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Use of *Saccharomyces cerevisiae* Cell Walls in Diets for Two Genetic Strains of Laying Hens Reared in Floor and Cages

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Abstract: Two trials were carried out to evaluate the productive response in Bovans white hens housed in cages and Isa Brown hens reared in floor fed with sorghum + soybean meal supplemented with *Saccharomyces cerevisiae* Cell Walls (CW) growth promoters or zinc bacitracin in the diets. In the first one, 216 Bovans hens, 45 weeks old, reared in pens, were allocated in a completely randomized design in three treatments with 6 replicates of 12 hens each one. In the second one, 600 Isa Brown hens, 43 weeks old, were used and allocated in floor with straw litter and three treatments with 4 replicates of 50 hens each one. For both trials, the following treatments were used: 1.- Diet without growth promoter, 2.-As 1 + Zinc bacitracin (30 ppm), 3.- As 1 + CW (500 ppm). Water and feed were given *ad libitum*. Feed intake records, egg production, egg weight, egg mass per bird per day, dirty egg and feed conversion ratio, were taken during 14 weeks. At the end of the trials, to the variables above mentionate, an analysis of time repeated observations was carried out. Results indicated for Trial 1, difference among treatments ($p<0.05$), with higher percentage of dirty eggs the treatment without promoter. In Trial 2, there was better egg production, feed conversion ratio, egg mass and less dirty eggs with CW, being these results similar to those of Zinc bacitracin treatment ($p<0.05$) and higher than treatment without promoter. The results obtained show a promoter effect on the production of hens reared in floor, when CW or zinc bacitracin were included in the diet.

Key words: Laying hen, S*accharomyces cerevisiae* cell walls, production system, zinc bacitracin

INTRODUCTION

Growth Promoter Antibiotics (GPA) have been used in chicks feeding as additives for more than 50 years ago; administration of low doses for long periods of time create ideal conditions for resistance induction (Jones and Ricke, 2003). Since 2006, GPA in animal diets has been banned in the European Community; this obliges to look for natural alternatives for GPA, such as prebiotics and probiotics (Patterson and Burkholder, 2003).

Yeasts and *Saccharomyces cerevisiae* cell walls are found among probiotics and prebiotics, respectively, which have been approved as safe microorganisms for animal feeding within the European Union and the FDA has granted the grade of safe microorganism or GRAS (Generally Recognized As Safe) grade (Nitta and Kobayashi, 1999). *Saccharomyces cerevisiae* yeast Cell Walls (CW) can represent from 10-25% of the total dry matter of the cell depending of the growth conditions. The oligosaccharide percentage in CW is 85-90% and the rest 10% or 15% is protein (Swennen *et al*., 2006).

In studies where different species of yeasts were evaluated, 26-32% values of cell wall dry matter were found, observing differences according to the yeast species (Nguyen *et al*., 1998). It has been estimated that the polysaccharide percentage that the yeast cell wall may contain can be about 85-90% and 10-15% of proteins. At structural scale, the yeast cell wall is constituted by three groups of polysaccharides: 1) mannose and mannoprotein polymers, 2) glucose polymers or $-glyucanes and 3) N-acetylglucosamine polymers or chitin (Aguilar-Uscanga and Francois, 2003; Klis *et al*., 2006).

It can be said that although the construction of the yeast cell wall is firmly controlled by yeast, the polysaccharides composition, structure and thickness, greatly depend on the imposed environmental conditions inside the fermenters, the cell's life cycle and the strain's origin (Aguilar-Uscanga and Francois, 2003).

Osborn and Khan (2000) and Kocher (2005) reported that $-glucans and Mannanoligosaccharides (MOS) are molecules that have vital functions in communication processes at intestinal and immune system scale. The benefits observed with the addition of MOS to birds diet, show to be similar to the obtained with GPA in productive and animal health parameter improvements (Pettigrew, 2000; Hooge, 2004).

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In tests performed in broiler chicks adding MOS to feed, results indicate that the use of MOS in feed represented improvements with regard to negative controls (without MOS) (Pettigrew, 2000; Hooge, 2004). The effects shown by the use of MOS include: increments in productive indexes, greater resistance to bacterial infections, lower mortality and modification of abdominal fat in chicks. In laying hens, the use of MOS showed greater productivity and better egg quality (Haugh units) (Dimovelis *et al*., 2004). With these backgrounds, the aim of this work was to evaluate the growth promoter effect of *Saccharomyses cerevisiae* cell walls in contrast to a GPA, in two egg production systems (floor and cage) and two genetic strains.

MATERIALS AND METHODS

Two trails were carried out simultaneously; in the first one, 216 white Bovans hens, 45 weeks old and 28 weeks in production were housed in a natural environment layer house in pyramidal type cages arrangement. The birds were allocated in three treatments and each treatment counted with 6 replicates of 12 birds each one. Water and feed were given *ad libitum* during all the trial.

In the second trial, 600 red Isa Brown hens, 43 weeks old and 25 weeks in production were reared in 12 pens, in a natural environment pen with straw litter. Birds were allocated in three treatments with four replicates of 50 birds each one (4 birds/m^2). Water and feed were given *ad libitum* during all the trial. Treatments or experimental diets in the two trials were as follows:

Treatment 1 = Diet without growth promoter (control).
Treatment 2 = As 1 + zinc bacitracin[3] (30 ppm).
Treatment 3 = As 1 + *Saccharomyces cerevisiae* cell walls[4] (500 ppm).

The diet used in both trials was sorghum + soybean meal type (Table 1). In each trial, weekly records were taken during 14 weeks of egg production, average egg weight, feed intake, egg mass, feed conversion ratio, dirty egg percentage, thin-shelled eggs percentage, soft-shelled eggs percentage and blind eggshells. At half way and end of trial, shell thickness (mm), egg yolk pigmentation using a DSM colorimeter and Haugh unities were measured, at 5 eggs per replicate of Trial 1 and 10 eggs per replicate of Trial 2.

At the end of the study of each trial, a statistical analysis was done to the obtained variables of the productive parameters according to a complete randomized design with time repeated measurements, using the computer pack of the University of Nuevo Leon see. 2.5 and the difference between means were analyzed by Tukey test with a $p<0.05$.

Table 1: Basal diet composition without promoters used for hens

Ingredients	Kgs.
Sorghum grain	565.95
Soybean meal 48%	269.10
Calcium carbonate	99.59
Vegetable oil	38.21
Dicalcium phosphate	16.49
Salt	4.65
DL-Methionine	1.79
Vitamins/minerals premix*	1.50
Yellow and red vegetable pigment	1.20
HCl L-lysine	0.87
Choline chloride 60%	0.50
Antioxidant	0.15
Total	1000
Calculated analysis	
ME (Kcal/Kg)	2,850
Crude protein (%)	17.90
Lysine (%)	1.00
Methionine + cystine (%)	0.75
Threonine (%)	0.71
Total calcium (%)	4.00
Available phosphorus (%)	0.44
Sodium (%)	0.19

*The vitamin and mineral premix contained per kg: Vitamin A 10 000 IU, Vitamin D^3 2 500 IU, Vitamin E 0.280 I.U, Vitamin K 2.5 g, Thiamine 1.6 g, Riboflavin 5 g, Cyanocobalamin 0.01 g, Fólic acid 0.50 g, Pyridoxine 1.5 g, Calcium pantothenate 10 g, Niacin 30 g, Iron 40 g, Manganese 80 g, Copper 10 g, Iodine 2 g, Zinc 60 g, Selenium 0.30 g

RESULTS

Table 2, shows the average data from the studied variables in Trial 1. It is observed that there was no difference among treatments ($p>0.05$) for egg production, feed intake, feed conversion ratio, thin-shelled egg percentage, soft-shelled egg percentage, blind eggshell percentage, Haugh units, egg shell thickness and yolk color with DSM colorimeter.

Nevertheless, it is shown that egg weight was greater ($p<0.05$) with the addition of zinc bacitracin promoter. It can also be observed that the percentage of dirty eggs significantly lowered with the addition of cell walls and zinc bacitracin ($p<0.05$).

Table 3 shows the average results for Trial 2. There were no significant differences among treatments ($p>0.05$) for the following variables: Feed intake, thin-shelled eggs percentage, soft-shelled egg percentage, blind eggshell percentage, shell thickness and yolk color with DSM colorimeter. Differences among treatments ($p>0.05$) were present in egg production, egg weight, feed conversion ratio, egg mass (bird/day), dirty egg percentage and favorable Haugh units with the addition of zinc bacitracin and cell walls.

DISCUSSION

Since some nutrients are directly obtained from the metabolites of bacteria, the luminal mucosa cell interchange rate is also favored by the administration of antibiotics or prebiotics, by reduction or modification of

Table 2: Average results in Bovans white hens fed with or without growth promoter antibiotics and yeast cell walls (Trial 1)

Variables	Control	Zinc bacitracin	Cell walls
Egg production %	92.6±0.85[a]	91.7±1.39[a]	91.2±1.81[a]
Egg weight (g)	61.0±0.41[b]	61.8±0.28[a]	61.3±0.49[b]
Feed intake (g)	112.8±0.51[a]	111.6±1.24[a]	112.2±2.16[a]
Feed conversion ratio(kg:kg)	2.00±0.02[a]	1.97±0.03[a]	2.01±0.03[a]
Egg mass/day (g)	56.4±0.62[a]	55.6±0.87[a]	55.8±1.28[a]
Thin-shelled egg (%)	0.3±0.24[a]	0.4±0.14[a]	0.6±0.37[a]
Dirty egg (%)	4.9±0.50[a]	3.3±0.65[b]	2.6±0.99[c]
Soft-shelled egg (%)	0.3±0.24[a]	0.3±0.13[a]	0.6±0.39[a]
Blind eggshell (%)	6.9±0.93[a]	8.0±0.77[a]	9.4±1.20[a]
Haugh Units	92.0±1.92[a]	96.0±0.90[a]	93.2±2.09[a]
Yolk color*	7.8±0.07[a]	8.3±0.08[a]	8.6±0.11[a]
Thickness (mm)	0.341±0.004[a]	0.347±0.004[a]	0.335±0.004[a]

Values with different letter are statistically different (p<0.05). Average ± standard error. *DSM colorimeter

Table 3: Average results of Isa Brown hens fed with or without growth promoter antibiotics and cell walls (Trial 2)

	Control	Zinc bacitracin	Cell walls
Egg production %	92.9±0.53[b]	96.16±0.73[a]	96.27±0.73[a]
Egg weight (g)	63.00±0.21[b]	63.56±0.18[a]	62.54±0.33[c]
Feed intake (g)	122.58±0.91[a]	124.33±1.15[a]	122.37±0.70[a]
Feed conversion ratio (kg:kg)	2.08±0.01[a]	2.04±0.01[b]	2.03±0.01[b]
Egg mass/day (g)	58.52±0.22[b]	61.13±0.63[a]	60.43±0.44[a]
Thin-shelled egg (%)	0.34±0.03[a]	0.27±0.07[a]	0.21±0.04[a]
Dirty egg (%)	8.66±1.0[a]	4.58±0.69[b]	3.62±0.39[b]
Soft-shelled egg (%)	0.04±0.02[a]	0.01±0.00[a]	0.03±0.01[a]
Blind eggshell (%)	4.22±0.31[a]	3.13±0.68[a]	1.91±0.47[b]
Haugh units	92.6±2.33[b]	87.0±1.13[a]	92.68±2.33[b]
Yolk color*	9.2±0.1[a]	8.8±0.2[a]	9.1±0.1[a]
Thickness (mm)	0.366±0.003[a]	0.374±0.004[a]	0.373±0.004[a]

Values with different letters are statistically different (p<0.05). Average ± standard error. *DSM colorimeter

the microflora (Donoghue, 2003); therefore, it has been demonstrated that MOS and glucans present in cell walls, decrease enteropathogenic bacteria that prevent increase the beneficial bacterial flora control (Santin *et al*., 2003).

Santin *et al*., 2001; Arce *et al*., 2008, found that by using CW, the length and number of intestinal villi increased; by having better intestinal health, the nutrients provided by the diet in both trials were absorbed, digested and distributed to the tissues in a suitable way; therefore, the use of bacitracin zinc or yeast cell walls in this study were production promoters being more effective in Trial 2 carried out in floor. This effect can be explained by the observations of Gomez *et al*. in 2009, who found an increase in the cellular and humoral response in chickens fed CW in a dirty environment. The use of CW can have a greater response in laying hens reared in floor than housed in cages.

In a similar way to other new additives, the action mechanisms of probiotic microorganisms and prebiotic substances are only half known. According to different researches, the action mechanisms that these additives can exert in the host digestive tract include the next effects: competition for bacterial substrate and places, compound production that inhibit pathogen microorganisms growth, pathogen bacteria colonization reduction, bacterial population modification, immune system modification, ammonium, skatole, indole, p-cresol and phenol reduction (Waldroup *et al*., 2003).

Products derived from yeast cells (*S. cerevisiae*) are known as extracts or yeast autolysates and yeast cell walls, products obtained from complete yeast cell autolysis. In the animal alimentary field, since the past decade, the interest on using yeast cell walls fractions as source of polysaccharides type $-glucans and Mannanoligosaccharides (MOS) has been increased. These types of polysaccharides are known as natural additives capable of exerting beneficial effects on the health and productivity of the individual (Hooge, 2004).

The benefits observed by the addition of CW in the diet, show to be similar to the obtained with GPA as in this study. This situation could suggest that these type of additives can represent a real tool to increase the productive efficiency of the bird when GPA are not present in feed. The MOS supplementation in diets for pigs and chickens has reported benefits in terms of productive and animal health parameters (Pettigrew, 2000; Hooge, 2004).

In laying hens, the use of MSO showed greater production and better egg quality (Haugh units) (Dimovelis *et al*., 2004) as observed in this research. Gracia *et al*., in 2004, while using MOS found better production behavior and egg yolk color in IsaBrown hens, similar response to the one in this research; nevertheless, in this research, no color response was shown like the one found by Gracia *et al*. (2004).

Fairchild *et al*. (2001), found a decrease in *E. coli* when using MOS or flavomicin and weight gain increased in hens.

From the obtained results and under the experimental conditions used, it can be conclude that the addition of S*accharomyces cerevisiae* cell walls in laying hens diet based on sorghum + soy meal are alternative to growth promoter antibiotics.

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