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Short communication

Productive performance and cecal microbial counts of floor housed laying hens supplemented with dry whey powder alone or combined with Pediococcus acidilactici in the late phase of production

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ABSTRACT

Probiotics, prebiotics, and synbiotics have been proposed as safe additives in animal feeding. The purpose of this study was to assess the effect of supplementing corn-soybean diets of laying hens with dry whey powder (prebiotic), *Pediococcus acidilactici* (probiotic), and the combination of both (synbiotic) on the productive performance, egg quality traits, and cecal microbial counts. A total of 300 laying hens, 57 wk of age, were randomly allocated to floor pens for 70 d. Pens were assigned to 1 of 4 experimental diets with 5 pens per treatment and 15 laying hens per pen. The experiment consisted of a 2×2 factorial arrangement of treatments with 2 levels of inclusion of dry whey powder (WP, 0 and 60 g/kg of diet) and 2 levels of *P. acidilactici* (PA, 0 and 2 g/kg of diet). Cecal counts of *Bifidobacterium* spp. were increased with the addition of WP (8.4 vs. 6.5 $\log_{10}$ cfu/g cecal content, P=0.012). An interaction between levels of WP and PA was found on egg production (P=0.008) and on cecal counts of *Clostridium perfringens* (P=0.047), so that the addition of WP increased egg production (82.5 vs. 75.6%) and reduced *Clostridium perfringens* colony counts (4.3 vs. 5.8 $\log_{10}$ cfu/g cecal content) only when PA was not used. In conclusion the joint addition of WP and PA in hens' diets during the late stage of production did not improve productive performance or change the cecal microbial population. However, the addition of WP increased *Bifidobacterium* spp. cecal counts and only reduced the *Clostridium perfringens* counts together with an increase on egg production, when PA was not added.

1. Introduction

It is well known that nutrition, age, and health status, as well as housing system, are key factors influencing the productive performance of laying hens (Ahmadi and Rahimi, 2011). Their health status, egg production, and quality will decrease after the laying peak (Liu et al., 2013). Because the use of medication is being minimized to avoid potential residues in eggs, producers rely on nutritional measures to improve the persistency on egg production and the resistance against intestinal disorders (Lensing et al., 2012; Yörük et al., 2004). As a consequence, the use of prebiotics, probiotics, and synbiotics in diets could be a safe alternative to improve animal performance and health (Janczyk et al., 2009; Patterson and Burkholder, 2003). Whey is a co-product of cheese-making process, with lactose being its major component (about 70% of dry matter; Aghaei et al., 2010). Lactose can be used as a prebiotic in non-mammalian animals, because they lack the enzyme lactase (Allaart et al., 2013). Lactose is not digested, and is thus fermented by the cecal microflora, which could decrease pH, promote lactic-acid bacteria growth, and suppress pathogenic bacteria (Gülşen et al., 2002; Van Der Wielen et al., 2002). Probiotics, such as *Pediococcus acidilactici*, are beneficial live micro-organisms that confer health benefits to the host mainly through the regulation of the intestinal microbial homeostasis (Gaggìa et al., 2010). Synbiotic is known as the combination of probiotics and prebiotics. Prebiotics beneficially affect the host because improve the survival and implantation of probiotics in the gastrointestinal tract (Awad et al., 2009).

We hypothesize that the beneficial effect of *Pediococcus acidilactici* could be enhanced by the simultaneous addition of dry whey powder so as to obtain additional benefits beyond those achieved when provided alone. Therefore, the purpose of this study was to assess the effect of diets supplemented with dry whey powder (prebiotic), *P. acidilactici* (probiotic), or their combination (synbiotics), on the productive performance, egg quality traits, and cecal microbial populations of

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floor-housed laying hens during the late phase of production.

2. Materials and methods

2.1. Animal housing

The experiment followed the European Union (2010/63/EU) and Spanish regulations (RD 53/2013) for animal experimentation, and was conducted at the experimental facilities of Neiker-Tecnalia (Vitoria-Gasteiz, Spain). A flock of 300, 57 wk-old hens (ISA Brown strain, Avigán Terralta S.A, Tarragona, Spain) with uniform body weight (2,035.4 ± 52 g) was used in an experiment lasting 70-d. Hens were randomly allocated, in groups of 15, to 2.5 m^2 floor pens with wood shavings. They had been fed with the same commercial diet, and had been subjected to the light program established by commercial guidelines previous to the experiment (ISA, 2010).

2.2. Experimental diets

Pens were randomly assigned to 1 of 4 experimental treatments, each with 5 replicates, consisting in 4 dietary treatments: no supplementation of dry whey powder (WP) or *P. acidilactici*/kg of diet (PA), inclusion of 60 g of WP/kg of diet, 2 g of PA/kg of diet, or a mixture of 60 g of WP and 2 g of PA/kg of diet. Dry whey powder was a commercial sweet powder (Sueromancha S.L, Toledo, Spain; 703 g of lactose/kg of product). The commercial probiotic (Bactocell, Lallemand, France) contained a live culture of *P. acidilactici* (strain MA 18/5, 10^{10} cfu/g). All experimental diets were formulated to meet laying hens' requirements (FEDNA, 2008), with ingredients and composition shown in Table 1. Feed and water were provided *ad libitum* and the light cycle program was 16 L:8D throughout the experiment.

Table 1
Dietary ingredients and composition of the experimental diets (as-fed basis).

Item	PA[a] (g/kg)	0	0	2	2
	WP[b] (g/kg)	0	60	0	60
Ingredients (g/kg)					
Yellow corn		413	455	409	451
Soybean meal		252	250	253	251
Wheat		100	100	100	100
Barley		100	0	0	0
Soybean oil		23.1	25.1	24.4	26.3
Dicalcium phosphate		17.5	15.8	17.5	15.8
Sodium chloride		3.3	2.2	3.3	2.2
Vitamin-mineral premix and pigments[c]		6	6	6	6
Other components[d]		85.2	85	85.2	85
Chemical composition					
AMEn[e], MJ/kg		11.5	11.5	11.5	11.5
Crude protein, g/kg		173	173	173	173
Ca, g/kg		38	38	38	38
Available P[f],g/kg		3.7	3.7	3.7	3.7

[a] PA=*Pediococcus acidilactici*. Bactocell (strain MA 18/5, 10^{10} cfu/g; Lallemand, Blagnac, France).
[b] WP=dry whey powder. Dry sweet powder (703 g of lactose/kg of product; Sueromancha S.L, Toledo, Spain).
[c] Providing the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3, 1,600 IU; vitamin E, 16 mg; thiamine, 1 mg; riboflavin, 3 mg; pyridoxine, 1 mg; vitamin B_{12}, 0.01 mg; vitamin K, 1 mg; pantotenic acid, 7 mg; nicotinic acid, 16 mg; Mn, 70 mg; ZnO, 50 mg; Fe ($FeSO_4$ H_2O), 30 mg; Cu ($CuSO_4$ $5H_2O$), 4 mg; I (KI), 1 mg; Co, 0.2 mg; Se (Na_2SeO_3), 0.1 mg; CL, 240 mg; phytase, 300 units; ethoxyquin, 110 mg; xanthine, 0.6 mg, and canthaxanthin, 0.4 mg.
[d] Providing the following per kilogram of diet: L-Methionine, 1.2; Sodium bicarbonate, 0.6; Calcium carbonate, 83.4.
[e] AMEn: Apparent metabolizable energy corrected by N, calculated according to FEDNA (2010).
[f] Calculated value.

2.3. Sample collection

The feeder from each pen was weighted weekly to calculate feed intake. Total eggs produced per pen were recorded daily to calculate egg production. Last day of the experiment, 3 hens per treatment were randomly selected and slaughtered by CO_2 inhalation. The cecal content was collected from each hen for bacterial counts.

2.4. Calculations and measurements

Feed intake was calculated as the difference between initial and final feeder weight. Feed conversion ratio (FCR) was expressed as the amount of feed (kg) to produce 1 kg of eggs or 12 eggs. Egg measurements started 2 wk after the beginning of the experiment. Egg production was calculated weekly as described by Ajakaiye et al. (2010). Eggs laid during the last day of each week were individually weighed and graded according to European Commission (2008). Egg quality traits were measured on 12 eggs per pen laid on three consecutive days. Measurements of egg weight, shell thickness and albumen height were made according to Keener et al. (2006). Yolk index was calculated as the ratio of yolk height to yolk diameter, while egg-shape index was calculated as the ratio of egg width to egg length. Haugh units score were estimated using the formula Haugh=100×log (T−1.7× $W^{0.37}$+7.57), where H=height of the albumen (mm) and W=egg weight (g). *Escherichia coli* culture and counts were determined on chromogenic medium (ChromID coli, BioMérieux, France) and *Clostridium perfringens* on tryptone sulphite neomycine agar (Scharlab, Spain). *Bifidobacterium* spp. and *Lactobacillus* spp. were cultured and enumerated on the man, rogosa and sharpe agar (Becton, Dickinson and Company, New Jersey, USA) according to O'Sullivan et al. (2011).

2.5. Statistical analysis

Pen was considered the experimental unit. Data were analyzed considering a 2×2 factorial arrangement of treatments using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, US). The results were considered significant if $P < 0.05$.

3. Results

No deaths occurred during the experiment. Results relative to productive performance, egg quality traits and cecal microbial counts are shown in Table 2. An interaction between levels of WP and PA was found on egg production (P=0.008) and on cecal counts of *Clostridium perfringens* (P=0.047), so that the addition of WP increased egg production (82.5 vs. 75.6%) and reduced *C. perfringens* (4.3 vs. 5.8 $\log_{10}$ cfu/g cecal content) only when PA was not used. Cecal counts of *Bifidobacterium* spp. was increased with the addition of WP (P=0.012). However, these microbial results should be viewed with caution because of the reduced number of replicate pens with 1 hen per pen. The remaining performance results, including egg quality traits, and cecal counts were not affected by the evaluated levels additives.

4. Discussion

To the best our knowledge no studies until today have reported the joint use of WP and PA in poultry diets. The mixture of WP and PA did not result in a synergic effect leading to a positive modulation of cecal bacteria and to better performance, as previously reported with different mixtures of prebiotics and probiotics in poultry diets (Gaggìa et al., 2010). PA are usually found in the gastrointestinal tract of healthy chickens (Ghareeb et al., 2012), and thus their adaptation to gut host conditions should not be a problem when is externally supplemented. However, the simultaneous presence of WP could stimulate the development of other bacteria populations that could