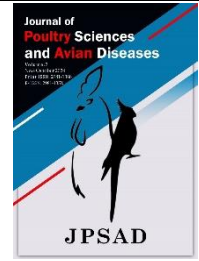






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## The Effect of Micronized Lignocelluloses on Calcium Metabolism, Egg Shell Quality and Performance of Hy-Line W-36 Laying Hens



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### ABSTRACT

Carbohydrates are the main macromolecule of poultry feed (40-70 %), categorized into digestible and indigestible forms. Poultry uses digestible carbohydrates to supply energy. Still, the indigestible part (water-soluble and non-soluble) has different roles in anatomy, histology, water loss, bacterial microflora, mucosal health, normal secretions, feed transport time, and digestion rate in the gastrointestinal tract. Cereals (like corn), the carbohydrate source in poultry feed, can contain mycotoxins or water-soluble indigestible fibers; thus, some mycotoxin-free commercial products contain insoluble indigestible carbohydrates. In this study, the effect of micronized Lignocelluloses (Under trade name ARBOCEL®) was evaluated on calcium metabolism, eggshell quality, and performance of commercial Hy-Line W-36 layers. The results showed that the product had a good effect on the evaluated factors, but in some cases, there was not any significant effect that compared to other groups. However, it is better to evaluate the impact of using this product in various concentrations, ages, periods, heat-stress conditions, and digestive tract microflora disturbance, which is beyond the aim of this study and can be subject to subsequent researches.

**Keywords:** Carbohydrates, Fiber, Micronized Lignocelluloses, Poultry, Macromolecules

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## 1 Introduction

Carbohydrates, the main macromolecule of poultry feed (40-70 %), are categorized into digestible and indigestible forms (1). The role of the digestible part is to provide energy for the body. The indigestible part (mostly fiber, including non-starch carbohydrates, oligosaccharides, and lignin), classified into water-soluble and non-soluble, has different physical, functional, and structural effects on anatomy, histology, water loss, bacterial microflora, mucosal health, normal secretions, feed transport time and digestion rate in the gastrointestinal tract (2, 3). Although fiber is essential in poultry feed, its high amount can have anti-nutritional effects, mostly related to the soluble part. This adverse effect is due to increased viscosity, accumulation of feed, and prevention of the absorbance of other nutrients (3, 4). However, the fiber is mainly influenced by fermentation in the large intestine (5); the insoluble fiber (primarily cellulose) can positively affect the gastrointestinal system's feed digestion and absorption (6), local immunity (7), and normal development of different parts that can indirectly influence layers performance factors. An example of this positive effect is improved calcium and other nutrient absorption affecting eggshell quality (8-10).

Poultry-feed carbohydrates are mainly from plant sources, especially cereals (corn). Using cereals to provide energy and fiber can cause problems like soluble fibers and possible mycotoxins (1, 11). In the recent years, some studies have been conducted to produce commercial feed additives containing fiber to remove these obstacles. This kind of production requires chemical, mechanical, and enzymatic processes that prevent the product from having the effects of soluble fiber (12).

ARBOCEL<sup>®</sup> is a commercial micronized lignocellulose product (JRS company, Germany) that supplies insoluble fiber in poultry feed. It is claimed that it can increase the absorption of other feed parts, improve litter quality, improve bacterial microflora of the digestive tract, and decrease the use of antibiotics. Above all, due to special processing, it is free from mycotoxin and other harmful components (13-15). This study evaluates the effect of ARBOCEL<sup>®</sup> supplementation on calcium metabolism, eggshell quality, and performance factors of Hy-Line W-36 commercial hens.

## 2 Materials and methods

This study was conducted on 90 Hy-Line W-36 commercial hens that were randomly distributed in 3 groups (G<sub>1</sub>: Control group with no wheat bran and ARBOCEL<sup>®</sup>; G<sub>2</sub>: Group with feed containing 0.7 percent of dry matter wheat bran and 0.8 percent of dry matter ARBOCEL<sup>®</sup>; G<sub>3</sub>: Group with feed containing only 1.5 percent of dry matter wheat bran, respectively) for one month. The chickens were maintained in galvanized cages with 1.5×1.5 m<sup>2</sup> length and width and 55 cm height in five replicates for each group (six hens for one cage). The needed light was supplied with 23-watt tungsten lamps in three rows, with a 2.5 m distance from each other and one meter from the ceiling, according to management guidelines of the breed, which was dimerizable. Four 140×140 cm<sup>2</sup> fans were used according to the Hy-Line W-36 manual to cover the essential air velocity and ventilation. This study's feeding system consisted of gutter feeders filled three times daily, and the drinking system consisted of 360-degree nipple lines (*ad libitum*). The feed composition used in the study was formulated with UFFDA software based on the minimum needs of the breed (summarized in Table 1). This study performed all the protocols for house preparation, vaccination, and biosecurity. The formulated feeds were fed to birds twice daily, and after each time, collecting, counting, and weighing the eggs was done. The factors related to egg weight, eggshell quality (summarized in Table 1.), and feed conversion ratio (FCR) were estimated every two weeks:

### 2.1 Determining the Eggshell Weight Percentage:

For this purpose, the weight of eggs was measured with an accurate weighting machine. Then, after drying, the eggshell was separated, and the percentage was calculated by dividing this weight by the total egg weight (16).

### 2.2 Assessment of Eggshell Stability:

In this regard, based on the required force for an eggshell break using the Sanovo Egg Shell Force Reader, the direct method was used according to the manufacturer's guidelines based on kg of force in square centimeters (16).

### 2.3 Measurement of Eggshell Thickness:

After drying the eggshell at 60 centigrade degrees oven for eight hours and detaching the eggshell membranes, a special micrometer measured the eggshell thickness at three points (two ends and the middle part). Then, the average of

these three numbers is calculated as the final eggshell thickness (17).

#### 2.4 Evaluating Blood Calcium Values:

At the end of the study, two hens were randomly selected from each repeat, and blood samples were collected from the brachial vein. Then, the samples were centrifugated in blood tubes without anticoagulant at 300 rpm for 15 minutes, and calcium values were determined by commercial kites (Zistshimy company, Iran) (18).

#### 2.5 Evaluating Eggshell and Droppings Calcium:

Droppings were collected from the cage floor weekly and dried in an oven. The calcium values of the droppings were measured using a special kit (Zistshimy company, Iran) and laboratory methods. Similarly, eggshell calcium content was measured with a highly accurate Flame Photometer (PFP7 model, JENWAY, England) after the eggshells were dried, ground, and mixed with special solutions (17).

#### 2.6 Egg Weight measurement:

For this purpose, eggs were collected and weighed every day after feeding time, and then the average of all numbers was calculated and used for comparison.

#### 2.7 Egg Production Efficiency:

The hen day egg production index is calculated daily by dividing the number of produced eggs by the number of live hens. This meticulous approach allowed us to compare the average of all numbers at the end of the study with other groups, ensuring a comprehensive data analysis.

#### 2.8 Feed Conversion Ratio:

It was estimated by dividing consumed feed into egg weight (both in grams) (19).

#### 2.9 Statistical Analysis:

Statistical analysis was performed using SAS software version 1.9. Data were analyzed using Analysis of Variance (ANOVA) and LSD Post Hoc tests. A  $p \leq 0.05$  was statistically considered significant.

### 3 Results

The results revealed a significant finding: the group with fiber (ARBOCEL®) and wheat bran (G<sub>2</sub>) demonstrated a higher eggshell weight percentage. This finding, detailed in Table 2, underscores the importance of this research. Regarding eggshell stability, the G<sub>2</sub> group showed higher resistance than the control (G<sub>1</sub>) and wheat bran (G<sub>3</sub>) groups, although these differences were insignificant. The study also found no significant difference in eggshell thickness between the groups, but the G<sub>2</sub> group showed higher eggshell thickness (Table 2). Blood, eggshell, and feces calcium were also measured on the last day, and the findings showed that apart from the difference in groups, they are not notable (Table 3). Table 4 demonstrates that egg weight is higher in the group with micronized insoluble fiber (ARBOCEL®) (G<sub>2</sub>), but it is not statistically significant ( $p > 0.05$ ). Regarding egg production efficiency, G<sub>2</sub> is better than other groups, but no significant difference was estimated ( $p > 0.05$ ) (Table 4). Furthermore, this study unequivocally determined that although the G<sub>2</sub> group has a better Feed Conversion Ratio, there is still no significant difference between the groups ( $p > 0.05$ ) (Table 4).

**Table 1.** Feed composition for different groups

Nutrient	Groups*		
	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>
Corn	67.7	66.2	66.2
Wheat Bran	Zero	0.7	1.5
ARBOCEL®	Zero	0.8	Zero
Soybean Meal	19	19	19
Barley	2.75	2.75	2.75
Alfalfa Powder	1	1	1
CaCO <sub>3</sub>	8	8	8
DCP	0.8	0.8	0.8
Mineral supp	0.25	0.25	0.25
Vitamin supp	0.25	0.25	0.25
NaCl	0.25	0.25	0.25
D-L Methionine	0.01	0.01	0.01
Metabolizable Energy (ME) (Kcal/Kg)	2780	2780	2780
Crude Protein (CP)	14.6	14.6	14.6

\*G<sub>1</sub>: Control group, without wheat bran and ARBOCEL®; G<sub>2</sub>: Group with feed containing 0.7 percent of dry matter wheat bran and 0.8 percent of dry matter ARBOCEL®; G<sub>3</sub>: Group with feed containing 1.5 percent of dry matter wheat bran

**Table 2.** The effect of micronized insoluble Lignocelluloses (ARBOCEL®) on Egg Shell weight Percentage; Egg Shell stability (kg/cm); and Egg Shell Thickness (mm) in different groups

Groups	Egg Shell weight Percentage		Egg Shell stability (kg/cm)		Egg Shell Thickness (mm)	
	2 <sup>nd</sup> week	4 <sup>th</sup> week	2 <sup>nd</sup> week	4 <sup>th</sup> week	2 <sup>nd</sup> week	4 <sup>th</sup> week
G <sub>1</sub>	11.03±0.37	11.17±0.42	2/76±0.10	2/81±0.09	0.2709±0.02	0.2740±0.01
G <sub>2</sub>	11.23±0.44	11.250.66	2/89±0.09	2/98±0.09	0.2813±0.28	0.2916±0.01
G <sub>3</sub>	11.18±0.52	11.20±0.52	2.83±0.12	2.92±0.11	0.2709±0.27	0.2760±0.02
<i>p</i> -value	0.77	0.97	0.09	0.26	0.50	0.16

**Table 3.** The effect of micronized insoluble Lignocelluloses (ARBOCEL®) on blood; egg shell and feces calcium in different groups

Groups	Feces Calcium	Egg Shell Calcium	Blood Calcium
G <sub>1</sub>	19.12±1.04	37±1.80	2±0.12
G <sub>2</sub>	19.21±1.17	37.36±1.43	1.93±0.95
G <sub>3</sub>	18.96±1.35	36.50±1.66	2.06±0.13
<i>p</i> -value	0.90	0.26	0.54

**Table 4.** The effect of micronized insoluble Lignocelluloses (ARBOCEL®) on egg weight (in grams); egg production efficiency (in percent); and feed conversion ratio in different groups

Groups	Egg Weight (gram)		Egg Production Efficiency (Hen Day)		Feed Conversion Ratio	
	2 <sup>nd</sup> week	4 <sup>th</sup> week	2 <sup>nd</sup> week	4 <sup>th</sup> week	2 <sup>nd</sup> week	4 <sup>th</sup> week
G <sub>1</sub>	60.16±3.84	62.10±4.78	77.10±4.30	78.04±3.78	1.99±0.08	2.01±0.09
G <sub>2</sub>	61.10±3.37	64.02±3.91	80.55±5.72	83.03±3.83	1.96±0.07	2.00±0.07
G <sub>3</sub>	59.56±4.28	62.56±4.33	80.54±7.35	80.04±4.78	1.97±0.07	2.07±0.04
<i>p</i> -value	0.88	0.77	0.38	0.19	0.81	0.14

#### 4 Discussion

This study evaluated the effect of using micronized lignocellulose fibers on different factors of a laying hen flock, like average egg weight, feed conversion ratio, eggshell weight, eggshell percentage to whole egg, and so on. The results showed that regarding egg weight average and egg production percent, there was no significant difference between treatments ( $p>0.05$ ). This result agrees with Hetland *et al.* (2005), who evaluated the effect of barley bran and chopped wood on feed digestion and related factors in broiler and layer flocks, which saw no significant difference in egg production and feed consumption among different groups. However, they relate their findings to the high diversity of flocks and the shortness of the study period (20).

This study showed no significant difference among groups regarding egg production conversion ratio. This finding is similar to that of Abdollahi *et al.* (2021), who evaluated the effect of fiber from different sources on egg production, egg weight, and feed conversion ratio. The results showed no significant difference between groups

regarding egg weight and feed conversion ratio; however, egg production showed a significant difference that is disagree of our findings (21).

The eggshell characteristics evaluated in this study showed no significant variation in thickness, eggshell percentage, or stability except for a difference between the control and feed-containing lignocellulose (G<sub>2</sub>) groups ( $p>0.05$ ). This finding is disagree Noroozi *et al.*'s study (2013) on a 47-week-old leghorn layer. In their research, a difference in feed fiber could affect the number of produced eggs, consumed feed, feed conversion ratio, eggshell percentage, and egg particular weight (22). The three groups' eggshell, dropping, and blood calcium values had no significant difference ( $p>0.05$ ). This data contrasts with Rath *et al.*'s study, which found that high-feeding fiber could decrease calcium absorption from the digestive tract and blood calcium. This study's findings showed no significant difference between blood and dropping calcium ( $p>0.05$ ) (23).

This study's data showed that although the ARBOCEL® has a positive effect on hy-line W36 commercial layers, they are not statistically significant in most cases ( $p>0.05$ ). However, we propose that different factors, such as the use

of this product containing insoluble fiber in various concentrations, age and period of use, heat-stress conditions, and digestive tract microflora disturbance were evaluated. For this purpose, we offer the ARBOCEL® use for a longer time or during stressful times, from the start of production to the peak. It is also practical to evaluate the effect of layer breeds, but these are beyond the aim of this study and can be a subject for subsequent researches.

### Acknowledgements

None.

### Conflict of Interest

The authors declared no conflicts of interest.

### Author Contributions

Raza Hassani Farahani, Morteza Mehri, and Arash Ghalyanchi Langeroudi wrote the first draft and performed different assays. Moein Khodayari wrote and reviewed the article and all the authors approved final version.

### Data Availability Statement

Data are available from the first author upon reasonable request.

### Ethical Considerations

All the experiments were done with regard to animal rights.

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