

# Neonatal stimulation improves egg production in laying hens

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## Abstract

**Cid, M.P.; Kirkwood, C.A.; Arce, A.; Salvatierra N.A.: Neonatal stimulation improves egg production in laying hens.** *Rev. vet.* 22: 1, 8–12, 2011. The development of behavioral and endocrine responses to acute stress is greatly influenced by the early postnatal rearing environment. These environmental effects persist throughout life, resulting in stable individual differences in fearfulness. Early stimulation, such as neonatal novelty exposure decreases behavioral reactivity. Previous reports also show that early-life stimulation, such as neonatal exposure to novelty, reduces behavioural reactivity. The aim of this study was to evaluate whether early stimulation increases egg production in adult laying eggs. One-day-old laying hen chicks were exposed to a T-maze (Method A) or a test based on the latency to peck (Method B), and then reared in an industrial poultry farm until adults. A group of non-stimulated hens was used as a control group. Weekly egg production, individual egg weight and weekly feed intake were measured in laying hens under farm conditions for 25 weeks. An increase in egg number was observed in stimulated laying hens by Method A ( $5.22 \pm 0.06$ ,  $p < 0.01$ ) and by Method B ( $5.33 \pm 0.08$ ,  $p < 0.001$ ) compared to the controls ( $4.78 \pm 0.24$ ). Likewise, the food conversion index was also greater for stimulated hens by Method A ( $0.356 \pm 0.042$ ;  $p < 0.05$ ) and Method B ( $0.363 \pm 0.053$ ;  $p < 0.01$ ), compared to the control group ( $0.330 \pm 0.085$ ). These results indicate that early stimulation could help to improve the adaptation of laying hens in industrial poultry farms.

**Key words:** chick, early stimulation, egg production, laying hen, stress.

## Resumen

**Cid, M.P.; Kirkwood, C.A.; Arce, A.; Salvatierra N.A.: La estimulación neonatal por un ambiente nuevo mejora la producción de huevos en gallinas ponedoras.** *Rev. vet.* 22: 1, 8–12, 2011. El desarrollo de las respuestas comportamentales y endocrinas al estrés agudo está fuertemente influenciado por el entorno postnatal temprano. Estos efectos ambientales persisten a lo largo de la vida, generando diferencias individuales estables en la temerosidad. La estimulación temprana, como la exposición a un ambiente nuevo, disminuye la reactividad comportamental. También se demostró en investigaciones previas que una estimulación temprana en la vida, tal como una exposición neonatal al ambiente nuevo, redujo la reactividad comportamental. El objetivo de este estudio fue evaluar si una estimulación temprana aumenta la producción de huevos en gallinas adultas. Pollitos de gallinas ponedoras de un día de edad se expusieron a un laberinto en T (método A) o una prueba basada en la latencia para picotear un objeto (método B), posteriormente se trasladaron a una granja avícola hasta la edad adulta. Se designó a un grupo de gallinas como control, las cuales no se estimularon por ninguno de los métodos anteriormente nombrados. Durante 25 semanas se registró la postura semanal de huevos, el peso individual de los huevos y el consumo de alimento semanal en las gallinas ponedoras criadas bajo condiciones de granja. Se observó un aumento en el número de huevos en las gallinas estimuladas por el método A ( $5,22 \pm 0,06$ ,  $p < 0,01$ ) y por el método B ( $5,33 \pm 0,08$ ,  $p < 0,001$ ) comparadas a los controles no estimulados ( $4,78 \pm 0,24$ ). Del mismo modo, el índice de conversión alimenticia también fue mayor para las gallinas estimuladas por el método A ( $0,356 \pm 0,042$ ,  $p < 0,05$ ) y el método B ( $0,363 \pm 0,053$ ,  $p < 0,01$ ) respecto al grupo control ( $0,330 \pm 0,085$ ). Estos resultados indican que la estimulación temprana puede ayudar a mejorar la adaptación de gallinas ponedoras a las condiciones de cría intensiva en las granjas avícolas industriales.

**Palabras clave:** pollito, estimulación temprana, producción de huevos, gallinas ponedoras, estrés.

## INTRODUCTION

Stress is known to reduce well-being, consistency and growth in poultries. The level of stress could affect egg production and the quality of hen life<sup>15</sup>. Furthermore, procedures involving human contact, such as catching and handling, can evoke both stressful and fearful reactions that can compromise the birds' welfare<sup>16</sup>. Environmental enrichment, defined as the use of objects and cage design to improve the quality of life of animals, may decrease certain measures of fearfulness and anxiety<sup>5</sup> and encourage a tendency to approach novel stimuli<sup>8</sup>.

In domestic chicks, early environmental manipulation or regular manipulation reduces behavioural reactivity (ambulation, escapes, vocalizations and other behavioural responses) in adults<sup>13</sup>. Manipulation of hatchlings (captures, transport, and process of categorization) and characteristic environmental cues (pebbles of colour and different size, a mirror) could reduce the fear to "novelty" and increase the adaptation to environmental changes in adult hens<sup>15</sup>.

The beneficial effects of several forms of environmental enrichment have been reported including improvements in egg production and a nutritional conversion index of up to 4.3%<sup>8</sup>. It has been suggested that early life stimulation, which takes place while many systems are still developing, might have a long-lasting impact<sup>7</sup>. Thus, neonatal stimulation and manipulation by the experimenter during the early post-hatch stage could reduce adverse reactions to new situations of stress during the bird's life and hence improve egg production.

The T-maze test has been designed as a paradigm of learning in young chickens<sup>6</sup>. It is also used for determining emotionality, based on the escape response that can be defined as a compromise between the tendency to reinstate contact with pairs and avoid predation<sup>17</sup>, or as an influential variable of behaviour. Another paradigm of learning in young chicks is the food discrimination task<sup>1</sup>. Latency to peck pebbles during the first pre-training session for this task has been used as an emotionality index because the inhibition to peck reflects the conflict between the tendency of the birds to explore and their neophobia of a new environment<sup>19</sup>.

The aim of the present work was to determine if adult laying hens (White Leghorn) exposed to a T-maze (Method A) or a cage to peck pebbles in a new environment (Method B) during their first day of life, show an improvement in egg production compared with control hens (non-stimulated).

## MATERIAL AND METHODS

**Animals.** A total of 15 000 newly hatched chicks (White Leghorn) arrived at the laying commercial farm on Day 0 from a commercial hatchery (ARTUSIN S.A., Córdoba, Argentina). On the same morning (Day 0), 300 chicks were randomly chosen for the experiments;

150 birds were socially housed in 5 white wood brooders of 100 x 100 cm (Group 1 for Method A) and another 150 birds were individually housed in white wood pens of 24 cm x 24 cm (Group 2 for Method B). A third group of 30 chicks was chosen randomly from total of 15 000 pullets and was socially housed and mixed with them (Group C, the naïve controls not stimulated). All groups were kept under constant temperature (31–32°C) and humidity with no further human contact until the following morning. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals as approved by the Animal Care and Use Committee of the Universidad Nacional de Córdoba, and efforts were made to minimize animal suffering and to reduce the number of animals used.

**Stimulation methods. Method A: Stimulation of one-day-old chicks in a T-maze.** Chicks from Group 1 were tested individually and only once in a T-maze apparatus as described in detail elsewhere<sup>6,18</sup>. Each T-maze consisted of a start box or isolation chamber (21 cm long x 21 cm wide) opening into a corridor (21 cm long x 7 cm wide) that linked the two open-end perpendicular arms (each measured 7 cm long x 7 cm wide) leading to equivalent open spaces within the T-maze section of the larger wooden white box containing the T-maze. A mirror (10 x 10 cm) was situated at the junction of the T-corridor to encourage chicks to move towards it. A hardware cloth wire separated the T-maze section of each wooden box from the remaining 60 x 60 cm brood area that contained other chicks (20 conspecifics).

Food and water were freely available in the brood areas. Light was provided by incandescent lamps (100 W) placed 150 cm above each brooder box. When a test chick reached the mirror, it could see the brood area and its companions as it looked down one of the two open perpendicular arms. Twenty-four hours after hatching (Day 1), 20 chicks (4 chicks chosen randomly from 5 brooders) were individually placed in the brood area of each of three identical T-mazes and allowed a 30-min acclimatization period before testing, which began at 8:00 h. The test began by removing a chick from the brooding area and placing it in the centre of the isolation chamber facing away from the entrance to the T corridor, and ended when the chick left the perpendicular arm facing the brood area. Each chick was then classified into one of three categories (for more details, see<sup>18</sup>) according to the time it took to escape.

**Method B: Stimulation of one-day-old chicks in a cage to peck pebbles.** A total of 150 birds were individually housed in 24 cm x 20 cm cages (made of white wood) on the morning of the hatching day (Day 0) and kept in quiet conditions under dim red light with constant temperature (31–32°C) and humidity, without food but with water freely available. Chicks were housed and categorised individually to avoid the social-isolation-stress as described for day-old chicks<sup>12</sup>.

During the first 5 days of life chicks can use two parallel sources of nutrients, the yolk sac and food<sup>11</sup>; therefore, they did not suffer any lack of food. Twenty–four hours after hatching (Day 1), each bird was cupped gently and without restraint in the palm of the hand and individually transferred to a testing cage and placed in an adjacent room identical to the housing cage except for a scattering of small pebbles (2–4 mm in diameter) glued to the floor. Each testing cage was illuminated with a lamp (60 W) suspended immediately above it. The time of the latency to peck at the pebbles was registered for each chick (for more details, see<sup>20</sup>) and used to categorize them.

**Housing conditions.** Pullets grew up in two different farms according to their age: from hatching to 14 weeks of age (rearing period), they were kept in a rearing barn in the locality of Los Reartes (Córdoba, Argentina). From 14 weeks of age to the end of the experiment (laying period) they were housed in a production barn in a farm located in Mi Granja (Córdoba, Argentina). The farm used extensive production systems. Brooding temperature was  $32 \pm 2^\circ\text{C}$  during the two first weeks of life followed by a weekly decline of  $2.5^\circ\text{C}$  until reaching room temperature ( $24.5$  to  $26.5^\circ\text{C}$ ). At 18 weeks of age an hour of artificial light was increased per week until completing 15–16 hours of light per day.

**Rearing period.** From hatching to four weeks of age, 300 stimulated chicks (Groups A and B) and 30 control birds (Group C) were mixed randomly with non–experimental chicks (a total of 15 000 chicks) and lodged in groups of 60 individuals per pen (about 250 pens). Each pen housed 15 chicks tested by Method A (5 of each category), 15 chicks tested by Method B (5 of each category), 3 naïve control chicks and 27 non–experimental birds. Pens were 300 cm long  $\times$  150 cm wide, so the stocking density was  $750 \text{ cm}^2$  per bird. From 4 to 14 weeks of age the birds were housed in cages with three pullets each assigned randomly. These cages measured 46 cm  $\times$  45 cm  $\times$  45 cm (length  $\times$  width  $\times$  height), providing an average space of  $675 \text{ cm}^2$  per bird. The pullets were identified with numbered leg rings.

**Laying period.** During the laying period (from 14 weeks onwards), pullets were individually housed to measure egg production and food intake per hen in cages of 46 cm  $\times$  39 cm  $\times$  44 cm (length  $\times$  width  $\times$  height) providing an average space of  $1794 \text{ cm}^2$  per bird.

**Feeding and vaccination.** Water and commercial laying hen feed were provided *ad libitum* according to the indications of the manufacturer (Pronut S.A., Argentina) and the composition of the food was varied according to age. Laying hens were vaccinated according to the sanitary plan of vaccination of Argentina. Prophylactic treatments against external respiratory diseases and parasites were also performed.

**Experimental design.** Birds were bred and maintained in rearing and laying sheds under the same conditions as the other birds of the industrial poultry farm (15 000 pullets). Weekly egg production was recorded once egg laying was uniform (at about 5 months of age). All experimental data were recorded for 25 weeks between the months of October and April. Individual egg weight and weekly consumed food were recorded with a digital weighing machine with 0.1 g of precision. A food conversion index was calculated as the ratio between the weekly weight of laid eggs and food intake (weight egg/food intake). During the rearing and laying period, any hens that died, escaped, or were removed for health reasons were not replaced, and the data from such these cages were not included in the final analyses. All experiments and animal husbandry were performed in accordance to institutional animal welfare guidelines.

**Statistical analysis.** Results were expressed as the mean  $\pm$  SEM. Data from egg production, body weight, food conversion index and food intake were analyzed using one–way analysis of variance (ANOVA). Whenever ANOVA indicated significant effects ( $p < 0.05$ ), a pairwise comparison of means was carried out using the Newman–Keuls test. ANOVA assumptions (homoscedasticity and normal distribution) were attained in all cases. For all the statistic analysis a  $p < 0.05$  was considered significant.

## RESULTS

Although we initially expected to observe differences in egg production between the different subpopulations categorized by both tests, these were nonexistent (data not shown). However, when the data from stimulated hens were pooled and compared against controls, a significant improvement in egg production was observed.

As shown in Table 1, one–way ANOVA of weekly egg production showed a significant effect of stimulation ( $F_{2,208} = 5.00$ ,  $p < 0.0075$ ). The Newman–Keuls test revealed that the weekly egg production of Group 1 ( $5.22 \pm 0.06$ ,  $p < 0.01$ ) and Group 2 ( $5.33 \pm 0.08$ ,  $p < 0.001$ ) was higher than in the control group ( $4.78 \pm 0.24$ ). However, there were no significant differences in egg number between Groups 1 and 2 ( $p = 0.3463$ ).

One–way ANOVA also revealed a significant effect of stimulation on egg weight ( $F_{2,208} = 12.54$ ,  $p < 0.0001$ ). The Newman–Keuls test showed that the average egg weight of Group 1 ( $56.63 \pm 0.13 \text{ g}$ ,  $p < 0.001$ ) was significantly higher than for the control group ( $55.04 \pm 0.40 \text{ g}$ ) (Table 1). No significant differences in average egg weight in Group 2 with respect control group were observed ( $55.67 \pm 0.21 \text{ g}$ ,  $p = 0.10$ ).

Table 1 also shows that early stimulation also had a significant positive effect on the food conversion index ( $F_{2,208} = 3.67$ ,  $p < 0.02712$ ). The Newman–Keuls test revealed that this index was significantly greater in Group

**Table 1.** Data of weekly egg number, egg weight, food conversion index and feed intake in laying hens categorized by a T-maze (Method A) and on the basis of their latency to peck pebbles (Method B) at 1 day of age.

	control (25)	method A (99)	method B (87)
body weight (g) at 18 weeks	1652 ± 101	1687 ± 81	1665 ± 91
body weight (g) at 45 weeks	1750 ± 150	1763 ± 82	1759 ± 69
egg number	4.78 ± 0.24	5.22 ± 0.06 <sup>b</sup>	5.33 ± 0.08 <sup>c</sup>
egg weight (g)	55.04 ± 0.40	56.63 ± 0.13 <sup>b</sup>	55.67 ± 0.21
food intake (g)	810 ± 12	849 ± 2 <sup>b</sup>	836 ± 3 <sup>b</sup>
food conversion index	0.330 ± 0.085	0.356 ± 0.042 <sup>a</sup>	0.363 ± 0.053 <sup>b</sup>

Values are expressed as mean ± SEM. Number of cases is indicated in parenthesis. <sup>a</sup>p < 0.05, <sup>b</sup>p < 0.01 and <sup>c</sup>p < 0.001 compared with the corresponding controls (non stimulated hens) (Newman–Keuls post hoc test).

1 (0.356 ± 0.042; p < 0.05) and Group 2 (0.363 ± 0.053; p < 0.01) compared to the control group (0.330 ± 0.085).

Likewise, a significant effect of stimulation on food intake was also evidenced ( $F_{2,208} = 18.45$ , p < 0.0001). The Newman–Keuls test showed that stimulated hens ate more food (849 ± 2 g, p < 0.01 and 836 ± 3 g, p < 0.01) than control hens (810 ± 12 g) (Table 1). On the other hand, there were no significant differences in body weight, measured at the beginning and the end of the recording period, between the different groups (Table 1).

## DISCUSSION

One-day-old laying hens were subjected to one of two methods of early categorization, a T maze test and a test based on the latency to peck, to evaluate whether early stimulation improves egg production in adult laying hens. Our results showed significant differences in egg number and food conversion index between stimulated groups and naïve birds, both exposed to same conditions of rearing and laying in the farm except the procedure of categorization. This suggests that neonatal events might cause physiological divergences that are then reflected in basic natural characteristics such as egg production in adulthood.

Authors reported that Japanese quails with contrasting adrenocortical responsiveness in the T-maze showed differences in egg production <sup>20</sup>. However, they worked with Japanese quails from two genetic lines and not with an entire population. Furthermore, early life events in domestic fowls, such as exposure to a novel environment, improved the response to the negative impact of an environmental change and the young chick's ability to cope with new stressful events <sup>21</sup>. In general, farm animals are particularly sensitive to human stimulation during the neonatal stage <sup>10</sup>. It is suggested that early life stimulation, while many systems of the chicks are still developing, may have a long lasting impact and could possibly modify the expression of their genetic potential <sup>7</sup>.

It is reported that regular treatments that involve experimenters placing their hands either on or in the chickens' cage, allowing the birds to observe other

birds being handled, reduces the subsequent avoidance behaviour of young chickens to humans <sup>14</sup>. Working with laying hens, it was noted that birds exposed to daily visual contact with humans were less fearful to humans <sup>3</sup>. Fear of humans has also been described as a factor that could limit the productivity of commercial laying hens <sup>2</sup>. A regimen of regular handling may have beneficial effects on physiology <sup>9</sup>, behaviour <sup>9, 13</sup> and productivity <sup>3</sup> of poultry.

In other trial it was demonstrated that human visual contact had a positive influence on body weight, feed

conversion and survival in broiler chickens <sup>22</sup>. These authors noted that regular visual contact seems to be less effective when evoked after three weeks of life. Despite the many desirable effects of tactile interaction on poultry, regular handling of every bird is obviously not a feasible and practical method in commercial flocks. However, in the present report we demonstrated that a single exposure to an early categorization test involving handling and human visual contact improves egg production in adult laying hens.

Early environmental manipulation as environmental enrichment or regular manipulation reduces the reactivity of adult poultries <sup>13</sup>. Environmental manipulation at hatching (captures, transport, process of categorization) as well as characteristic environmental cues (pebbles of colour and different size, a mirror) might reduce the fear to "novelty" in pullets, increasing their adaptation to environmental changes and producing an improvement in subsequent production <sup>15</sup>. The beneficial effects of several forms of environmental enrichment have already been reported for egg production <sup>4</sup> and for a nutritional conversion index <sup>8</sup>. Our results showed that stimulation, during the categorization, of one-day-old laying hen chicks had an important long-lasting impact increasing egg production in adults compared to the naive controls.

In conclusion, both methods increased the egg number about 9% and 11%, Methods A and B respectively, compared to unstimulated hens. In addition, we observed an increase about 8% (by Method A) and 10% (by Method B) in food conversion index compared to the controls. Hence, present study shows that a single procedure of early stimulation can improve egg production in laying hens. We hope further research on this topic will increase the awareness of farmers on the influence of human-animal interactions and animal stimulation on animal production.

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