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# Effects of Graduated Levels of Dietary Bentonite on the Zootechnical and Biological Performance of Laying Hens under North African Conditions

**Djelailia Sofiane[1*], Boussaada Djelloul[2]**

[1]Agricultural Sciences Department, Faculty of Sciences, Mohamed Boudiaf University, Address: PO Box 166 Echbilia, M'Sila 28000, Algeria, Email: sofiane.djelailia@univ-msila.dz

[2]Department of biology, University of Tiaret, 14000, Algeria, Email: djelloul.boussaada@univ-tiaret.dz

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## Abstract:

This study aimed to evaluate the effects of adding a local food supplement to the diet of laying hens in a semi-industrial facility in a semi-arid region (M'Sila). The experiment was designed to determine the effect of calcium bentonite, which is used as a mycotoxin binder in laying hen diets, on their zootechnical performance. This includes feed conversion ratio, laying rate, egg weight, body weight and mortality.

A total of 144 Novogen Brown laying hens, aged 22 weeks, were randomly assigned to four groups receiving graded doses of bentonite: 0%, 0.5%, 0.75% and 1%. The different doses were distributed among four groups, and each group was further subdivided into six cages containing six hens per cage, i.e. 36 hens per treatment. At the start of the experiment, the hens were 22 weeks old, and the monitoring period lasted 26 weeks, covering the productive cycle. The standard diet mainly consisted of corn (maize) and soybean meal.

The results relating to the zootechnical parameters revealed a significant positive impact when 1% bentonite was included: Feed conversion ratio: $2.12 \pm 0.10^{b}$ ($p < 0.001$), Laying rate (%): $89.05 \pm 2.45^{a}$ ($p < 0.001$), Egg weight (g): $57.60 \pm 3.43^{a}$ ($p < 0.001$), Body weight (g): $1856.98 \pm 42.79^{ac}$ ($p < 0.043$) - Mortality rate (%): $2.12 \pm 1.34$ ($p < 0.010$).

Moreover, the results demonstrated a significant effect of bentonite on biological parameters. Supplementation at 1% led to significant reductions in triglycerides (0.8787 g/L) and cholesterol (0.7985 g/L) compared with the other treatments ($p < 0.05$). This improvement in the lipid profile

may be attributed to the adsorptive properties of bentonite, which facilitate the elimination of bile acids and contribute to the regulation of lipid metabolism. Despite the slight potential increase in feed costs associated with the inclusion of bentonite, the gains achieved largely compensate for this investment, making the 1% inclusion level an economically optimal strategy under the conditions of this study.
These results highlight the positive effects of bentonite as a dietary additive in improving the feed conversion ratio, laying rate, egg weight and survival rate of laying hens. Furthermore, bentonite demonstrated remarkable safety as a feed additive for laying hens.
**Keywords:** laying hens, bentonite, biological parameters, zootechnical parameters.

## 1. Introduction

In Algeria, the rearing of laying hens is a key part of the animal production sector. It effectively contributes to the country's food security. However, the performance of laying hens is directly influenced by the quality of their feed. This dependency is particularly pronounced in intensive production systems, where nutritional and health constraints are most severe. The sector's reliance on imported raw materials poses a significant risk of mycotoxin contamination at production farms. This situation justifies the adoption of nutritional strategies aimed at improving food safety and zootechnical performance.
Modern poultry production, especially table egg production, faces numerous challenges related to animal health, production system quality, and feed efficiency (Hidalgo et al., 2022). In this context, managing the nutrition of laying hens is key to improving performance while ensuring animal welfare. Among the various approaches explored in terms of mineral additives, natural clays such as bentonite have recently attracted considerable attention. These natural clays are used as mycotoxin adsorbents and an increasing body of research reports beneficial effects on intestinal health and stabilisation of laying performance (Sarica et al., 2013; Hussein et al., 2019).
Bentonite is a colloidal clay belonging to the smectite group that is mainly composed of montmorillonite. Its physicochemical properties — cation exchange capacity, swelling upon contact with water and a high specific surface area — provide it with numerous applications in animal nutrition (Tajick et al., 2021). Essentially made up of montmorillonite, it exhibits the characteristics of a natural clay, including strong adsorption capacity, catalytic activity, and ion-exchange ability (Montayeva et al., 2023). Bentonite can bind various undesirable feed components, including mycotoxins, heavy metals, nitrates, and nitrogenous compounds (Montayeva et al., 2023). Dietary

bentonite can also bind aflatoxins and neutralise their toxicity (Mgbeahuruike et al., 2021), with similar effects reported for ochratoxin (Ghazalah et al., 2021).

Dietary bentonite is recognised as a multipurpose feed supplement. It acts as an adsorbent of mycotoxins, supports intestinal health and helps stabilise production performance at different levels (Gül et al., 2017; Choi, 2018). It is also considered a binding agent that improves nutrient digestibility and feed efficiency. Furthermore, several studies have highlighted its role in reducing dietary toxins and promoting intestinal microbiota health (Abdel-Wahhab et al., 2021; Prasai et al., 2016).

Numerous studies have investigated the use of bentonite as a feed additive for poultry with the aim of improving production performance, nutrient digestibility and overall health. According to Gül et al. (2016) and Chen et al. (2020), incorporating 0.5 g/kg of bentonite into laying hen diets enhances intestinal stability and boosts egg production. Furthermore, the dietary inclusion of 0.50% bentonite can mitigate the adverse effects of contaminated feed and promote weight gain (Ghazala et al., 2021).

Bentonite is generally used as a feed additive for poultry to improve nutrient digestibility and zootechnical performance. It has been shown to reduce feed intake and increase egg production and weight (Yenice et al., 2015; Ezzat et al., 2016; Prasai et al., 2017; Chen et al., 2020). It may also affect the digestibility of certain nutrients or egg quality (Bertin et al., 2016).

The beneficial effects of bentonite are thought to be due to one or more of the following mechanisms: (i) an increase in the volume of gastrointestinal contents, associated with water adsorption, which slows digesta transit and enhances digestion and nutrient absorption (Gilani et al., 2021); (ii) the adsorption of mycotoxins present in feed ingredients, which reduces mycotoxin toxicity (Darmawan et al., 2022); and (iii) a potential role in intestinal health, since bentonite can stabilise the epithelial barrier (Quisenberry, 1968).

In addition, according to the scientific literature, the reported effects of dietary bentonite inclusion vary depending on the levels used. However, many studies are short-term (with an exposure duration of 8–16 weeks) and the tested inclusion rates differ widely (from 0.5% to 5%).

Nevertheless, due to specific farming conditions, rations that may differ considerably, and distinct climatic conditions from one region to another in Algeria, there is a strong rationale for conducting scientific studies to establish more rational feeding plans, particularly to determine the optimal dose of bentonite that would maximise performance.

Against this backdrop, the present study examines the impact of moderate bentonite supplementation at various inclusion rates (0.25%, 0.5%, 0.75%, and 1%) over an extended period, beginning with the initial laying period and continuing throughout the laying cycle (26 weeks). This study will help determine whether these dose levels are truly effective, and whether any effects emerge and/or accumulate over time. Several zootechnical and biological parameters of laying hens were measured in this study: body weight, egg weight, feed conversion ratio, laying rate and mortality rate. However, it is necessary to identify the dose at which productive performance can be optimised.

Note that the European Food Safety Authority (EFSA, 2017) recommends a maximum incorporation of 2% bentonite in animal diets to ensure efficacy and safety. This recommendation is particularly relevant to our experiment since the study design uses moderate doses of 1% or less.

## 2. Materials and methods

### 2.1 Experimental site and housing conditions

The study was conducted in 2021 in an intensive (semi-industrial) poultry house in a semi-arid climate area in M'Sila, Algeria. The facility was designed for laying hens and housed them in furnished cages. The building was closed and equipped with a controlled ventilation system. The environmental conditions (temperature, relative humidity and ventilation) were maintained within optimal ranges to minimise the effects of heat stress on animal performance. Lighting was gradually adjusted to achieve a photoperiod of 16 hours of light per day.

### 2.2 Animals and experimental design

This study was carried out in accordance with the ethical research guidelines for the care and use of animals at Mohamed Boudiaf University in M'Sila, Algeria. To assess the dietary impact of bentonite, 144 Novogen Brown laying hens, aged 22 weeks at the start of the trial, were used.

To evaluate the effects of different doses of bentonite (0%, 0.5%, 0.75% and 1%), the hens were randomly allocated to four groups. Each group consisted of six replicates (experimental units), with six hens per replicate, for a total of 36 hens per treatment. The experimental design was completely randomised to minimise the potential effects related to the position of cages within the building.

The experimental procedure was as follows:

- A control group (T0) received only the standard diet.

The remaining groups received the standard basal diet supplemented with bentonite according to the following treatments:

T1: basal diet + 0.5% bentonite

- T2: basal diet + 0.75% bentonite

T3: basal diet + 1% bentonite

The experimental period lasted 26 weeks, with monitoring from weeks 22 to 48 of the hens' age. The hens were housed individually in wire cages. The temperature inside the building ranged from 22 to 25 °C throughout the production phase. Water and feed were provided ad libitum. All comfort parameters were set according to the Novogen Brown rearing guide. All groups received preventive management, including vaccinations against infectious bronchitis and Newcastle disease.

For the analysis of biochemical parameters, particularly triglycerides and cholesterol, in relation to bentonite doses, 120 hens were allocated to four experimental groups, each receiving a different dose: 0%, 0.5%, 0.75% and 1%.

**2.3 Diet and supplementation**

The hens were fed a basal ration formulated to meet the nutritional requirements of laying hens during production, in accordance with standard poultry nutrition recommendations. The feed was supplied by the Office National des Aliments du Bétail (O.N.A.B.). The diet composition included: fine bran, maize, soybean meal, a mineral and vitamin supplement, as well as phosphorus and calcium.

In this study, bentonite was incorporated into the feed at levels defined for each treatment (0%, 0.25%, 0.5% and 1%). Feed and water were provided ad libitum throughout the entire experimental period.

Table 1 shows the feed composition of the provided diet and the bentonite inclusion rates in the diet.

| **Ingredient** | **T0 (0%)** | **T1 (0.5%)** | **T2 (0.75%)** | **T3 (1%)** |
|---|---|---|---|---|
| Corn | 62 | 61,5 | 61,25 | 61 |
| Soybean meal | 25 | 25 | 25 | 25 |
| Wheat bran | 3 | 3 | 3 | 3 |
| Cacarbonate | 7 | 7 | 7 | 7 |
| Phosphate | 1,5 | 1,5 | 1,5 | 1,5 |
| Salt | 0,3 | 0,3 | 0,3 | 0,3 |
| Premix | 1,2 | 1,2 | 1,2 | 1,2 |
| Bentonite | 0 | 0,5 | 0,75 | 1 |
| Total | 100 | 100 | 100 | 100 |

**2.4 Chemical composition of bentonite**

Maghnia bentonite is of natural origin. It is a calcium-type bentonite (Ca-bentonite) with a pH of 6.2, which is slightly acidic. It is classified as calcium montmorillonite and contains a low percentage of sodium. Its relatively high content of $SiO_2$ and $Al_2O_3$ indicates that it has an aluminosilicate structure typical of smectite clays.

Its chemical composition is presented in the following table:

Table 2: Chemical composition of Maghnia bentonite.

| **Constituants** | **%** |
|---|---|
| $SiO_2$ | 69,4 |
| $Al_2O_3$ | 14,7 |
| $Fe_2O_3$ | 1,2 |
| CaO | 0,3 |
| MgO | 1,1 |
| $Na_2O$ | 0,5 |
| $K_2O$ | 0,8 |
| $MnO_2$ | 0,2 |
| **As** | 0,05 |

### 2.5 Measured parameters

#### 2.5.1 Body weight of the hens

The body weight of the hens was measured weekly using a precision electronic scale.

#### 2.5.2 Egg production

The number of eggs produced was recorded daily to calculate the laying rate (%). Egg weight was measured using a precision balance.

#### 2.5.3 Laying rate

The laying rate was calculated on a weekly basis as follows:

Laying rate (%) = Number of eggs produced / Number of hens x 100 (where applicable to the weekly recording scheme used).

Egg mass was calculated as follows:

Egg mass = laying rate (%) × average egg weight.

#### 2.5.4 Feed conversion ratio (FCR)

Feed intake was recorded per cage, enabling the calculation of the feed conversion ratio (FCR) using the following formula:

FCR = feed intake (kg)/egg mass (kg).

Feed intake calculation: Total feed intake (kg):

Egg mass calculation:

Egg mass (kg) = number of eggs × average egg weight (kg).

#### 2.5.5 Mortality

Mortality was recorded daily throughout the experimental period. When possible, the likely cause of death was noted.

### 2.5.6 Economic calculation

$$\text{Gain } (\%) = \frac{\text{Valeur } (1\%) - \text{Valeur } (0\%)}{\text{Valeur } (0\%)} \times 100$$

### 2.6 Statistical analysis

The zootechnical and biochemical parameters were analysed using SPSS software. One-way analysis of variance (ANOVA) was used to evaluate the effect of different levels of bentonite on the various measured parameters. When significant differences were detected, the means were compared using the Tukey test. Results were considered statistically significant at $P < 0.05$. Results are reported as means ± standard error of the mean (SEM).

### 3. Results and discussion

Table 3: Effect of different bentonite doses on various biological parameters measured

| Parameter | 0 % Bentonite | 0,5 % Bentonite | 0,75 % Bentonite | 1 % Bentonite | F | P-value |
|---|---|---|---|---|---|---|
| **Body weight (g)** | 1842,40 ± 52,05$^{b}$ | 1844,76 ± 51,78$^{b}$ | 1848,19 ± 45,70$^{b}$ | 1856,98 ± 42,79$^{a}$ | 2,737 | 0,043 |
| **Egg weight (g)** | 55,13 ± 3,72$^{b}$ | 55,03 ± 3,66$^{b}$ | 55,18 ± 3,70$^{b}$ | 57,60 ± 3,43$^{a}$ | 18,324 | 0,000 |
| **Feed conversion ratio (FCR)** | 2,22 ± 0,13$^{a}$ | 2,23 ± 0,13$^{a}$ | 2,22 ± 0,13$^{a}$ | 2,12 ± 0,10$^{b}$ | 26,706 | 0,000 |
| **Laying rate (%)** | 88,32 ± 3,60$^{b}$ | 86,69 ± 7,77$^{c}$ | 86,63 ± 2,87$^{c}$ | 89,05 ± 2,45$^{a}$ | 10,351 | 0,000 |
| **Mortality rate (%)** | 2,33 ± 1,36$^{b}$ | 2,68 ± 1,57$^{a}$ | 2,38 ± 1,67$^{b}$ | 2,12 ± 1,34$^{c}$ | 3,850 | 0,010 |

### 3.1 Effect on egg weight

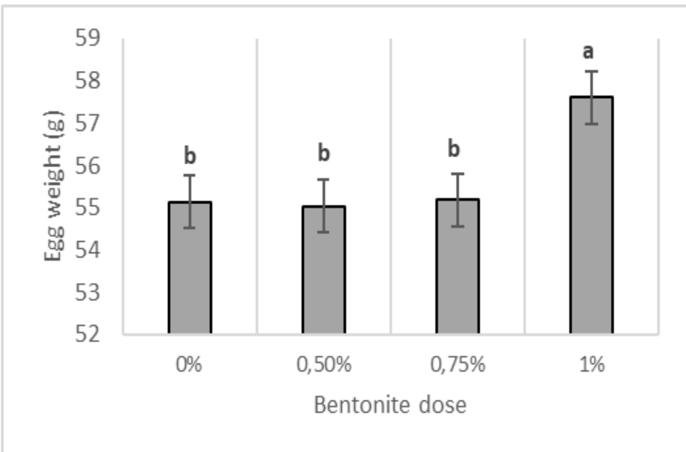


Figure 1: Effect of bentonite dosage on egg weight

A significant increase in egg weight was observed at a 1% dose compared with the control group:

+2.47 g (57.60 g vs. 55.13 g), corresponding to an increase of +4.48% ($P < 0.001$) (Figure 1 and Table 3).

These results are consistent with those of Safaeikatouli et al. (2011), who demonstrated that incorporating bentonite into poultry diets can significantly enhance egg quality and weight. This suggests that at the optimal dose, bentonite promotes the better assimilation of essential nutrients involved in egg formation, particularly proteins and minerals. Additionally, the adsorption of mycotoxins present in the feed, even at subclinical levels, could mitigate their adverse effects on metabolism and the physiology of laying hens (Darmawan et al., 2022). These authors also reported a linear increase in egg weight.

This is in line with the meta-analysis by Öztürk and Darmawan (2022), which showed a linear increase in egg weight with increasing bentonite incorporation ($P < 0.05$). Similar findings were reported by Quisenberry (1968), who observed a significant improvement in egg weight in hens receiving different levels of bentonite (1.25% to 5%). Furthermore, in their meta-analysis based on nine studies, Darmawan et al. (2022) showed a linear increase in egg weight with increasing bentonite inclusion, with gains ranging from 2% to 6%, depending on the dosage. More recently, Gilani et al. (2021) observed increased egg weight in laying hens supplemented with natural clays and attributed this to improved amino acid digestibility.

Our results are consistent with those of Ait Saada et al. (2013), who conducted studies in Algeria and reported significant improvements in egg weight with a 2% inclusion rate of locally sourced sodium bentonite.

There are several mechanisms that may explain this effect. Due to its ability to absorb water, bentonite slows intestinal transit, thereby increasing the contact time between nutrients and the digestive mucosa (Quisenberry, 1968). In addition, Tajick et al. (2021) confirm this finding. The increase in egg weight observed from the first weeks of the study and maintained throughout suggests that the 1% dose is sufficient to induce a beneficial and sustained effect without any evidence of habituation or depletion of the effect. Furthermore, bentonite contributes to certain qualitative aspects of the egg (Inal et al., 2000).

This study’s original element is investigating the persistence of bentonite’s effect over 26 weeks, since most previous studies conducted in Algeria were of shorter duration or focused on well-defined production sequences. However, unlike some studies in which no significant effect of bentonite on this parameter was observed, Inal et al. (2000) showed in this classical study on laying hens that the inclusion of 1.5–3.5% bentonite did not significantly affect egg weight. For this parameter, a clear improvement was recorded at 1% in our study. This further supports the hypothesis of a dose- and context-dependent nutritional effect. From a physiological standpoint, this improvement may be related to the diet being less contaminated or more balanced, which leads to improved availability of the essential nutrients, particularly proteins and minerals, required for egg formation. Alternatively, this effect may be due to the high efficiency of the bentonite used in this study.

**3.2 Effect on laying rate**

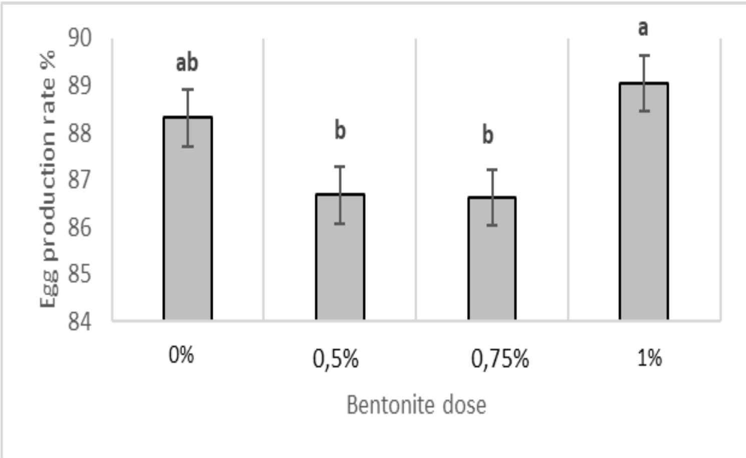


Figure 2: Effect of bentonite dose on laying rate

Indeed, the laying rate also improved significantly at the 1% bentonite dose, increasing by +0.73 percentage points (from 88.32% to 89.05%), which corresponds to an increase of +0.83% ($P < 0.001$). While the increase is small, it is still statistically and economically significant at the farm level (see Figure 2 and Table 3).

While this improvement is modest, it is economically important in large-scale, intensive production systems. The non-linear pattern suggests that only an optimal level of inclusion (1%) yields a zootechnical benefit for egg production, whereas suboptimal levels do not provide any significant advantage. This finding is in perfect agreement with the results of Darmawan et al. (2022), who confirmed through meta-analyses that the laying rate increases quadratically.

The observed laying rate of 89.05% lies within the upper range of values reported in the literature (80–88%). In this context, Gul et al. (2017) and Diaz et al. (2021) reported improved laying rates, primarily when mycotoxins were present. In the present study, however, the improvement occurred without any apparent stress conditions, suggesting that bentonite acts as both a detoxifying agent and a modulator of digestive efficiency. The improvement in laying rate at 1% may be related to a reduction in metabolic and toxic stress, allowing the animals to fully express their genetic potential. In contrast, intermediate doses may be insufficient to produce a complete beneficial effect, or may slightly interfere with nutritional balance. Moreover, monitoring the effect of bentonite over a long period of 26 weeks may indicate a cumulative effect, potentially linked to a progressive improvement in intestinal health or a long-term protective effect against nutritional or environmental stressors, as suggested by Tajick et al. (2021). The laying-rate response observed in this study, with an optimum of 1%, differs from that in the meta-analysis by Öztürk and Darmawan (2022), which estimated the optimum to be 2.19%. This discrepancy may be attributed to various factors, including the strain employed (Novogen Brown in the present study versus a variety of strains in the meta-analysis), the extended experimental period (26 weeks versus an average of 11 weeks) and the distinct properties of the bentonite utilised. However, our results are consistent with those obtained in Algeria by Ait Saada et al. (2013), who reported a significant improvement in laying rate with a 2% inclusion level over a prolonged period (22–74 weeks).

The beneficial effect of bentonite on the laying rate may be explained by three mechanisms: (i) improved nutrient digestibility (Quisenberry, 1968); (ii) reduced intestinal pathogenic bacterial load (Olver, 1989); and (iii) a protective effect against mycotoxins (Darmawan et al., 2022).

However, the results obtained in this study do not corroborate those of Safaei-Khorram et al. (2010), who reported no significant effect of bentonite on laying rates in hens fed a healthy diet. Similarly, Eraslan et al. (2005) demonstrated that the improvement in laying performance observed with adsorbents is only evident under conditions of mycotoxin contamination. Furthermore, Olver (1989) observed no significant effect on egg production at 2%, 4% and 8% bentonite levels, although a negative quadratic response was noted. This discrepancy may be related to the type of bentonite used (sodium vs. calcium) and the experimental conditions. Furthermore, Pasha et al. (2008) reported that bentonite has a weaker effect on egg production than on the feed conversion ratio.

**3.3 Effect on feed conversion ratio (FCR)**

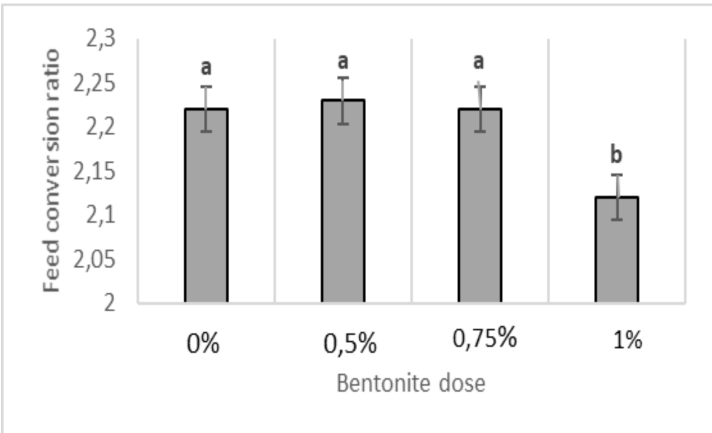


Figure 3 shows the effect of the bentonite dose on the FCR.

The FCR showed a clear and statistically significant improvement, decreasing from 2.22 in the control group to 2.12 in the 1% bentonite group. This corresponds to a reduction of 0.10 points (approximately 4.5%, $P < 0.001$). This decrease indicates improved feed efficiency, meaning more production with less feed, which represents a major economic advantage (see Figure 3 and Table 3). This result is consistent with the findings of Khanedar et al. (2012), who reported a significant reduction in FCR in broilers fed dietary clays. They attributed this reduction to improved digestibility and reduced nutritional losses.

The values obtained in this study are substantially lower — thus better — than those reported in several previous studies, particularly in the Algerian context, where FCR may exceed 2.5. This places the results of the present study within a high-performance range. Improved feed efficiency may be explained by several mechanisms, including the chicken strain used, toxin adsorption, slowed intestinal transit promoting better digestion and stabilised intestinal microflora. Together, these effects allow for a more efficient use of nutrients.

This result is consistent with Darmawan et al. (2022), who reported an improvement in feed efficiency with bentonite inclusion, although the magnitude of the effect can vary across studies. The quadratic decrease in feed conversion ratio (FCR), with an optimum value of 2.12%, is consistent with the meta-analysis by Öztürk and Darmawan (2022), which identified an optimal value of 1.63%. This reduction is associated with increased egg production. Additionally, Pasha et al. (2008) observed a significant impact of bentonite on the feed conversion ratio. Our findings are also consistent with those of Ait Saada et al. (2013), who reported an improvement in the feed conversion ratio at a 2% inclusion rate of bentonite.

One of the most striking results of this study is the FCR value of 2.12, which is lower than that reported in most international studies (Inal et al., 2000; Miazzo et al., 2005; 2.4–2.6). This difference indicates superior feed efficiency and suggests that the bentonite enabled improved nutrient utilisation and highly effective digestion. This may also be explained by the fact that slower intestinal transit increases contact time with digestive enzymes (Quisenberry, 1968). Additionally, it could result from reduced endogenous losses and improved intestinal barrier integrity (Gilani et al., 2021). This finding is particularly important because the FCR is a major economic indicator in poultry production. The improvement in feed efficiency can be explained by two mechanisms: firstly, increased egg weight without an increase in feed intake, and secondly, improved nutrient digestibility due to slower transit (Gilani et al., 2021). However, these results differ from those reported in the meta-analysis by Öztürk and Darmawan (2022), which found no significant effect of bentonite on the feed conversion ratio. This discrepancy may be related to the longer duration of our study (26 weeks versus an average of 11 weeks), which allowed the beneficial effects to be expressed more completely.

**3.4 Effect on body weight**

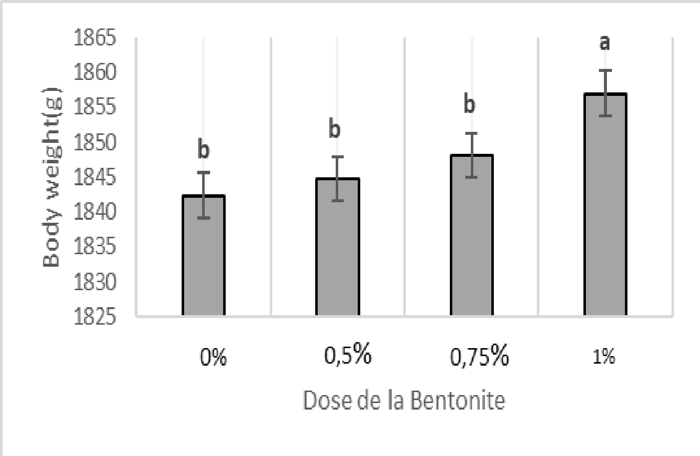


Figure 4: Effect of bentonite dose on body weight

An increase in body weight of +14.58 g was observed in the 1% bentonite group (1856.98 ± 42.79 g) compared with the control group (1842.40 g). This represents a relative gain of approximately +0.79% ($P < 0.05$). Although modest, this improvement indicates a positive effect of bentonite on hen growth (see Figure 4 and Table 3).

This effect may be attributed to bentonite's capacity to adsorb certain toxins and stabilise intestinal microflora, thereby promoting more efficient nutrient absorption. Similar observations have been reported by Dalié et al. (2010), who emphasised the importance of adsorbent agents in mitigating the adverse effects of mycotoxins and enhancing digestive health. Unlike the other groups (0%, 0.50%, 0.75%), the pullets' body weight showed a significant increase in the 1% treatment (letter b). This pattern of group results suggests a threshold effect, whereby bentonite only exerts a significant impact from a sufficiently high inclusion level.

The values obtained for changes in body weight are also higher than those reported in some Algerian rearing conditions, where variations in management and feed quality can have a significant impact on the outcome. In this regard, Quisenberry (1968) observed an increase in body weight with higher levels of bentonite. Our moderate inclusion of 1% appears sufficient to influence laying metabolism without significantly affecting live weight by altering the overall energy balance.

Improvements in live weight may be explained by the adsorptive properties of bentonite, which bind mycotoxins and improve intestinal mucosal integrity, thereby enhancing nutrient absorption. These findings are consistent with several studies showing improved body weight in the presence of dietary clays. However, some studies have reported no significant effect on body weight, which is consistent with previous research (Öztürk & Darmawan, 2022; Olver, 1989) and suggests that the energy and nutrients saved through improved feed efficiency may be directed towards egg production rather than live weight gain.

### 3.5. Effect on mortality

Finally, the mortality rate decreased by -0.21 percentage points (2.12% vs. 2.33%), corresponding to a -9.01% reduction (p = 0.010). This suggests that bentonite at a 1% inclusion level has a beneficial effect on the health and survival of the animals (see Table 3). Similar findings were reported by Hassan et al. (2010), who observed a reduction in poultry mortality when they were given mineral additives. Furthermore, the levels recorded in this study are comparable to those observed in certain rearing systems in Algeria by Aït Saada et al. (2013).

These results are consistent with those of Darmawan et al. (2022) and Gilani et al. (2021), who also reported no adverse effects on animal survival. This effect may be attributed to bentonite's ability to neutralise mycotoxins, improve the intestinal barrier and enhance the immune response. Furthermore, our results confirm the safety of bentonite at the tested levels.

### 3.6 Effect of Bentonite on Blood Biochemical Parameters

The results concerning the effects of bentonite incorporation on blood triglycerides and cholesterol are presented in Table 4.

Table 4 shows the effect of different doses of bentonite on blood triglycerides and cholesterol in laying hens towards the end of the laying cycle.

| Parameter | 0 % Bentonite | 0,5 % Bentonite | 0,75 % Bentonite | 1 % Bentonite | F | p-value |
|---|---|---|---|---|---|---|
| **Triglycerides (g/L)** | 1,0347 ± 0,15$^{a}$ | 1,0347 ± 0,15$^{a}$ | 1,0330 ± 0,15$^{a}$ | 0,8787 ± 0,14$^{b}$ | 9,225 | <0,001 |
| **Cholesterol (g/L)** | 0,9389 ± 0,20$^{a}$ | 0,9389 ± 0,20$^{a}$ | 0,9302 ± 0,20$^{a}$ | 0,7985 ± 0,19$^{b}$ | 3,939 | 0,010 |

The values are expressed as mean ± standard deviation (n = 30). According to Tukey's test (p < 0.05), means followed by different letters (a, b) on the same row are significantly different.

Analysis of variance shows a highly significant effect of bentonite on triglycerides (p < 0.001) and a significant effect on cholesterol (p = 0.010). Tukey's test indicates that the 1% bentonite dose induced a significant reduction in both parameters compared with the other treatments, which did not differ from one another. These results highlight a significant improvement in the blood lipid profile of laying hens following the incorporation of bentonite, particularly at the 1% dose. This was associated with a significant decrease in triglycerides (0.8787 g/L) and cholesterol (0.7985 g/L) relative to the other treatments ($p < 0.05$). This decline reflects favourable modulation of lipid metabolism (Table 4).

The reduction in blood cholesterol can be attributed to bentonite's ability to adsorb bile acids in the digestive tract, resulting in their increased excretion. This stimulates the use of hepatic cholesterol for synthesising new bile acids, thereby decreasing its concentration in the blood. This phenomenon has been widely reported in the scientific literature, particularly by Trckova et al. (2004). Similarly, the decrease in triglycerides observed at 1% bentonite suggests improved utilisation of dietary lipids and reduced intestinal absorption. According to Miazzo et al. (2005), dietary clays stabilise the digestive environment and improve enzymatic efficiency, resulting in reduced circulating lipids.

Finally, the improved lipid profile may also be related to bentonite's detoxifying properties, as it adsorbs mycotoxins and other undesirable compounds. This reduces metabolic stress and improves liver function (Abdel-Wahhab et al., 2013). Moreover, these findings are consistent with trends observed in several international studies in which clay additives, such as bentonite, lead to a significant decrease in blood lipid parameters. This agreement strengthens the biological relevance of the results obtained in the present study.

In summary, incorporating 1% bentonite into the diet of laying hens significantly improves blood biochemical parameters by reducing triglycerides and cholesterol. These results confirm the usefulness of bentonite as a nutritional additive capable of optimising lipid metabolism and consequently enhancing overall performance in poultry production.

**3.7 Economic assessment**

Incorporating 1% bentonite significantly improved zootechnical performance, with egg weight increasing by 4.48% and feed efficiency improving by 4.50%, alongside a marked reduction in the mortality rate (-9.01%), compared with the control group.

This significant improvement in zootechnical performance resulting from the inclusion of 1% bentonite directly translates into favourable economic benefits. The increase in egg weight (+4.48%) represents a significant commercial advantage, as larger eggs are generally more highly valued in the market, thereby increasing income per egg. This qualitative improvement strengthens the overall profitability of the production system.

Additionally, the improvement in feed efficiency (+4.50%) indicates a better conversion of feed into marketable products, resulting in reduced feed costs per unit produced. Since feed constitutes the main cost component in poultry farming, optimising this is a major economic lever, significantly lowering egg production costs.

Furthermore, the decrease in the mortality rate (-9.01%) enables a higher productive stock to be maintained throughout the rearing period. This increases the total number of eggs produced per batch, improving overall revenue while reducing fixed costs. Consequently, reducing biological losses enhances the system's economic efficiency.

## Conclusion and recommendations

The results obtained show that incorporating 1% bentonite into the diet of laying hens significantly improves zootechnical performance, particularly body weight, egg weight, feed efficiency and laying rate, while reducing mortality. These effects are mainly due to bentonite's adsorptive, digestive and detoxifying properties. Therefore, bentonite appears to be an effective and economically profitable nutritional additive, providing an optimal balance between cost of incorporation and productivity gains. It is also well-suited to the framework of efficient and sustainable poultry production, especially in rearing systems facing sanitary and nutritional constraints.

Furthermore, the incorporation of 1% bentonite significantly improves blood biochemical parameters by reducing triglycerides and cholesterol. These findings confirm the value of bentonite as a nutritional additive capable of optimising lipid metabolism and consequently improving overall poultry production performance. From an economic perspective, the combined effects — namely improved productive performance, lower feed costs, and reduced losses — lead to an increase in net profit and the profitability index.

Furthermore, additional studies could be conducted to: (i) evaluate the effect on internal and external egg quality, (ii) analyse the impact on intestinal microbiota and (iii) test the effect in combination with other additives (e.g. probiotics and enzymes).

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## Author contributions

- **Djelailia Sofiane:** manuscript writing, study design, experimental planning, data collection and experimental analyses.
- **Boussaada Djelloul:** statistical analysis, interpretation of results and manuscript revision.

**Conflicts of interest**

The authors declare that they have no financial or personal conflicts of interest that could have influenced the results or interpretation of the data presented in this article.

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