Effect of Using Different Resources of Premix in Diet on Some Egg Quality of Layer Hen

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Abstract

A total of 84 laying hens, ISA Brown, 60 weeks age, were used, randomly distributed into four treatment groups with 3 replicates for each treatment. The treatments were distributed as follows: T1: added the premix to diet supplied by Nusscience Company. T2: added the premix to diet supplied by Provimi Company. T3: added the premix to diet supplied by Max Care Company. T4: added the premix to diet supplied by INTRACO Company. The results show that there were no significant differences in the relative weight of the shell, except for the mean, a significant superiority of T2 and T4 compared T3, a significant differences in the shell thickness. There were no significant differences in the relative weight of albumin, and a significant superiority in the general average, where T2 outperformed on T4. There were no significant differences in the yolk index. T3 and T2 were significantly superior to T1.

Keywords: Premix, Egg, Layer Hen.

1. Introduction

Premixes were a mixture or mixture of a group of nutrients represented by vitamins, amino acids, mineral elements, medicines, feed supplements and diluents that can be added to diets [1]. The idea of producing premix (premixes) was started by the experts of the German company BASF [2] and some international companies such as the American company Adisseo [3] and Pioneer [4], the Swiss company Zagro [5], the Turkish company Kartalkimya [6] and the German Association for the Processing of Feed Additives [7]. By developing the premix industry in terms of the materials included in the composition of the feed, these percentages allowed to be added to the feed have been reduced from 15% to 3% and then to 1% per ton of feed currently [8]. It contains micro-components that are added to the carrier materials and added at a rate of no more than 1.5-1 kg/ton of feed, then this ratio was raised to 3-2% in order to reduce the impact of heat stress in most months of the year [9]. Premix can be manufactured so that it is added to the main mixture in any possible proportion that is suitable for feed making conditions between 2-10% [10]. Al-Attar and Tawfiq [11] mentioned several benefits of premixes, including that they are not considered as sources of energy and protein, and are necessary auxiliary factors for building metabolic compounds to sustain the body and production, and their deficiency causes reduced production and continued deficiency causes pathological conditions that vary in severity according to the severity of the deficiency and according to the type of element and its addition improves the condition Health of the body and increase production in addition to the possibility of processing them individually or as a group in the form of ready-made preparations in different packages and trade names, such as the word Premix, which is currently in the market with the need for it according to the type of animal and the nature of nutrition and according to production and proportions. The premixes, with both parts (the carrier and the active substance), are either natural in origin, such as wheat, barley, corn, starch, flour and other foodstuffs, or unnatural [12].

A nutritional characteristic for its ability to carry nutrients for the purpose of making some adjustments to the levels of these nutrients in the diet to raise its percentage and reach the actual needs of birds in feed [13]. To achieve a balance in each of the amino acids, minerals and vitamins, where protein can be used [14]. Because the bird needs nutrients that help it grow, produce and reproduce, they are obtained from a balanced diet [15]. The addition of alternative materials used in place of protein concentrates is expressed as (mixture of vitamins and minerals) or premix [16]. Its effect can be observed on the qualitative and productive characteristics of laying hens, such as the ISA Brown strain [17]. In order to achieve the requirements that must be met in the diets that lack these nutrients in vitamins, minerals and amino acids [18]. Premixes are added to the diet of birds in order to fill the shortage [19]. The fluctuations that occur in the natural content of nutrients in order to ensure that the bird obtains sufficient quantities of the nutrients required to be available [20]. Many countries of the world have tended to use premixes instead of protein concentrate in bird diets in order to get rid of the pathological infections that occur and to ensure that the bird gets its needs of nutrients and vitamins [21]. Some feed factories purchase the premix components individually to make the basic mixture, where these components are mixed according to the required...
specifications and according to the productive status of the birds and the age and type of birds [22]. This study aims to determine the effect of four types of premix on the qualitative characteristics of the eggs produced.

2. Materials and Methods

2.1 Experiment site

This experiment was conducted in the field of laying hens, Agricultural Research and Experiment Station, College of Agriculture, Al-Muthanna University, from 1/7/2020 to 22/9/2020 for a period of 12 weeks, using 84 laying hens (ISA Brown) of age 60 weeks, which were divided into three treatments distributed to three places (3x3m) and each was divided into three equal sections so that each section contained 7 laying hens (21 laying hens/treatment) and the treatments were as follows:

- T1: Added the premix to diet supplied by Nuscience Dutch Company by 2.5%.
- T2: Added the premix to diet supplied by Provimi Jordanian Company by 2.5%.
- T3: Added the premix to diet supplied by Max Care Belgian Company by 2.5%.
- T4: Added the premix to diet supplied by INTRACO Belgian Company by 2.5%.

2.2 Experimental diet

The birds were fed upon their arrival at the station at the age of (60) weeks on a ration containing 2,800 kilocalories, metabolism energy/kg feed, crude protein 17.5%, and the feeding continued for 12 weeks, after which the diets used in the experiment were mixed (Table 1), and the feed was provided to the birds in the form of Mash, finely ground groats, amounting to 115 g/fowl/day (specified feed), divided into 4 nutritional treatments, as follows:

- The first treatment (control treatment): the laying hens were fed on a basal diet, and 2.5% of the Dutch Nuscience premix was added.
- The second treatment: the laying hens were fed on a basic diet (Basal diet), and 2.5% of the Jordanian Provimi premix was added.
- The third treatment: the laying hens were fed on a basic diet (Basal diet), and 2.5% of the Belgian Max Care premix was added.
- Fourth treatment: the laying hens were fed on a basic diet (Basal diet) and 2.5% of the Belgian INTRACO premix was added.

Table 1. Basal Diet in four treatments during the study periods (60-72 weeks).

| Items                  | T1   | T2   | T3   | T4   |
|------------------------|------|------|------|------|
| Yellow corn            | 38.2 | 38   | 38   | 36.05|
| Soya bean meal         | 23.0 | 23   | 23.5 | 24.2 |
| Bran                   | 5.3  | 6    | 5.5  | 5.8  |
| Wheat                  | 10   | 9    | 10   | 10   |
| Barley                 | 10   | 10   | 10   | 10   |
| Plant oil              | 2.1  | 2    | 2.8  | 3.4  |
| Dicalcium              | 0.8  | 2    | 0.8  | 0.75 |
| Limestone              | 7.8  | 7.5  | 6.6  | 7.3  |
| Salt                   | 0.3  | ---- | 0.3  | ---- |
| Dutch Nuscience premix | 2.5  |      |      |      |
| Jordanian Provimi premix| 2.5  |      |      |      |
| Belgian Max Care premix|      |      | 2.5  |      |
| Belgian INTRACO premix |      |      |      | 2.5  |
| Total                  | 100  | 100  | 100  | 100  |
| Chemical analysis      |      |      |      |      |
| Crude protein (%)      | 17   | 17.1 | 17   | 17.1 |
| metabolism energy kilocalories /kg | 2775 | 2775 | 2770 | 2774 |
| Methionine (%)         | 0.35 | 0.48 | 0.40 | 0.41 |
| Lysine (%)             | 0.95 | o.95 | 0.96 | 0.97 |
| Calcium (%)            | 3.44 | 3.45 | 3.44 | 3.64 |
| Phosphorus (%)         | 0.39 | 0.39 | 0.39 | 0.39 |
2.3 Prepare the diets

The experimental feed for laying hens was prepared at the research station of the College of Agriculture, Al-Muthanna University, Umm Al-Aqaf. The treatments were prepared by adding different sources of imported premixes for each of the four treatments at a rate of 2.5%. The premixes were brought from the holy Karbala governorate and Al-Hamza district of Diwaniyah governorate, where a premix was added to the first treatment (control treatment) T1 of the Nuscience type of Dutch origin at a rate of 2.5%, and a premix of the type of Provimi of Jordanian origin was added to the second treatment T2 at a rate of 2.5%, and a premix of the Max Care type of Belgian origin was added to the third treatment T3 at a rate of 2.5 %, and a premix type INTRACO of Belgian origin at a rate of 2.5 % was added to the treatments.

2.4 Traits studied

2.4.1 Egg quality measurements

2.4.1.1 External measurements of egg

2.4.1.2 Shell thickness

The thickness of the shell was measured by means of a micrometer by taking three readings (the first from the wide end, the second from the pointed end and the third from the middle of the egg) while retaining the inner and outer shell membranes, and then taking an average of three readings for each egg [23]:

\[
\text{Shell thickness rate} = \frac{\text{Spire shell thickness (mm)} + \text{Convex shell thickness (mm)}}{2}
\]

2.4.1.3 Shell relative weight

According to the relative weight of the shell of samples of eggs in each replicate of each treatment by applying the following equation [23]:

\[
\text{Relative weight of the shell} = \frac{\text{Shell Weight (g)}}{\text{egg weight (g)}} \times 100
\]

2.4.2 Internal Measurements of Egg

2.4.2.1 Yolk relative weight

According to the relative weight of the yolk according to the equation provided by Al-Fayyadh and Naji [23]:

\[
\text{Relative weight of the yolk} = \frac{\text{Yolk Weight (g)}}{\text{egg weight (g)}} \times 100
\]

2.4.2.2 Albumin relative weight

According to the relative weight of the albumin, according to the equation provided by Al-Fayyadh and Naji [23]:

\[
\text{Relative weight of the albumin} = \frac{\text{Albumin Weight (g)}}{\text{egg weight (g)}} \times 100
\]

2.4.2.3 Yolk index

The yolk index was measured for all replicates of the transactions, on average, once every 14 days, according to the following two equations [23]:

\[
\text{Yolk index} = \frac{\text{Yolk height}}{\text{Yolk diameter}}
\]
2.4.2.4 Haugh Unit (H.U.)

To extract the value of a unit, the following equation was used by Al-Fayyadh and Naji [23]:

\[
\text{Haugh Unit} = 100 \log \left[ H - \frac{G \times (30W0.37 - 100)}{100} + 1.9 \right]
\]

whereas:

\[H = \text{egg height (mm)}, \ W = \text{egg weight (g)}, \ G = \text{fixed number of 32.2}\]

2.5 Statistical analysis

Complete Random Design (CRD) was used to study the effect of different sources of imported premixes in laying hens’ diets on the studied traits, and significant differences between means were compared with Duncan [24] multiple range test under the 0.05 level of significance. The program SPSS [25] was used in the statistical analysis.

3. Results and Discussion

3.1 Outer egg traits

3.1.1 Relative weight of Shell

Table 2. show that the effect of different sources of imported premixes included in the ration of ISA Brown chicken eggs. The table indicates that there are no significant differences (P<0.05) between all the treatments in this trait for all production periods in which the measurement was carried out, which extends between (60-62, 62-64, 64-66, 66-68, 68-70 and 70-72) weeks. When calculating the general average of this trait, it was noticed that there was a significant superiority (P<0.05) of T2 and T4 compared to T3, while we did not find significant differences between the second, fourth and first treatments (T2, T4, T1), on the one hand, as well as T1 and T3 on the other hand. Perhaps it explains the differences between the transactions over the length of the experiment. They were very simple differences that did not rise to the degree of morality or the moral difference, but in the final these differences gathered to appear significantly in favor of the second and fourth treatments. The reason for this may be due to a recovery in the body’s stock of vitamins and mineral elements [25,26], which seemed to show an effect with the extension of the trial period, meaning that the cumulative effect of these elements played a role in an improvement in this characteristic.

While Bohwmik [27] noted that the premix was distinguished by its containing vitamins and essential and important mineral elements for the purpose of meeting the needs of domestic birds in terms of maintenance and production requirements, which contributed to the improvement of this characteristic.

These results were in agreement with Al-Machi [14], which included the use of some local limestone sources as a carrier in the production of premixes in the hen egg diets in the qualitative characteristics of the eggs produced, which indicated that there were no significant differences for the relative weight of the egg shell For the periods (20-21, 23-24, 24-25, 26-27, 28-30 and 29-30).

| Treatments | 60-62 | 62-64 | 64-66 | 66-68 | 68-70 | 70-72 | General average |
|------------|-------|-------|-------|-------|-------|-------|----------------|
| T1         | 64.79 | 66.66 | 66.72 | 66.77 | 67.02 | 66.91 | 66.48 ab       |
| T2         | 68.10 | 67.71 | 67.50 | 68.91 | 65.36 | 66.80 | 67.40 a        |
| T3         | 67.36 | 66.61 | 66.54 | 67.10 | 66.33 | 67.73 | 66.94 ab       |
| T4         | 63.53 | 65.18 | 66.89 | 66.70 | 65.84 | 65.52 | 65.61 b        |
| Sig.       | N.S   | N.S   | N.S   | N.S   | N.S   | N.S   | 0.05           |
3.1.2 Shell thickness

This characteristic is one of the most important qualitative characteristics of the eggs produced because it controls the extent to which the eggs reach the consumer without being broken. Table 3. indicates the effect of different sources of premixes included in the ration of ISA Brown table eggs, as the table shows that there are significant (P<0.05) differences between all treatments in the thickness of the shell during the first production period (62-60). A week, as the fourth treatment (T4) outperformed the second and first treatment (T2, T1), while we found no significant differences between the fourth and third treatments (T4, T3) on the one hand, as well as the third, first and second treatments (T3, T1, T2) on the other hand. As for the second production period (62-64) weeks, the second treatment (T2) outperformed all treatments (T3, T1, T4), while in the third production period (66-64) weeks, it was noticed that the fourth treatment (T1) outperformed the rest of the treatments. During the fourth production period (68-66) weeks, the first treatment (T1) outperformed the rest of the treatments, and in the fifth production period (70-68) weeks, it was noticed that the fourth treatment (T4) was superior to the rest of the treatments as it was in the first and third periods, and during the production period The sixth treatment (72-70) weeks, noted the superiority of the first treatment (T1) over the rest of the treatments.

However, there were no significant differences (P<0.05) when calculating the general average (60-70, 62-72). The results agreed with Mirza [28], who found a significant superiority for this trait when using the prepared protein concentrates and comparing it with the protein concentrate on the productive performance of laying hens. The reason may be due to the fact that the premix provided the bird with all its needs of vitamins and minerals, which led to an improvement in the thickness of the shell thickness [29, 30], and these results do not agree with [31, 32, 33], who indicated that the use of premix did not have a significant effect on the thickness of the crust. The reason for this may be due to the role of premix in terms of containing vitamins and mineral elements in terms of their impact on production without the level of addition, and this confirms the fact that a very low or very low percentage of most vitamins and mineral elements is sufficient to perform their functions [26].

Table 3. Effect of different sources of imported premixes included in the diet of ISA Brown hens on the average of the eggshell thickness during the weeks of production (60-72).

| Treatments | 60-62 | 62-64 | 64-66 | 66-68 | 68-70 | 70-72 | General average |
|------------|-------|-------|-------|-------|-------|-------|-----------------|
| T1         | 0.31 b| 0.31 b| 0.31 b| 0.33 a| 0.31 b| 0.33 a| 0.32            |
| T2         | 0.31 b| 0.33 a| 0.31 b| 0.32 b| 0.32 b| 0.32 b| 0.32            |
| T3         | 0.32 ab| 0.31 b| 0.31 b| 0.32 b| 0.31 b| 0.31 b| 0.31            |
| T4         | 0.33 a| 0.31 b| 0.33 a| 0.31 b| 0.33 a| 0.31 b| 0.32            |
| Sig.       | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  | N.S             |

3.2 Inside Egg traits

3.2.1 Relative Yolk Weight

Table 4. showed that there were no significant differences among all treatments for this trait during the first (62-60) weeks of chicken life, the second (62-64) weeks, and the third (64-66) weeks, the fifth (68-70) weeks and the sixth (70-72) weeks. While there were significant (P<0.05) differences between treatments in the fourth productive period (64-68) weeks in favor of the third treatment (T3) at the expense of the second treatment (T2), with no significant differences (P<0.05) between the third, fourth and first treatments. (T3, T4, T1) on one side and between the fourth, first and second (T4, T1, T2) on the other. These results were in agreement with [14], who indicated the presence of significant superiority through the utilization of some sources of local limestone as a carrier in the production of premix in the diets of egg hens and broilers. As well as Ezzat [31] when comparing it to a premix with protein concentrate in the diets of table eggs, as the treatments showed a significant superiority over the control treatment, and the reason for this was attributed to the increased availability of minerals and vitamins necessary for the formation of yolk [34]. And Bohwmik [27] noted that the premix contain all the nutrients for the purpose of meeting the necessary needs that the domestic birds need in terms of their content of mineral elements, vitamins and amino acids, which led to an improvement in this characteristic in laying hens.

When calculating the general average (60-72) weeks of chicken age for this trait, a significant superiority was observed for the fourth treatment (T4) at the expense of the second treatment (T2), while no significant differences appeared between the fourth, first and third treatments (T4, T1, T3) from one side and between the first, third and second (T1, T3, T2) on the other. The reason for this may be due to the presence of vitamins and minerals in the feed in proportions necessary to improve its quality and to secure the physiological needs of the chicken from these elements because they are metabolic catalysts,
meaning that some basic metabolic processes cannot occur without a sufficient amount of these elements and this is related to the general health status of the bird’s body So that it is in a positive balance and therefore more able to benefit from the nutrients found in the diet [35].

Table 4. Effect of different sources of imported premixes included in the diet of ISA Brown hens on the average of the relative yolk weight during the weeks of production (60-72).

| Treatments | Periods (weeks) | General average |
|------------|-----------------|-----------------|
|            | 60-62 | 62-64 | 64-66 | 66-68 | 68-70 | 70-72 |       |
| T1         | 25.44 | 22.92 | 23.52 | 23.47 | 23.44 | 23.61 | 23.73 ab |
| T2         | 21.29 | 21.96 | 22.66 | 22.28 b | 24.61 | 23.72 | 22.75 b |
| T3         | 22.36 | 23.56 | 24.38 | 24.17 a | 24.13 | 23.24 | 23.64 ab |
| T4         | 25.85 | 24.65 | 24.09 | 24.00 ab | 24.27 | 24.69 | 24.59 a |
| Sig.       | N.S   | N.S   | N.S   | 0.05   | N.S   | N.S   | 0.05    |

3.2.2 Relative weight of albumin

Table 5. shows that there are no significant differences on the relative weight of egg albumin between all treatments during all the different production periods (60-62, 62-64, 64-66, 66-68, 68-70 and 70-72). When calculating the general average (60-62 and 70-72) weeks of age for the characteristic of the relative weight of egg albumin for each treatment, there are significant differences (P<0.05) between the treatments, where the second treatment (T2) outperformed the fourth treatment (T4). We did not notice any significant differences between the second, third and first treatments (T2, T3, T1) on the one hand, and between the third, fourth and first treatments (T3, T4, T1) on the other hand, and this explains the presence of very simple differences between the transactions over the periods past to gather at the end of the period and appear as significant differences. The reason for this may be due to a recovery in the stock of the bird’s body in terms of vitamins and mineral elements, which seemed to show an effect with the extension of the experiment period, meaning that the cumulative effect of these elements played a role in the improvement of this characteristic [26], and this result is consistent in that the quality of the eggs produced is related if the premix contains important vitamins and minerals that meet the needs of laying hens [27]. These results do not agree with what was indicated by [14], who indicated that there are no significant differences for this trait by making use of some sources of local limestone as a carrier in the production of premix in the diets of egg hens and broilers.

Table 5. Effect of different sources of imported premixes included in the diet of ISA Brown hens on the average of the relative albumin weight during the weeks of production (60-72).

| Treatments | Periods (weeks) | General average |
|------------|-----------------|-----------------|
|            | 60-62 | 62-64 | 64-66 | 66-68 | 68-70 | 70-72 |       |
| T1         | 64.79 | 66.66 | 66.72 | 66.77 | 67.02 | 66.91 | 66.48 ab |
| T2         | 68.10 | 67.71 | 67.50 | 68.91 | 65.36 | 66.80 | 67.40 a |
| T3         | 67.36 | 66.61 | 66.54 | 67.10 | 66.33 | 67.73 | 66.94 ab |
| T4         | 63.53 | 65.18 | 66.89 | 66.70 | 65.84 | 65.52 | 65.61 b |
| Sig.       | N.S   | N.S   | N.S   | N.S   | N.S   | N.S   | 0.05    |

3.2.3 Yolk Index

It is one of the common standards in expressing the shape of the yolk, as the consumer prefers high and round yolks and does not prefer low and flat yolks [21]. The results shown in Table 6. indicated that there were no significant differences (P<0.05) between the treatments for this trait in the egg yolk index rates of the different treatments during all production periods (60-62, 70-72) weeks, and these results were in agreement with Ezzat [31] indicated that there was no significant effect on this trait when feeding chickens on different levels of the premix in the ration. Al-Fayyadh and Naji [23] mentioned that the value of the yolk index is mainly affected by the conditions and duration of egg storage. And Ezzat and Al-Mashhadani [21] who did not notice the absence of significant differences in the yolk characteristic of table chicken eggs when using the premix to replace the imported concentrates in laying hens' diets.

As for calculating the general average (60-62,70-72) weeks of age for the yolk index for each treatment, it is noted from Table 6. that there are significant differences (P<0.05) between the treatments, where the third and second treatment (T3, T2) outperformed the treatment The first (T1) while we did not find significant differences between the third, second and fourth treatments (T3, T2, T4) on the one hand, and the fourth and first treatments (T4, T1) on the other hand, as mentioned
previously in Table (14). This result does not agree with [21, 31] who indicated that there are no significant differences in calculating the general average. This is consistent with Tsiaqbe et al. [36] indicated, which is represented in feeding laying hens on diets fortified with nutrients with vitamins and minerals, which led to a noticeable improvement in this characteristic of laying hens. The imported premix, as explained by Bohwminik [27], may be distinguished in terms of its content by the presence of vitamins and essential mineral elements that met the needs of laying hens and contributed to the improvement of this characteristic.

Table 6. Effect of different sources of imported premixes included in the diet of ISA Brown hens on the average of the yolk index during the weeks of production (60-72).

| Treatments | Periods (weeks) | 60-62 | 62-64 | 64-66 | 66-68 | 68-70 | 70-72 | General average |
|------------|----------------|-------|-------|-------|-------|-------|-------|----------------|
| T1         |                | 0.43  | 0.41  | 0.44  | 0.43  | 0.47  | 0.43  | 0.44 b         |
| T2         |                | 0.46  | 0.45  | 0.44  | 0.43  | 0.48  | 0.42  | 0.45 a         |
| T3         |                | 0.46  | 0.43  | 0.45  | 0.43  | 0.48  | 0.45  | 0.45 a         |
| T4         |                | 0.44  | 0.44  | 0.47  | 0.42  | 0.46  | 0.43  | 0.44 ab        |
| Sig.       |                | N.S   | N.S   | N.S   | N.S   | N.S   | N.S   | 0.05           |

3.2.4 Hough Unit

The Hough unit is one of the most important and wide scales used to express the quality of egg albumin. This scale was created by Roymand Haugh in 1937, and that is why it was named after him [23]. It is noticed from Table 7. that there are no significant differences in the values of the egg unit of all experiment treatments during all production periods (62-60, 72-72) weeks. As well as when calculating the general average for this trait throughout the experiment period (62-60, 70-72) weeks, and perhaps the reason for the equality of the adjective unit is that the four treatments have pushed the trait in the right direction for all transactions, and accordingly, the premixes (premixes) according to the experts of the German company BASF They are substances with natural specifications that are added to poultry feed due to the lack or deficiency of those feeds containing them, and they have specific goals. Vitamins and minerals have been classified within the first category of feed additives based on the classification of the European Union. These results are consistent with Ezzat [31], where no significant differences were observed between the treatments when calculating the general average for this trait when using a premix. This result was confirmed by Abdel-Abbas [32] that he did not obtain significant differences between the different treatments that included feeding table-egg chickens on a protein concentrate and comparing it with two plant and animal protein concentrates. As for the effect of the production periods on the unit, it was also not observed that there were significant differences between the different production periods for this trait.

Table 7. Effect of different sources of imported premixes included in the diet of ISA Brown hens on the average of the Hough unit during the weeks of production (60-72).

| Treatments | Periods (weeks) | 60-62 | 62-64 | 64-66 | 66-68 | 68-70 | 70-72 | General average |
|------------|----------------|-------|-------|-------|-------|-------|-------|----------------|
| T1         |                | 95.66 | 87.36 | 82.80 | 78.40 | 90.06 | 78.70 ab | 85.50         |
| T2         |                | 95.20 | 84.00 | 82.73 | 78.63 | 86.96 | 75.76 b | 83.88         |
| T3         |                | 92.20 | 83.63 | 88.83 | 79.46 | 92.36 | 85.36 a | 86.97         |
| T4         |                | 94.30 | 81.76 | 83.26 | 80.93 | 85.30 | 80.16 ab | 84.28         |
| Sig.       |                | N.S   | N.S   | N.S   | N.S   | N.S   | N.S   | 0.05          | N.S           |

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