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Lignocellulose Improves Protein and Amino Acid Digestibility in Roosters and Egg Hatchability in Broiler Breeders

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The present work assessed the effect of supplementation of 0.8% dietary Arbocel® RC Fine, a readily available commercial lignocellulose, to poultry feed. In a complete randomized design using 36 individually caged mature dubbed Hy-Line roosters (aged 55 weeks) grouped in 4 treatments with 9 birds per treatment, a digestibility trial was performed to determine apparent and true metabolizable energy values along with digestibility coefficients of protein and amino acid in Arbocel® containing diets. Results showed that 0.8% Arbocel® supplemented diets improved protein digestibility by 6% ($P<0.05$). Additionally, Arbocel® caused an increase in apparent and true amino acid digestibility in roosters when compared to control diets and controls with 0.8% wheat bran (WB) supplementation. In a second experiment, 26,000 layers and 2,600 roosters aged 33 weeks (Ross 308 broiler breeder strain) were maintained in 6 poultry houses at a commercial breeding farm, with an average of 4330 layers and 433 roosters per house. Performance, egg grade, and hatchability rate were assessed over a post peak period of 6 months. Compared to the control group fed the 0.8% WB diet, the 0.8% lignocellulose dietary supplementation resulted in a decrease ($P<0.05$) in percent infertility leading to an average increase of 4.07% ($P<0.05$) in egg hatchability. The Arbocel® fed group had 3.8 more eggs per housed hen compared to control birds. Overall, Arbocel® supplementation at 0.8% resulted in the production of 5.7 more saleable chicks per housed hen during the 6 months trial, a sizeable profit to the farmer.

Key words: digestibility coefficients, hatchability, lignocellulose

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Introduction

A major cost item in the price of poultry production is diet. It is generally known that climate change and the increase in production of corn based ethanol as well as competition from other animal husbandry industries have resulted in an increase in prices of conventional feed ingredients such as corn and soybean meal. These price increases tend to be asymmetrical, affecting producers in developing nations more than their counterparts in developed nations that have better markets and farm subsidies. Accordingly, any technology or feed additive that would decrease the production cost and improve or at least maintain good production performance, would be welcome to poultry farmers that have to compete on an international market since hatching eggs are frequently exported to neighboring countries (personal communication, Poultry Syndicate in Lebanon, 2015).

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Dietary fiber or non-starch polysaccharides (NSP) are considered as non-nutritive feed additives in monogastric diets and are found in two distinct forms, soluble and insoluble, with each having its own set of characteristics and effects. Soluble dietary fibers are notorious for their detrimental effects on poultry because they contain anti-nutritional factors (ANF) which could trigger a variety of mechanisms that substantially hinder the ideal function of the gastrointestinal tract by altering its physiological functions (Jørgensen *et al*., 1996; Iji *et al*., 2001). Such alterations limit digestion and absorption of major nutrients like proteins, lipids and starch (Carré and Leclercq, 1985; Smits *et al*., 1997) and consequently lead to poor performance of birds. Alternatively, cellulose and some NSP, such as lignocellulose and commercially available Arbocel® RC Fine are insoluble and typically classified as crude fiber (Wilson and Beyer, 2000). Arbocel® RC Fine is a natural fiber produced by J.RETTENMAIER & SÖHNE GmbH + Co.KG, Rosenberg, Germany, basically from fresh spruce trees (Picea species). It is mainly composed of insoluble fibers (cellulose, hemicellulose and lignin) and reported by the manufacturing company to have the following on as is basis:

moisture (7.70%), crude protein (1.00%), crude fiber (65.3 %), crude fat (0.30%), ash (0.50%) and Nitrogen Free Extract (25.1%) with a water holding capacity of 700%. The company recommends that Arbocel® RC Fine be incorporated in poultry diets at a rate of 0.8%. These non-soluble fibers are reported to have beneficial effects on nutrient digestion by poultry (Svihus and Hetland, 2001; Hetland *et al*., 2005). The concentration of lignocellulose in the diet at a rate of 0.8% has been shown to significantly improve apparent protein digestion accompanied with a significant decrease in litter moisture of broilers (Farran *et al*., 2013). It also results in significant improvement in feed conversion ratio and weight gain in broilers (Oke and Oke, 2007) and a significant increase in egg production in layers (Lim *et al*., 2013). The present work was performed to assess potential benefits on ME values and digestibility coefficients of amino acids, and on egg production and reproductive performance of broiler breeders raised under commercial conditions by supplementing their diets with Arbocel® RC Fine.

Materials and Methods

The present work was performed at the American University of Beirut (AUB) agriculture research station and a commercial farm. Regulations and guidelines of the Institutional Animal Care and Use Committee of the AUB were followed in the management and care of the experimental birds.

Rooster Digestibility Trial

Experiment 1 was performed at the Agricultural Research and Education Center (AREC) of AUB in the Beqaa plain, Lebanon. The method of Sibbald (1986) was followed, where 36 mature dubbed Hy-Line roosters (aged 55 weeks) were housed in individual cages and received feed and water *ad libitum* for a two week adaptation period. Afterwards, they were randomly divided into 4 groups of 9 birds each. One group was given the control diet, another group given the Control plus wheat bran (WB) and one group given the Control plus Arbocel (Table 1). The 3 diets were formulated to have the same levels of ME (3200 kcal/kg), crude protein (20%), all other essential nutrients, and to meet nutrient requirements of broilers during the finisher period. A lighting program of 16 hours per day was applied throughout the trial. Forty-eight hours prior to precision feeding, feed was removed and each bird was intubated with 40 m*l* of aqueous glucose solution (50% wt/vol) at 8 and 24 hours after the start of fasting (McNab and Blair, 1988).

The diets were ground to 2 mm particle size and 9 birds in each of the 3 groups were individually precision intubated 30 g of each diet. The remaining 9 birds in the fourth group, were individually precision fed 30 g glucose powder to estimate endogenous and metabolic energy and amino acid losses. Water was provided *ad libitum* and each bird was intubated with 50 m*l* water 32 h post precision feeding. Total excreta of each rooster was collected in a clean plastic tray during the whole 48 h post-feeding period, oven dried at 105℃ until constant weight and ground. Both dried feed and feces samples were analyzed for gross energy determination using an adiabatic bomb calorimeter (C5300 control, IKAWerke GmbH and Co. KG, Staufen, Germany). Correction to zero nitrogen balance was done using the factor 8, 220 cal/g of uric acid nitrogen (Hill and Anderson, 1958). Feed and excreta samples were also analyzed for proximate chemical composition following the methods of the AOAC (1998). The concentration of amino acids, including total sulfur amino acids (TSAA) and tryptophan in the test feed and excreta samples, was determined using HPLC 2690 (Waters Co., Milford, MA). Except for tryptophan, all amino acids in feed and excreta samples were quantified after acid hy-

Table 1. **Composition of the experimental diets used in the digestibility trial**

Ingredients (%)	Control	Control + Wheat Bran	Control + Arbocel®
Corn	62.176	61.172	60.566
Soybean Meal (48% CP)	30.091	30.017	30.375
Arbocel®	—	—	0.800
Wheat bran	—	0.800	—
Sunflower oil	3.558	3.838	4.085
Salt	0.443	0.443	0.444
Limestone	1.71	1.172	1.166
Dicalcium Phosphate	1.856	1.851	1.862
DL- Methionine	0.250	0.250	0.251
L-Lysine HCL	0.094	0.094	0.088
Vitamin & Mineral Mix	0.300	0.300	0.300
Coccidiostat	0.0625	0.0625	0.0625
Calculated Analysis			
ME (Kcal/Kg)	3200	3200	3200
CP (%)	20.0	20.0	20.0
TSA (%)	0.88	0.88	0.88
Lysine (%)	1.10	1.10	1.10

Table 2. **Composition of the broiler breeder diets used in the second experiment**

Ingredients (%)	Control Male	Arbocel® Male	Control Female	Arbocel® Female
Yellow Corn	67.8	68.1	64.15	63.45
Soybean 48%CP	15.0	15.4	25.5	25.7
Wheat Bran	14.0	12.5	0.8	—
Arbocel®	—	0.8	—	0.8
Limestone	2.0	2.0	7.3	7.3
Salt	0.3	0.3	0.32	0.32
DL Methionine	—	—	0.03	0.03
Vit& Min Mix*	0.3	0.3	0.3	0.3
Mono Calcium Phosphate	0.6	0.6	0.6	0.6
Soybean Oil	—	—	1.0	1.5
Calculated Composition				
ME (kg/kcal)	2850	2850	2900	2900
CP (%)	15	15	17.5	17.5
Methionine + Cysteine (%)	0.50	0.50	0.60	0.60
Lysine (%)	0.88	0.88	0.90	0.90

* Provided per kilogram diet for male and female breeders: Vitamin A (retinyl acetate), 11000 IU, Vitamin D3 3500 IU, Vitamin E (DL-α-tocopheryl acetate), 100 IU, Vitamin K (Menadione) 5 mg, Thiamin (B1) 3 mg, Riboflavin (B2) 12 mg, Nicotinic Acid, 55 mg, Pantothenic Acid, 15 mg, Pyridoxine (B6) 4 mg, Biotin 0.25 mg, Folic Acid 0.25 mg, Vitamin B12 0.03 mg, Choline, 1000 mg, Cu (copper sulfate) 10 mg, I (potassium iodide) 2 mg , Fe (ferrous sulfate monohydrate) 50 mg, Mn (manganous oxide) 120 mg, Se (sodium selenite) 0.3 mg, Zn (zinc oxide) 110 mg.

drolysis in 6 *N* HCl using the 982.30E and 982.30Ea methods of AOAC (1998) in the presence of phenol at 110℃ for 24 h. For TSAA determination, samples were subjected to performic acid oxidation before acid hydrolysis as in AOAC (1998) official method 982.30Eb. Tryptophan was quantified after sample hydrolysis in barium hydroxide at 120℃ for 16 h according to AOAC (1998) official method 982.30Ec. All amino acids, except for tryptophan, were derivatized using the AccQ-Tag method, whereas all amino acids were separated by Waters HPLC column (AccQ-Tag 3.9×150) and then identified and quantified using Waters 474 scanning fluorescence detector at a range between 285 and 345 nm for tryptophan and 250 to 395 nm for the other amino acids (Waters Co., Milford, MA). The apparent and true amino acid digestibility of the test diets was calculated using the method of Likuski and Dorrell (1978). Data were analyzed as one way ANOVA using the GLM procedure and means were compared by Tukey's Studentized Range Test at $P<$ 0.05 where appropriate (SAS, 1992).

Broiler Breeder Trial

In the second experiment, 26,000 layers in their post-peak period (33 weeks of age) and 2,600 roosters were used to test the effects of Arbocel® on egg production, mortality rate and hatching performance of Ross 308 parent broiler breeders. All birds were reared in a commercial farm, following guidelines set by the Ross PS Management Handbook (Ross, 2013), Parent Stock Nutrition Specifications (2013), and Performance Objectives (2011) from hatch to 21 weeks of age. They were then randomly transferred with the corresponding males to 6 adjacent layer houses standing in parallel to each other. Houses had wood shaving as litter materials on the floors and were all equipped with laying nests, automatic nipple drinkers and separate automatic conveyor and pan feeders for males and females. After finishing the daily meal, both male and female feeders would be adjusted at a higher level to allow for proper natural mating. Thus a complete randomized design was used with 2 treatments and 3 replicates (houses) per treatment with an average of 4,330 hens and 433 roosters per house. At the start of the trial, birds were 33 weeks of age and data collected over a 6 month period.

Corn-soybean meal rations were formulated (Table 2), to meet the specifications of parent breeders provided by the Ross Company (Ross, 2013). The layer rations were either mixed with 0.8% wheat bran and fed to control birds raised in 3 houses or with 0.8% Arbocel® for the experimental birds in the remaining 3 houses. The control rooster diet contained 14% WB and Arbocel® was included at 0.8% in the other diet which contained 12.5% WB (Table 2). Both diets per gender were formulated to have the same specifications in terms of energy, crude protein, amino acids and other essential nutrients; hence the two experimental diets were isocaloric and isonitrogenous. All birds followed a light schedule of 15 hours per day and were fed daily at amounts set by the breeder manual whereas water was provided *ad libitum*.

Egg production was recorded daily and eggs were graded into different categories of undersized, oversized, cracked, and soiled eggs that were all discarded. Eggs for hatching were counted and set for incubation. On a monthly basis,

Table 3. **Averages[1] of apparent and true metabolizable energy (AME and TME) and those corrected for zero nitrogen balance AME_n and TME_n along with protein digestibility coefficients (TCP) in the three diets tested in experiment 1.**

Treatment	AME (Kcal/kg)	AME_n (Kcal/kg)	TME (Kcal/kg)	TME_n (Kcal/kg)	TCP (%)
Control	3035	3232	3769	3619	54.3[b]
Control + Wheat Bran	3049	3245	3788	3627	54.0[b]
Control + Arbocel®	3056	3254	3795	3641	60.3[a]
SEM[2]	51.2	41.3	51.2	41.3	1.32

[ab,] Means in the same column with no common superscripts differ significantly ($P<0.05$).
[1,] There were 9 individual roosters (replicates) per treatment
[2,] Pooled standard error of means.

hatching eggs (864) were randomly selected from each breeder house (replicate), labeled and set apart in the common collective incubator for 18 days and then moved to a hatcher for 3 days. Infertility was determined in percentages of total eggs set after candling these selected eggs on the 10th day of incubation. Day-old chicks were counted, graded and percent hatchability was computed. All data pertaining to egg production, infertility, and hatchability along with mortality rates were compared using Student's *t*-test.

Results and Discussion

In the digestibility trial, dietary Arbocel® at a level of 0.8 % had no effect on AME, AME_n, TME, and TME_n (Table 3). However, Arbocel® inclusion caused a significant increase ($P<0.05$) in true digestibility of protein manifested by a 6% increase in comparison with the control and the control plus 0.8% wheat bran treatments. Similar results were reported by Farran *et al*. (2013) where a 5.5% increase in apparent protein digestibility in Ross 308 broilers fed diets supplemented with 0.8% Arbocel®, when compared to a control group (0% Arbocel®). Additionally, Arbocel® supplementation resulted in an average increase of 6% ($P<0.05$) for both apparent and true digestibility coefficients of almost all amino acids (Tables 4 and 5). This positive effect of Arbocel® was also observed for the digestibility coefficient of all essential amino acids. Previous research work (Boguslawska-Tryk, 2005) demonstrated the association of Arbocel® supplementation in Cobb chicken diets with an increase in proteolytic activity of the pancreatic trypsin and chymotrypsin. Such observations support our findings of improved protein digestion in the current experimental birds. Recently, the work of Yokhana *et al*. (2016) corroborated results showing increased trypsin activity as well as proteolytic activity of the pancreas. Furthermore, they reported significant increases in both pepsin and intestinal aminopeptidase activities in layer pullets offered control diets supplemented with 1.5% levels of dietary Arbocel®. Although the present work did not test gut enzymatic activity, the improved protein and amino acid digestibility coefficients observed are strongly suggestive of improved digestion and assimilation of nutrients (Boguslawska-Tryk, 2005; Yokhana *et al*., 2016).

The total numbers of eggs produced in the second experiment per housed hen with a breakdown into various categories, along with percent hen mortality during the six month experimental period are presented in Table 6. All data collected over a six month experimental period were not significantly different between the two treatments. Lignocellulose dietary supplementation for a six month period had the tendency to increase the total number of eggs by 2.68% and hatching eggs by 3.60% which correspond to 3 and 3.8 more eggs per housed hen, respectively. Similarly, a non-significant increase of 1.33 and 2.30% in hen-day egg production was reported by Inchaoren and Maneechote (2013) when H & N Brown Nick hens were offered, for a 12 week feeding trial, 3 and 6% dietary white rice hull as a source of insoluble fiber, respectively. On the other hand, Lim *et al*. (2013) reported a significant increase in egg production by 3.43% in Dekalb layers fed a dietary insoluble raw fiber concentrate for 16 weeks. Therefore, the lack of statistical significance in the effect of lignocellulose on egg production of Ross 308 broiler breeders in the present work and the results of Inchaoren and Maneechote (2013), as opposed to the results of Lim *et al*. (2013), may have resulted from differences in the type of birds used in these experiments and/or the period of the feeding trials as indicated above.

Hatchery data on sampled eggs in terms of percent hatchability and infertility, collected on a monthly basis throughout the experiment are summarized in Table 7. Percent infertility in the Arbocel® treatment group was consistently lower than that of the control in every month the eggs were sampled, but statistical significance ($P<0.05$) was only observed 3 months out of the six. A significant decrease in infertility was observed in the second, third, and fourth months of the trial, whilst the differences were lower but not significant during the remaining three months of the investigation. On the other hand, percent hatchability of sam-

Table 4. **Effect of Arbocel® on apparent dietary amino acid digestibility coefficients[1] (%) of diets and that of essential amino acids**

Treatment	Control	Control + Wheat Bran	Control + Arbocel®	SEM[2]
Aspartate	72.8[b]	72.1[b]	78.5[a]	1.43
Threonine	58.8[ab]	58.6[b]	65.7[a]	2.60
Serine	61.4	60.1	66.4	2.10
Glutamate	78.3[b]	78.0[b]	82.6[a]	1.14
Proline	69.6	70.3	73.3	1.61
Alanine	65.4[b]	66.1[b]	72.6[a]	1.81
Cystine	48.5	48.5	54.6	2.39
Valine	63.5[b]	62.6[b]	71.0[a]	2.16
Methionine	80.8[b]	81.1[b]	87.6[a]	1.17
Isoleucine	68.3[b]	67.3[b]	75.0[a]	1.87
Leucine	73.4[ab]	73.2[b]	78.5[a]	1.59
Tyrosine	67.0[ab]	66.5[b]	73.4[a]	1.87
Phenylalanine	73.2[ab]	72.5[b]	78.4[a]	1.61
Lysine	76.5[b]	74.3[b]	81.9[a]	1.32
Histidine	76.3[b]	75.9[b]	81.6[a]	1.60
Arginine	77.1[b]	76.5[b]	81.6[a]	1.32
Tryptophan	81.1[ab]	79.8[b]	83.9[a]	1.21
Essential amino acids	72.9[b]	71.8[b]	78.5[a]	1.60

[ab,] Means in the same row with no common superscripts differ significantly ($P<0.05$).
[1,] There were 9 individual roosters (replicates) per treatment
[2,] Pooled standard error of means.

Table 5. **Effect of Arbocel® on true dietary amino acid digestibility coefficients[1] (%) of diets and that of essential amino acids**

Treatment	Control	Control + Wheat Bran	Control + Arbocel®	SEM[2]
Aspartate	85.7[b]	86.0[b]	91.5[a]	1.43
Threonine	85.2[ab]	85.0[b]	92.3[a]	2.60
Serine	86.7	86.6	90.8	2.10
Glutamate	88.0[b]	88.5[b]	92.5[a]	1.14
Proline	86.8	88.0	90.8	1.61
Alanine	83.6[b]	84.9[b]	90.9[a]	1.81
Cystine	90.5	87.7	90.5	2.39
Valine	84.9[b]	86.0[ab]	92.4[a]	2.16
Methionine	89.7[b]	90.4[b]	94.9[a]	1.17
Isoleucine	85.4[b]	86.0[ab]	92.1[a]	1.87
Leucine	86.8[b]	87.2[ab]	92.1[a]	1.59
Tyrosine	89.2[b]	89.9[ab]	96.2[a]	1.87
Phenylalanine	87.4[b]	87.7[ab]	92.8[a]	1.61
Lysine	86.0[b]	85.9[b]	91.7[a]	1.32
Histidine	86.8[b]	86.9[ab]	92.1[a]	1.60
Arginine	89.5[b]	90.2[ab]	94.5[a]	1.32
Tryptophan	90.2[ab]	89.0[b]	93.6[a]	1.21
Essential amino acids	87.2[b]	87.2[b]	93.0[a]	1.60

[ab,] Means in the same row with no common superscripts differ significantly ($P<0.05$).
[1,] There were 9 individual roosters (replicates) per treatment
[2,] Pooled standard error of means.

Table 6. **Average number of eggs produced per grade per housed hen (HH) and cumulative hen mortality (%) for control and Arbocel® treatments[1] during the six month period of the second experiment There were 3 breeder houses (replicates)/ treatment with 4330 hens and 433 roosters per house**

	Treatment		
Egg Grade and number per HH	Control	Arbocel®	SEM[2]
Total	111.8	114.8	1.52
Hatching	105.6	109.4	1.56
Table	3.5	3.3	0.16
Large	0.5	0.5	0.05
Cracked	1.3	0.8	0.21
Discarded	0.9	0.8	0.06
Cumulative Hen Mortality (%)	11.4	9.4	1.31

[1,] There were 3 breeder houses (replicates)/ treatment with 4330 hens and 433 roosters per house.
[2,] Pooled standard error of means.

Table 7. **Percent hatchability and infertility of eggs sampled[1] from broiler breeder hens fed control and Arbocel® diets over the six months trial**

	Collection period (month)					
	First	Second	Third	Fourth	Fifth	Sixth
	Hatchability (%)					
Control	73.0[b]	71.8[b]	74.2[b]	66.7[b]	62.9[b]	58.0[b]
Arbocel®	77.1[a]	77.7[a]	76.9[a]	71.1[a]	66.3[a]	62.1[a]
SEM[2]	1.63	0.90	0.60	1.40	0.66	0.78
	Infertility (%)					
Control	7.21	6.52[a]	6.54[a]	4.59[a]	3.53	2.32
Arbocel®	6.32	5.73[b]	4.41[b]	2.88[b]	3.31	2.12
SEM[1]	0.891	0.232	0.358	0.246	0.114	0.130

[ab,] Within a column, for each criterion, averages with no common superscripts are significantly different ($P<0.05$).
[1,] Each month, a total of 864 eggs were sampled from each house. There were 3 breeder houses (replicates)/treatment with 4330 hens and 433 roosters per house.
[2,] Pooled standard error of mean

pled eggs was continuously greater by an average of 4.07% ($P<0.05$) in Arbocel® fed birds than in control birds every month of the trial. The greatest individual difference (Table 7) being observed during the second month, where hatch proportion of eggs of fowl fed the Arbocel® was 5.9% greater than in the control, whereas the lowest significant difference was seen during the third month at 2.7%. The current findings agree well with those of Mohiti-Asli *et al*. (2012) who reported an increase in egg fertility rate in broiler breeders fed dietary inulin or cellulose at a rate of 3%. The beneficial aspects of lignocellulose in improving the performance of the breeder hens under investigation in terms of increased total egg production and number of hatching eggs accompanied by a reduction in average mortality rate among the breeders and a reduction in infertility accompanied by an increased rate of hatchability in addition to increased broiler carcass yield (Farran *et al*., 2013), could be attributed to the increased protein and amino acid digestibility observed in the current rooster study (Tables 3, 4 and 5), and improved nutrient absorption reported by Hetland and Svihus (2001). In addition, the improvement in fertility and hatchability observed in the breeder's trial as a result of feeding lignocellulose may be explained by having a dryer litter (Farran *et al*., 2013) in Arbocel houses which could have positively reflected on the health of birds, especially the males that could have less feet problems, which was not evaluated in the current trial, thus allowing them to mate more frequently with females.

As previously mentioned, all hatching eggs produced during this trial were labeled by treatment, set in the same incubator and their hatching proportion recorded. An average increase in hatchability of 2.81% in the Arbocel® treatment as compared to the control WB treatment was observed during the 6-month trial. Consequently, taking into consideration the 3.8 increase in hatching egg production along with the 2.81% increase in egg hatchability, one can con-

Table 8. **Economics of using Arbocel® in broiler breeder diets taking into consideration the performance results obtained and the current market price in USD ($) of feed ingredients and day-old chicks**

Variables	Control	Arbocel®	Difference
Hatching Eggs in 6 months/Hen Housed	105.6	109.4	+ 3.8 eggs
Hatchability of Total Eggs (%)	68.7	71.5	+ 2.81%
Number of Chicks Hatched/Hen Housed	72.6	78.3	+ 5.7 chicks
Cumulative feed consumed in 6 months (kg/Hen Housed plus 10% of feed consumed per male)	31.65	31.65	0
Cost of feed consumed in 6 months ($)	11.10	11.65	+ 0.55
Revenues from the extra 5.7 chicks with a market price of 0.50 $ per chick ($)	0	2.85	+ 2.85
Net profit from chick sale in 6 months ($/Hen Housed)			+ 2.30

clude that 0.8% dietary Arbocel® resulted in 5.7 more saleable chicks (Table 8) per housed hen during a 6 month period. Considering the market price of a day-old chick is $0.5 (USD) and market price of Arbocel® is 1.60 $/kg, then a net profit of 2.30 $ per housed hen is obtained in this commercial operation.

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