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*Original Research Article*

# Effect of Environmental and Dietary Enrichment on Production Performance, Digestive Process and Some Physiological Parameters in chickens

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**ABSTRACT:**
Improving the habitat of farmed chickens through environmental enrichment is considered advantageous compared to barecages, sincean outdoor or free-range environment enables and encourages chickens to express their behaviour. In addition, chickens that are kept outdoors or in a free-range environment can incidentally ingest sand, especially if they frequently bath in soil or sand. As a result, the daily diet of chickens fed outdoors or in free-range housing contains an average of 10-20% sand. In the field of poultry science, various enrichment strategies (visual, auditory, olfactory, and tactile) have been applied. In this study, sand and perch structures were used as environmental enrichment because (1) they are less expensive than other forms of enrichment, (2) they are mainly made of local materials, and (3) they can form the basis for a comprehensive system of environmental enrichment for chickens. Thus, in our studies, experiments were carried out in 3 stages to determine the effect of environmental enrichment methods on the well-being of chicken breeds specialized for egg production. In the first stage, when the living environment of chickens was enriched with sand and perch structure, basic microbiological variables and physico-chemical blood parameters (blood count, haemoglobin, viscosity, erythrocytes sedimentation rate, and pH) were improved ($p=0,05$).The second stage of our experiment consisted of four treatment: 1-sand (S), 2-perch (P), 3 both -sand and a perch (SP), and 4-control (C) condition with no enrichment. We determined the ratio of heterophils to lymphocyte in chickens aged 22, 30 and 38 weeks, and we also observed that total egg production was increased by the experimental conditions ($p=0.05$). In the third stage, the effect of chicken breeds in the egg direction on productivity (egg production, egg mass) and nutrient absorption in laying-breed chickens was determined ($p=0.01$).
**Keywords:** Environmental enrichment, perch structure, sand, heterophil to lymphocyte ratio, nutrient adsorption.

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## INTRODUCTION

In recent years our country has seen, the development of its poultry industry and the expansion of both the total production and the variety of finished poultry products intended for export, the distribution of products environmental enrichment on local poultry farm, and the development of scientifically based norms to improve the living conditions of poultry and ensure the biological and environmental soundness of their housing: particular attention has been paid to increasing in the number of poultry produced and improving the quantitative and qualitative characteristics of the resulting products. Poultry breeders aim to encourage caged birds to express their behaviour as if they were in the wild or left alone with no confinement by adding environmental enrichment in chicken cages (Leone and Estevez 2008). Through a strategy of enrichment in the form of nest boxes, dust baths, and perches, caged chickens can be encouraged to express natural behaviour such as dust bathing, even in captivity. This behaviour is important for caged chickens because they express this behaviour when they are reared either outdoor or in a free-range housing system. Hence, boxes, dust baths, and perch structures are defined as the basis for all forms of enrichment (Appleby, Hughes 1995). Environmental enrichment is defined as"the addition of biological properties to the habitat of animals for the development of natural behaviour"(Leone and Estevez 2008).The strategy of environment enrichment cannot be discussed without mentioning the European Union's agenda on the welfare of chickens. The use of enriched housing was required by the European Union (EU) in 1999, and there was an instruction that should be fully implemented by 2012. According to the directive of the European Union in 1999, the requirements for living of space for chickens are as follows: (1) 750 $cm^2$ space for each chicken (incuding at least 600 $cm^2$ of floor space) and at least 45 cm vertical space, (2) a minimum total cage area of 2000 cm2, (3) a nest, (4) litter, and (5) a perch with a length of 15 cm. Some forms of environmental enrichment lead to stressful situations and anxious behaviour or a decrease in physiological adaptation (Maxwell 1993), while others increase the ability of poultry to produce eggs and procreate (Leone and Estevez. 2008). Currently, the Human Society of the United States (HSUS) and United Egg Producers (UEP) are working to ensure an easier and more controlled transition from traditional cages to enriched cages. These orginizations demand that their technical specifications for poultry production be fully incorporated into federal laws 2029 so that they will apply to every state in the country (Smith 2011).

Perch structures typically vary in height, shape, and materials. A beam with a perch squares, round or rectangular cross-section can serve as a perch. Compared to round perches, a rectangular design reduced the incidence of planter injuries in chickens (Appleby1998, Duncan, Appleby, and Hughes 1992, Faure and Jones 1982a). Subsequent studies have shown that introducing perch structures to birds at a young age allowed the birds to learn and become familiar with the use of perches more quickly (Gunnarsson et al., 2000). Perches also improves bone strength (Appleby et al., 1992) and maximizes the use of different locations in poultry housing (LeVan et al., 2000, Newberry and Shackleton1997). In addition, perch structures serve as refuges for birds attacked by aggressive cagemates (Appleby and Hughes 1991), increase the available space for birds (Gunnarsson et al., 2000), and reduce the number of eggs laid directly on the floor (Appleby et al., 1983).

Chickens raised outdoor or under free-range conditions have access to sand and can perform several actions on the sand. Under these circumstances, the chickens can eat the sand as grit, forage for food in it, and dust-bathe in it. These activities are also performed by caged birds (Appleby 2003; Shields et al., 2004), especially as they grow older (Shields et al,. 2005). In the absence of sand, birds express these behaviours in other litter materials. Additionally, frequent biting and scratching of the sand keeps the beaks and claws of the birds worn down to a normal length, thereby reducing the damage which they inflict on other birds (Fickenwirth et al., 1985). Adding large amounts of sand (approximately 20-30%) to the feed diluted the feed and increased consumption (Van Dar Meulen et al., 2008).

In terms of physiological responses, an increase in stress hormones leads to a decline in well-being (Bareham1972). An increase in the ratio of heterophils to lymphocytes is a positively associated with stress (Gross and Siegel 1983). Increased plasma corticosterone leads to an increase in the ratio of heterophils to lymphocytes (Gross, Siegel and Dubose, 1980). Heterophils in birds are produced in the bone marrow and serve the same function that neutrophils serve in human blood. Lymphocytes are formed in the spleen. There is a correlation between immune function and stress when animals are under stress, an increase in various diseases is observed (Moberg and Mench, 2000). Heterophils accumulate in large numbers at the site of infection and break down microorganisms by phagocytosis (Montali, 1988).

## MATERIALS AND METHODS

***Living conditions and diet composition:*** For this study, 48 chickens were singly housed in cages withthe same basic structure, measuring 2500 cm² each. The chickens were supplied with nutrients and water in a room at 22°C with a ventilation system for controlled. After the adaptation period, the chickens were given a control diet that met the standard requirements and was adapted to take advantage of locally available products (barley, wheat, and maize).

**Table 1:** Ingredients and composition of the diet

| № | Products | Grams |
|---|---|---|
| 1. | Maize | 231 |
| 2. | Maizeglutenfeed | 10 |
| 3. | Crushedpeas | 100 |
| 4. | Crushedcorn | 500 |
| 5. | Crushedbarley | 100 |
| 6. | Wheat | 200 |
| 7. | Wheatflour | 150 |
| 8. | Vitamin-mineralpremix* | 5.3 |
| 9. | Soybeanoil | 10 |
| 10. | Animalfat | 20 |
| 11. | Salt | 2.6 |
| 12. | Limestone | 100 |
| 13. | Calciumphosphate | 7.5 |
| 14. | $NaHCO_3$ | 2 |
| 15. | DL-methionine | 1.8 |
| 16. | P | 5.8 |
| 17. | Ca | 39 |

(Table 1, Research Diet Services BV, adapted to local conditions based on Wijk bij Duurstede).
*One kilogram of vitamin-mineral premix contains 8000 IU of vitamin A, 2500 IU of vitamin D3, 10 IU of vitamin E, 1 IU of vitamin K3, 0.5 mg of vitamin B1, 4 mg of vitamin B2, 6 mg of d-pantothenate, 6 mg of niacin, 20 mg of vitamin B12, 0.3 mg of folic acid, 2 mg of vitamin B6, 200 mg of choline chloride, 70 mg of Fe, 15 mg of Cu, 55 mg of Zn, 80 mg of Mn, 0.25 mg of Co, 0.2 mg of Se and 50 mg of antioxidants.

***Environmental enrichment:*** In the second stage, each cage was assigned to one of four treatments. Chickens aged 22, 30, and 38 weeks were allocated to receive one of the following four treatments: (1) sand (S), (2) a perch (P), (3) sand and a perch (QP) and (4) control (C) environment. In the experiment, plastic boxes (67.2 × 48.1 × 14.6 cm) were used to hold sand. To ensure that there was enough sand for the chickens to dust-bath, the sandbox was filled every day. The perches in this experiment, had rectangular polyvinyl chloride (PVC) bars and measured 64.4 × 47.6 × 24.2 cm.

***Blood counts:*** Blood samples were taken from the brachial vessels of each chicken. For blood collection, each bird was laid on its side on a table, one of its wings was stretched out to identify a blood vessel, and blood was drawn through a needle.

The following methods were used in our research:

- Measurement of haemoglobin concentration by spectrophotometry (EMC-30PC-UV);
- Erythrocyte and leukocyte counts by direct microscopy in a Goryaev chamber;
- pH measurement using– Nevodov's method;
- Blood viscosity measurement (HAAKE Viscotester 2 plus);

- Measurement of the erythrocyte sedimentation rate with a Panchenkov apparatus.

***Productivity indicators:*** The eggs of the birds under study were weighed on a pharmacy scale. Daily egg production was calculated the ratio of the total number of eggs laid per day to the total number of hens and expressed as a percentage (%). The following formula was used:

Egg production (%) = (total number of eggs laid/ hens) * 100.

***Effects of sand on digestion:*** For wildlife, sand consumption is estimated according to the soil ingestion equation: the same equation was used in this study. The soil ingestion equation for wildlife.

$$x = \frac{b - y + ay}{ay - c + b}$$

In this formula, $x$ is the fraction of soil in the feed, $y$ is the acid-insoluble ash concentration in the feed (g/kg dm), $a$ is the digestibility of the dry matter in the feed, $b$ is the acid-insoluble ash concentration in the feed (g/kg dm), and $c$ is the concentration of acid-insoluble ash in the soil (g/kg dm) (Beyer et al. 1994).

**RESULTS AND DISCUSSION**

In the first phase of the study, the effect of perch structures and sand on some physiological indicators in fattened chickens was studied (Table 2). Analysis of the data (presented in the table) showed that, during our study, blood physiochemical and morphological indicators were within the normal physiological ranges in both the control group and the enrichment group that was housed with sand and perches. This study also showed that the experimental group had 1.6% more erythrocytes and 1.13% more thrombocytes than the control group.

**Table 2:** The effect of environmental enrichment on some physicochemical and morpho-biochemical parameters in the blood of chickens

| No. | Blood indicators | Treatment groups | | Difference % |
|---|---|---|---|---|
| | | Control | Sand& Perch | |
| 1 | Erythrocytes, million/1 mm$^3$ | 3.15±0.16 | 3.20 ±0.24 | 1.6 |
| 2 | Leukocytes, million/1 mm$^3$ | 26.02±0.81 | 25.61±1.25 | -1.58 |
| 3 | Thrombacytes, thousand/1 mm$^3$ | 32.75±0.66 | 33.10±1.04 | 1.13 |
| 4 | Haemoglobin, g/l | 73.10±0.38 | 74.18±0.42 | 1.47 |
| 5 | Viscosity | 4.71 | 4.75 | 0.85 |
| 6 | ESR, mm/hour | 2.80±0.15 | 2.76±0.22 | 1.42 |
| 7 | pH | 7.43 | 7.44 | 0.13 |

Leukocytes, which play an important role in the processes of protection and recovery in the body of animals, perform the functions of phagocytosis, antibody production, neutralization, and removal; leukocyte counts increase under some pathological conditions. The leukocyte counts of the experimental group were 1.58% lower than those of the control group, suggesting that the immune system of the experimental group faced fewer challenges.

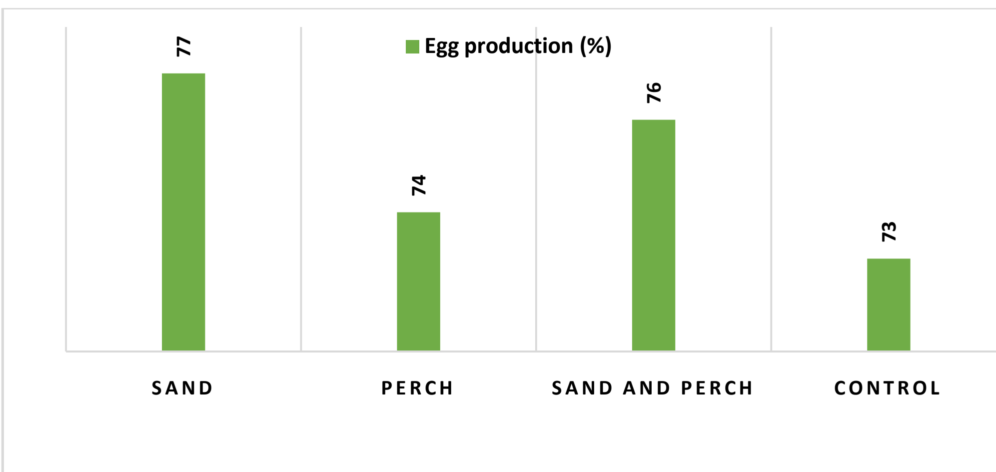
Compared to the control group, the experimental group was found to have small increases in haemoglobin (by 1.47%), blood viscosity (by 0,85%), ESR (erythrocytes sedimentation rate, by 1,42%), blood pH ( by 0,13%) was determined; the small magnitudes of these changes are evidenced of a stable physiological state in the experimental group (Table 2).

In our experiments, the heterophil to lymphocyte ratios were calculated separately for each of the three examined age groups..For 22-week-old hens, the ratios in the treatment groups were 0.32 in the first group (sand (S)), 0.38 in the second (perch (P)), 0.40 in the third (sand and a perch (SP)), and 0.34 in the fourth (control (C)).For 30-week-old hens, the ratios were 0.48 in the first group (sand (S)), 0.60 in the second (perch (P)), 0.52 in the third (sand and a perch (SP)), and 0.50 in the fourth (control (C)).Finally, for 38-week hens, the ratios were 0.48 in the first of the 4 treatmentgroups (sand (S)), 0.53 in the second (perch (P)), 0.50 in the third (sand and a perch (SP)), and 0.49 in the fourth (control (C)) (Table 3).

**Table 3:** The effect of enrichment strategies on the heterophil-to-lymphocyte ratio in chicken blood

| Treatments | 22 weeks | 30 weeks | 38 weeks |
|---|---|---|---|
| Sand (S) | 0.32 | 0.48 | 0.48 |
| Perch (P) | 0.38 | 0.60 | 0.53 |
| Sand & Perch (SP) | 0.40 | 0.52 | 0.50 |
| Control (C) | 0.34 | 0.50 | 0.49 |

The table shows that for laying hens of all three ages, the ratios were slightly increased in the second and third treatment groups compared to the control group, while in the first group there was a slight decrease. In treatment group 2, which had perches for environmental enrichment, the heterophil-to-lymphocyte ratio exceeded that of the control group by 0.04 in 22-week-old hens, 0.10 in 30-week-old hens, and 0.04 in 40-week-old hens. In the third group, which had both sand and perches for enrichment, the ratio was increased over that of the control group by 0.01 in 22-week-old hens, 0.02 in 30-week-old hens, and 0.01 in 38-week-old hens (Table 3).




**Graph 1**: Effect of enrichment on total egg production

Based on the above formula, daily egg production was calculated separately for each hen. The production rate was 77% in the first of the 4 treatment groups (sand (S)), 74% in the second (perch (P)), 76% in the third (combination of sand and perch (SP)) and 73% in the fourth (control (C)) (graph 1). So, in our experiments, there was an increase in the percentage of production in the experimental groups compared to the control group.

In the first group (sand), there was an increase of 4% compared to the control group, in the second group (perch), a 1% increase; and in the third group (combination of perch and sand), a 3% increase (p=0.05). Thus, the methods of enrichment tested in this study led to an improvement in the production of eggs (graph 1).

In our experimental described above, the feed was prepared according to the standard requirements for nutrient content. In our current experiments, pure sand was added to the standard nitrogen in various amounts during preparation. There were no significant differences in egg productionamong the five groups of chickens receiving 0 to 30% of sand added to nitrogen; specifically, the maximum difference in egg production between hen receiving sandless and sand-supplemented nitrogen was 1% (Table 4).

**Table 4:** Influence of feed sand content and on indicators of productivity

| Amount of sand (%) | 0 | 10 | 20 | 25 | 30 | max* |
|---|---|---|---|---|---|---|
| Laying eggs (%) | 96 | 95 | 96 | 97 | 97 | 1 |
| Egg mass (g) | 67.6 | 67.7 | 67.8 | 68.3 | 68.8 | 1,2 |
| Total nutrient intake (g/hen/d)* * | 137 | 146 | 152 | 161 | 174 | 37 |
| Ratio of total feed to egg mass (g/g) | 2.03 | 2.16 | 2.24 | 2.36 | 2.53 | 0,5 |
| Nutritive feed (sandless feed) (g/hen/d)* * | 137 | 123 | 122 | 121 | 122 | 16 |
| Ratio of nutritious feed (sandless feed) to egg mass (g/g) | 2.03 | 1.82 | 1.80 | 1.77 | 1.77 | 0.26 |

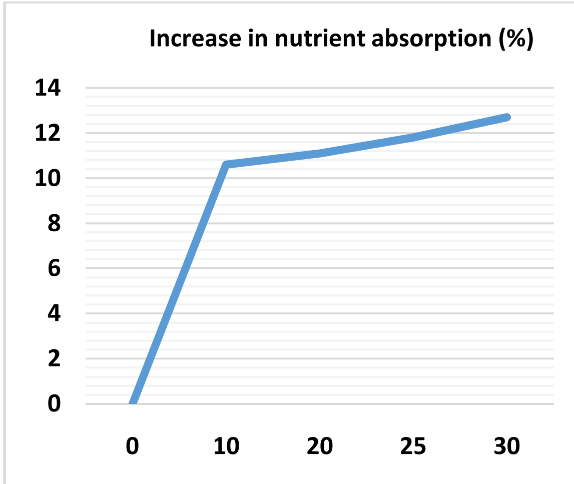
* max - The largest difference between control and experimental groups.
** (g/hen/d) - The mass of feed consumed by one chicken per day in grams.

There was almost no difference in egg weight among groups (the maximum difference was 1.2 g). Each hen consumed a daily average of 137 g of feed with 0% sand or up to 174 g of feed with 30% sand. Hence, nutrient intake was greater in hens that received sand-supplemented feed than in those that received sandless feed. Across all groups, there was almost no difference in the ratio of total feed mass or nutritive (sandless) feed mass to the mass of eggs (the ratio of nutritive feed to egg mass was 0.26). The net daily consumption of nutritive (sandless) was reduced by 16 g in chickens receiving sand-supplemented feed compared to sandless feed. Hens that were fed 0% or 10% sand, gained more than 120 g of body mass. For hens fed 20% and 25% sand in their feed, the increase in body mass was much smaller. In the group of chickens fed 30% sand, the average increase in body mass was only 37 g.

As the amount of sand in the feed increased, the utilization of nutrients in the feed also increased. These results were compared with the initial standard (sand-free feed) (graph 2). Compared to standart feed, the feed was found to have a 10.6% increase in nutrient availability when the feed supplemented with 10% sand, an 11.1% increase at 20% sand, an 11.8% increase at 25% sand, and a 12.7% increase at 30%.

The fact that sand had a larger effect than perch availability in this study was likely related to the age of the chickens. Perching behaviour decreases as chickens age (LeVan et al., 2000). The sandboxes were consistently filled with sand every day or as necessary, but the structure of the perches did not change. It was observed that the chickens receiving S or SP enrichment, used their sandboxes most often when the boxes were freshly filled. When a sand-filled vessel (made of solid wood) was placed on top of the nest box in a prior study, rarely used the sand (Barnett and Smith, 2003). In our study, the chickens used the sand more frequently if the cage was also enriched with a perch.

When we enriched the environment of chickens with S or SP, we found that egg production increased significantly. When a perch was used as the only enrichment, the production of eggs increased less. In a previous study, the use of perches and sand in chicken cages for environmental enrichment increased the number of eggs compared to an unenriched control condition (Pohle & Cheng, 2009). Another study found that the addition of different proportions of sand to the diet of chickens did not have a significant effect on egg production (Van Dar Meulen et al., 2008). Our experience confirmed that there was no difference in egg production across various levels of sand supplementation.




**Graph 2:** The effect of sand supplementation on nutrient

It was found that the addition of a large amount of sand to the feed also influenced productivity by increasing the efficiency of nutrient uptake. However, the addition of sand to the diet reduced weight gain. Chickens whose feed contained more than 10% sand gained less body weight than chickens whose feed contained no sand or 10% sand. Nonetheless, dietary supplementation with sand increases the digestibility of the nutritive portion of the feed (Moran, 1982).

The evaluation of soil consumption according to the soil ingestion equation for wildlife is a suitable method for determining the soil intake of chickens under outdoor and indoor experimental conditions. For this purpose, it is possible to collect the litter of chickens only from the place where they spend the night, since the ash content in the dry matter of the litter does not differ between the light and dark periods, but the dry matter content of the litter is different, perhaps as a result of differences in fluid consumption. When litter is sampled in this manner, it is necessary to use the standard procedure (Mahaney & Krishnamani, 2003) and the digestibility properties of dry nutrients in order to predict soil ingestion in accordance with the soil ingestion equation for wildlife. Chickens in this study consumed more sand than wild turkeys or chickens reportedly consume (Beyer et al. 1994).

## CONCLUSION

After assessing the impact of perches and sand on the well-being of chickens in this experiment, we drew the following conclusions:
(1) By enriching the habitat of laying hens with perches and sand, it is possible to increase the productivity (egg production) of the chickens. This effect is mediated by improvements in several physiological parameters of blood.
(2) As the amount of sand in the diet increased (0%, 10%, 20%, 25%, and 30%), nutrient absorption was improved, resulting in a certain increase in the productivity of the chickens.

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