al., 2002). In addition, it is relevant to note that although for data analyses BW were averaged per pen, they were registered individually and therefore discrimination between the individuals with a given PA and their groupmates with another PA was possible. However, egg data were collected per pen and any potential reduction effect on egg production that may occur in hens that were representatives of the lower PA proportion could be "diluted" within the egg production of the groupmates that represent the

Table 4. Summary of hen-day-egg production statistical results after artificial phenotypic appearance (PA) changes[1]

	1st phenotypic change (30% changed)			2nd phenotypic change (50% changed)			3rd phenotypic change (70% changed)		
Effect	df	*F*-value	*P*-value	df	*F*-value	*P*-value	df	*F*-value	*P*-value
PA treatment	3, 24	13.72	0.000	2, 18	3.52	0.050	3, 24	0.47	0.708
GS	2, 24	6.41	0.006	2, 18	3.58	0.049	2, 24	0.19	0.830
Time	4, 96	12.56	0.000	4, 72	9.49	0.000	4, 96	9.64	0.000
PA treatment × GS	6, 24	3.64	0.010	4, 18	0.67	0.622	6, 24	1.16	0.362
PA treatment × time	12, 96	4.62	0.000	8, 72	2.48	0.019	12, 96	0.97	0.483
GS × time	8, 96	3.21	0.003	8, 72	0.89	0.526	8, 96	0.65	0.736
PA treatment × GS × time	24, 96	1.84	0.103	16, 72	1.00	0.470	24, 96	1.02	0.453

[1]GS = group size; time = time after the PA change (before changes, and 1, 2, 3, or 4 wk after changes).

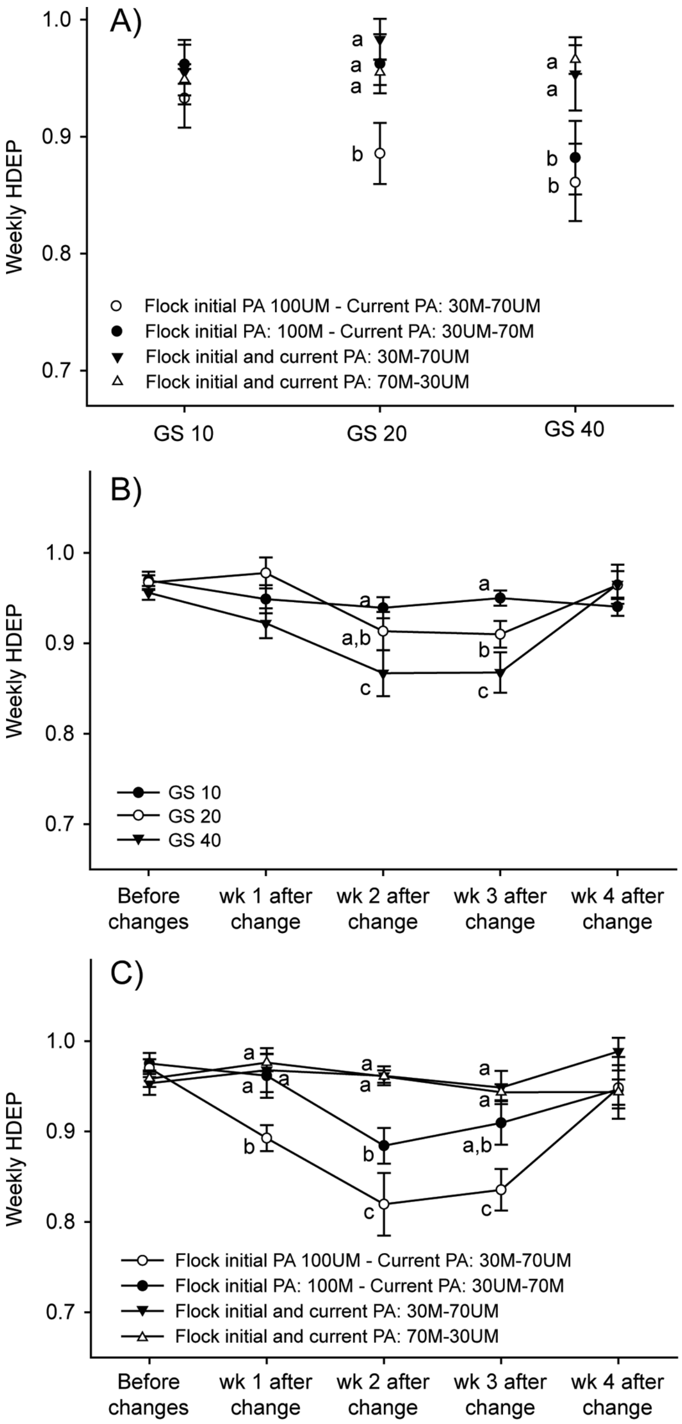

Figure 4. Weekly mean hen-day egg production (HDEP; ±SE) after the phenotypic appearance (PA) of 30% of the group was artificially changed. M = marked; UM = unmarked; 30, 70, and 100 = 30, 70, and 100% of the birds within the group either marked or unmarked. Groups with no common letters (a–c) differ significantly ($P < 0.05$).

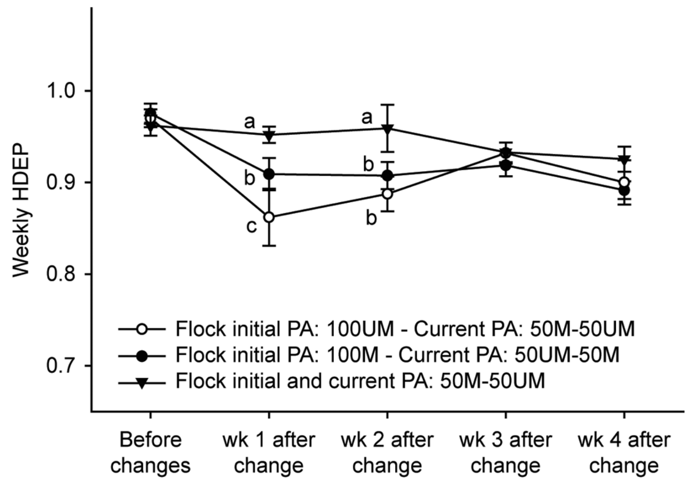

Figure 5. Weekly mean hen-day egg production (HDEP; ±SE) after the phenotypic appearance (PA) of 50% of the group was artificially changed. M = marked; UM = unmarked; 50 and 100 = 50 and 100% of the birds within the group either marked or unmarked. Groups with no common letters (a–c) differ significantly ($P < 0.05$).

larger PA proportion. Thus, egg production and BW results should not be directly compared. Unfortunately, we had no means to collect eggs from individual hens.

Results from phase II provide a different picture. When changes in PA occurred in homogeneous groups (100UM and 100M) during adulthood, BW gain and egg production declined in both 30M and 30UM birds when in groups of 40. It also declined in groups of 20 but in a less pronounced manner for 30UM. On the contrary, no changes in BW gain or HDEP were detected among different PA groups housed in groups of 10 individuals. Importantly, the impact of the PA change on HDEP was greater when the phenotype was changed by marking the hens (30M) than when the marks were eliminated (30UM), and the intensity of the impact was higher in larger groups of 40 individuals. In all cases HDEP recovered to initial values 4 wk after the change. The explanation of these findings is complex, but suggests that birds discriminated both types of PA changes, and that in both cases such changes produced sufficiently important social challenges, with severe consequences at a physiological level, to affect BW gain and egg production (see further considerations below). However, birds were able to return to initial levels of BW gain and egg production within a few weeks, perhaps due to social plasticity that would allow “alien phenotypes” to be fully incorporated in the social dynamics. This idea can be further supported by the findings that demonstrate that subsequent PA changes to complete 50 or 70% of the group changed showed no further impact on BW gain, and only a transient reduction in HDEP was detected predominantly in 50M and with lower impact in 50UM. These results suggest that once the new phenotype is recognized within groups, further phenotype changes of additional group members have a limited effect that reflects exclusively on HDEP. In addition, results of the changes in BW gain, according to the order in which each hen’s PA was altered (i.e., control never altered vs. altered (marked or unmarked) during either the 1st, 2nd, or 3rd PA changes), show that negative effects in BW gain were only observed in the hens that were first submitted to changes. This occurred regardless of the new phenotype assigned (marked or unmarked) and within the groups of 20 and 40 individuals but not within the groups of 10. Nevertheless, BW gains did not differ between groups after the 2nd or 3rd changes.

Considering all these findings, it is clear that the effects of phenotypic diversity are distinct when they occur at different developmental stages (near hatch time, during or after sexual maturation, and on adult birds). This is to be expected as social bonding among group members occurs early in life and is based on recognition and discrimination (Doyen, 1987; Zayan, 1987; Jones et al., 1996), even in the absence of individual recognition mechanisms. For example, kin recognition theory suggests that cooperation and reduced aggression may be controlled by perceived relatedness (Keller, 1997; Frank, 2013), which in some species appears to be based on the degree of phenotypic similarity (Hamilton, 1964a,b; Jaisson, 1991). Therefore, phenotypic diversity when occurring at early stages would be generally incorporated as part of the normal phenotype. When in adulthood, sudden changes in phenotypic diversity would create a (at least temporal) major social disruption possibly because the phenotype fails to be recognized as kin, a situation that may lead to more severe aggressive interactions in an attempt to exclude individuals that are not recognized as group members or to minimize interpopulation competition for resources, or even as a mechanism to avoid risk of diseases from unknown populations (Fugle et al., 1984, Järvi et al., 1987).

It has been proposed that situations such as crowding, alteration of group membership, or large groups exceeding their social recognition capacity may induce disturbances or completely prevent natural social relationships with potentially harmful effects for birds (Jones et al., 1996; Hughes et al., 1997). On the contrary, Pagel and Dawkins (1997) proposed that in large groups, badges of status can take over active dominance relationships, suggesting that, although the individual itself may not be identified, an identifiable feature might increase the likelihood of recognition of the birds' social status. Preliminary analyses of the birds' behavior in this study showed that after PA alterations, changed hens received more aggressions, and spent less time eating and more time resting than unchanged counterparts (M. G. Liste, I. Campderrich, and I. Estevez, unpublished data). Therefore, considering these behavioral differences between changed and unchanged hens, and the impaired performance observed herein, it can be suggested that altered birds in initially homogeneous groups were proactively targeted as aliens possibly assigning them to a low social status, which is also known to engender stress (Senar et al., 2000). In addition, alien phenotypes whose appearance was changed by black feathers induced a stronger response, which would agree with the hypothesis of the high cost of conspicuous signals in terms of social challenge (Tibbetts and Dale, 2004), and would explain why the consequences were far more severe for phenotypes altered by the presence of a conspicuous mark.

Interestingly, although reductions in HDEP were detected, those changes were not accompanied by changes on egg weight, length, and breadth, suggesting that the social mismatch engendered by the phenotype changes can affect the number but not the weight or size of eggs. However, because those measurements were taken over a random sample of 5 eggs per group, it is also possible that the samples contained primarily the eggs laid by those birds that were unaffected by the social disruptions of PA changes.

Regarding the observed differences related to GS during phase II, the findings are consistent with studies proposing that the social organization of laying hens is dynamic and can change from a hierarchical system for small groups to a tolerant system for larger groups (Estevez et al., 1997, 2003; Keeling et al., 2003). In the case of hens reared in groups of 10 individuals, it is possible that the high social disruptions induced by the PA changes in the 30% of hens would have included the reestablishment of a new social hierarchy, which would normally be resolved in a short period of time (Zayan, 1987; Bradshaw, 1992). Therefore, under the rearing conditions used in this study, with high resource availability (food and water ad libitum), and the sampling time frame used, adult individuals in small groups did not appear to be affected, at least at the level of accessing the resources needed to keep body mass. In the groups with higher number of individuals and particularly within GS 40, it is possible that even if a tolerant social system was primarily modulating interindividual aggressive interactions (Estevez et al., 1997), the impact of PA changes led to a social dynamic mismatch that is known to engender chronic stress responses (Zayan, 1991) and to damage productivity (Guhl and Allee, 1944; Siegel and Hurst, 1962; Muller, 1970; Jones, 1996). Indeed, the reestablishment and adaptation to the new social situation clearly affected the hens' performance. These findings are consistent with the work by Keeling et al. (2003) showing that raising hens in GS of around 30 birds could be problematic in terms of social interactions and production. Considering that the lowest HDEP values were observed 2 wk after the PA changes were imposed, it is conceivable that not only was an acute stress response induced by the PA changes but also that a chronic social stress response affected the changed birds during at least part of that period of time. Numerous researchers have already shown negative associations between stress, growth, and egg production (Gross et al., 1984; Bessei, 1985; McFarlane et al., 1989; Jones, 1996; Marin et al., 2002), which is not surprising if we consider that the activation of the avian defense mechanisms to cope with stressors require the expenditure of energetic resources that otherwise could be used for growth and egg production (Puvadolpirod and Thaxton, 2000). The reduced performance could also be associated to further energy costs due to attempts of avoiding aggressions as PA changed birds, and particularly PA birds with black marks, were proactively targeted (M. G. Liste, I. Campderrich, and I.

Estevez, unpublished data). Interestingly, 4 wk after the 1st PA change, altered groups were showing BW gain reductions; nevertheless, HDEP levels were similar to the control groups, suggesting that when affected, egg production can be recovered sooner than BW. A similar phenomenon is evident when hens recover egg production after stressful molting procedures (Berry, 2003). The results also suggest that less than 4 wk are needed to reestablish social group dynamics that do not affect egg lay.

Taken together, the results indicate that when PA changes occur within adult groups of hens, they affect primarily the performance of the birds whose appearance is first changed but not the performance of the birds that are changed in subsequent stages. Thus, the groups appear to reduce their reactions (or even stop them) to the introduction of birds with a repeated PA. This phenomenon, and the observed BW recovery shown by hens that were PA changed in the 1st change, can be the consequence of learning processes where the members of the group habituate to the presence of their new phenotype groupmates, therefore weaning down their social reactions toward them. At the same time, the PA changed hens can also be adapting to the social disturbances originated, improving their coping abilities. This could lead in return to minimize the impact toward the hens that are PA changed within the groups later on. However, because the 1st PA changes were made on a low proportion of the hens of the groups, and the subsequent changes also increased the frequency of the changed phenotypes within the groups (i.e., 50% altered and 50% unaltered after the 2nd change and 70% altered and 30% unaltered after the 3rd change), it is possible that the change in the frequency of the phenotypes may have also played an important role in the modulation of the social interactions. This situation could have helped the hens that were first changed to recover their BW. Indeed, as mentioned before, Dennis et al. (2008) showed that stress-related hormones were only altered when a small rather than a high proportion of the individuals were PA changed. With the 3rd PA change, after reaching 70% of change, the hens that remain unchanged (not altered during phase II) became the minority phenotype in the group (30%); thus, they would be in similar social conditions to the birds that were initially changed and a reduction in their performance could be expected. However, no effect was detected, suggesting that prior social interactions and learning processes during early stages modulate the social dynamics, allowing all birds, regardless of their phenotype proportion, to keep a similar BW.

In conclusion, our findings suggest that a hen's appearance is relevant enough to affect bird growth and egg production. However, the consequences of PA alterations depend on when in the development process they occur, the conspicuity of the phenotypic change, and the number of birds in the group. It is therefore a factor to consider for productive and welfare management practices.

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