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Effect of Dietary Natural Capsaicin on Experimental *Salmonella enteritidis* Infection and Yolk Pigmentation in Laying Hens

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Abstract: This study evaluated capsaicin extracted from chili pepper and its prophylactic effect on *Salmonella enteritidis* (SE) experimental infection, feed conversion, egg production, egg weight and yolk pigmentation in laying hens. Dekalb hens (30/treatment) were fed for 28 days with two different levels (18 and 36 ppm) of dietary capsaicin from paprika oil. Both levels (18 and 36 ppm) of dietary capsaicin did not affect the feed conversion, egg production or egg weight. At 25 days, hens were challenged with 10^8 cfu mL^{-1} of SE. Three days after inoculation, liver and spleen were collected aseptically and cultured as a combined sample. The higher capsaicin treatment significantly decreased ($p<0.05$) SE organ invasion (43.44%; 13/30) when it was compared with the low capsaicin treatment (56.67%; 17/30) and control group (76.67%; 23/30). Eggs were collected on day 20 of the trial and the yolk pigmentation was measured directly with a chroma meter CR-300 (Minolta) in the CIE*Lab* scale. Both concentrations of dietary capsaicin significantly increased the deposition of red pigment on egg yolk (14.11±1.40 and 17.44±1.90) compared with control group (-1.58±2.65). The results of the present investigation suggest that the natural capsaicin, extracted from paprika seeds at 36 ppm in the diet, had a prophylactic effect on experimental SE infection in laying hens and both concentrations of capsaicin increased red pigmentation of the yolk.

Key words: Capsaicin, *Salmonella enteritidis*, hens and pigmentation

Introduction

The recent increase in the incidence of *Salmonella enteritidis* infection in poultry flocks has been observed in the United States, the United Kingdom and other countries (Dreesen *et al*., 1992). In humans this problem is estimated to occur in approximately 1-2% of the U.S population each year (Potter, 1987). The adherence of bacteria to epithelial mucosal cell surface has been recognized to be of primary importance in the pathogenesis of *Salmonella* and other bacteria enteric pathogens (Droleskey *et al*., 1993). Because *Salmonella spp*. is notoriously resistant to many antibiotics and is capable to rapidly developing resistance when they are exposed (Smith and Tucker, 1975), new tools to avoid its dissemination are necessary.

One of them is the use of capsaicin. Capsaicin, a homovanillic acid derivative (8-methyl-N-vanillyl-6-moneamide), is an irritant and vasoactive component from chili pepper (*capsicum annuum*) and its effect on nerve tissue have been well documented. In addition to transmission of sensory impulses to the central nervous system, capsaicin-stimulated peripheral nerve endings release peptides, which may cause a variety of "local effector" action (Holzer, 1990). Capsaicin-neuron receptor in gut and brain released some peptides such as substance P, Neurokinin A and vasoactive intestinal polypeptide (Holzer, 1990; Rawdon, 1984). These neuropeptides induce local effects on blood flow, vascular permeability, smooth muscle activity, tissue growth and repair, immunologic processes (Croitoru *et al*., 1990; Felten *et al*., 1985) and as protection against gastrointestinal mucosal injury (Sterner and Szallasi, 1999). Furthermore, capsaicin has been reported to protect against gastrointestinal mucosal injuries via an ill-defined afferent neuron mediated mechanism (Holzer, 1990; Maggi, 1990; Szolcsanyi *et al*., 1986). Although neither correlative nor causative investigations of enteric pathogen resistance and these capsaicin-induced changes have been reported, capsaicin clearly has a number of known actions on enteric innervation and function in mammals.

Previous studies with different levels of dietary capsaicin, either natural or synthetic, has demonstrated reductions in *Salmonella enteritidis* organ invasion with no adverse effects on body weight and feed consumption on 11, 16 or 19 day old broilers and leghorn chickens (McElroy *et al*., 1994; Tellez *et al*., 1993). However, there are no reports on the effect dietary

Table 1: Effect of dietary natural capsaicin on feed conversion, egg production and egg weight on laying hens

	Mean±standard deviation		
Treatment	Feed conversion (kg)	Egg production/ group (kg)	Egg weight (g)
Control	2.818±0.179	8.52±0.860	62.67±2.60
Capsaicin 18 ppm	2.881±0.243	8.17±0.839	62.98±2.01
Capsaicin 36 ppm	2.539±0.201	9.05±0.861	63.19±2.20

Table 2: Effect of dietary natural capsaicin on *Salmonella enteritidis* organ invasion in laying hens

	No. positive/ total (%)
Treatment	Liver-spleen
Control	23/30 (76.67)
Capsaicin 18 ppm	21/30 (56.67)
Capsaicin 36 ppm	13/30 (43.33)*

*Values followed by asterisks are significantly different ($p<0.05$) from the control values

natural capsaicin in laying hens on *Salmonella enteritidis* organ invasion and on yolk pigmentation. The objectives of the present study were to evaluate the effect of natural capsaicin added in the diet on *Salmonella enteritidis* infection, yolk pigmentation, feed consumption, egg production and egg weight in laying hens on second cycle.

Materials and Methods

Salmonella: A serotype of *Salmonella enteritidis* was selected for resistance to novobiocin-nalidixic acid (NO/NA) and maintained on nutrient agar. Medium used to culture the resistant isolate in experimental studies contained 25 µg novobiocin/mL and 20 µg nalidixic acid/mL to inhibit the growth of other bacteria. Challenge innoculum for oral gavage was prepared in sterile phosphate-buffered saline. The viable cell concentration of the inoculum was determined by colony counts on brilliant green agar (BGA) plates.

Capsaicin: Capsaicin[1] was extracted from seeds and pericardium of the different kinds of chili peppers (*Capsicum sp.*) and suspended in oil. This product contained 24,475 scoville heat units of capsaicin and 3.457 grams of total xanthophyll (red and yellow pigments) per kilogram.

Experimental design: Ninety one-hundred-twenty week old Dekalb hens were obtained from a commercial farm and placed randomly in individual cages located in the Avian Department of the Instituto Nacional de Investigacion Pecuaria y Forestales (INIFAP-Mexico) divided in three groups (two replicates each one). Fifteen days before the experiment started, all hens received a pigment-free balanced unmedicated sorghum and soybean diet and water *ad libitum*. Before use, the feed ration was cultured for salmonellae using a standard culture method (Andrews *et al.*, 1978) and was found to be negative. On day 1 of the experiment, the experimental diets were administered and fed for 28d. Experimental diets were as follows: 1) Control diet without capsaicin); 2) Dietary capsaicin at 18ppm; 3) Dietary capsaicin at 36 ppm. The diet contained 10ppm of yellow pigment from Marigold flowers (Florafil 93[@]). Yolk pigmentation was performed at day 20 of the experiment using a chroma meter Minolta CR-300[2]. On day 24, hens were transported to the isolation units of Avian Medicine Department of the College of Veterinary Medicine at the Universidad Nacional Autonoma de Mexico, where they were challenged with 10^8 cfu mL^{-1} of *S. enteritidis* three days after arrival. Hens were killed and cultured three days post-challenge according to the National Poultry Improvement Plan (NPIP) guideline (USDA/APHIS, 1989).

***Salmonella enteritidis* organ colonization:** Briefly, specimens of liver and spleen were collected aseptically and cultured as a single combined sample. Organs were incubated for 24 hr at 37°C in tetrathionate broth. After incubation, the broth was agitated and streaked with a flamed loop on BGA plates, incubated for an additional 24 hr at 37°C and examined for the presence of lactose-negative, NO/NA resistant colonies.

Pigment evaluation: At 20 days, 15 eggs per group were collected. Pigment deposition were measured directly on yolk eggs by a Chroma Meter CR-300 in the *CIELab* scale according to the International Commission on Illumination. Variables of lightness, yellow pigments and red pigments were recorded.

Statistical analysis: Culture data were analyzed using chi-square analysis to determine significant differences in organ invasion (Zar, 1984). Significance was reported at $p<0.05$. Differences between groups in the variables of feed conversion, egg weight and pigmentation were determined by one way analysis of variance using the General Linear Models procedure. Significant differences were further separated using Duncan's multiple range test and commercial statistical analysis software (SAS Institute, 1989).

Results and Discussion

In the present study, continued dietary administration of capsaicin did not affect the feed conversion, egg production or egg weight (Table 1). Previous publications also reported that different levels of capsaicin on the diet did not affect the feed consumption

Table 3: Effect of dietary natural capsaicin on deposition of pigments (red and yellow) and lightness on yolk eggs evaluated by using a Chroma Meter Minolta CR-3000 (CIE L*a*b*. CIE 1976)

	Delta units (Chroma meter CR-300)[A]		
Treatment	Lightness	Red pigment	Yellow pigment
Control	65.17±2.54[a]	-1.58±2.65[b]	41.70±1.61[a]
Capsaicin 18 ppm	55.87±1.79[b]	14.11±1.40[a]	37.01±3.68[ab]
Capsaicin 36 ppm	51.58±2.73[c]	17.44±1.90[a]	34.03±4.02[b]

[A]Mean±S.D. *n* =15 yolks egg per group. Value followed by a different letter in the same column is statistically significant (p<0.05)

and the body weight (McElroy *et al*., 1994; Tellez *et al*., 1993). There is no previous information about the effect of capsaicin on egg production and egg weight, but our results indicated that capsaicin does not affect these productive parameters. Previous studies have determined that birds are relatively insensitive to capsaicin as an irritant, as capsaicin has long-term inhibitory effects on sensory neurons (Harti *et al*., 1991; Sann *et al*., 1987). The absence of perceived pain in birds exposed to capsaicin may explain the ready consumption of capsaicin-treated fed in the present study as well as previous publications (Harti *et al*., 1991; Sann *et al*., 1987), so that feed conversion remained unaffected by capsaicin consumption in this study.

The total number of *S. enteritidis*-organ-culture positive was significantly lower (p<0.05) in hens fed with 36 ppm dietary capsaicin compared with controls (Table 2). Previous research indicated that capsaicin-induced resistance to *S. enteritidis*-organ infectivity is associated with an increase in the lamina propria thickness based on morphometric analysis due to an infiltration of mononuclear cells and heterophils (Tellez *et al*., 1993). A possible mechanism for increased resistance to *S. enteritidis* as well as the observed infiltration of inflammatory cells in the intestinal mucosa following oral challenge may involve the release of neuropeptides, such as substance P, by the action of capsaicin (Holzer, 1990; Rawdon, 1984). This substance is contained in the brain and intestinal tissues that modulates the inflammatory and immune responsiveness of the host (Bartho and Holzer, 1985; De Simon *et al*., 1989; Payan *et al*., 1983). Specifically, substance P has been shown to stimulate polymorphonuclear cell chemotaxis, enzyme release from lysosomes, oxidative burst, ingestion of cells by phagocytes and also increases the natural killer cell activity (Croitoru *et al*., 1990; De Simon *et al*., 1989; Fiocchi, 1997). Substance P also acts on the repair processes in inflammatory areas by promoting smooth muscle and fibroblast proliferation, as well as lymphocyte proliferation (Harti *et al*., 1991; Payan *et al*., 1983) and stimulator in the gastrointestinal tract where contracts all muscle layers and stimulate peristalsis (Bengt, 1985). One of the advantages of dietary capsaicin administration is that this compound is not absorbed systemically following dietary consumption (Sann *et al*., 1987; Stanisz *et al*., 1986).

An additional benefit of natural capsaicin is its pigmenting capability (Fletcher and Halloran, 1983). Pigmentation evaluation showed that high capsaicin level increased significantly the deposition of red pigment on the yolk egg as well as the low-level compared to the control (p<0.05). Marusich *et al.* (1960) detected traces of increased pigmentation in egg yolks 48 hours after the addition of pigment to the diet and reached the maximum concentration 8 days after the experiment began. Williams *et al.* (1963) evaluated a diet with red pigment from capsicum and yellow pigment from marigold. Two days after addition of pigment to the diet, the pigment deposition in the yolk was detected at 3 days and the high level was reached at 10 days. Yellow pigment values were higher (p<0.05) in the control group than in either capsaicin-treated group. The level of capsaicin added (high or low) did not result in a difference in yellow pigmentation (Table 3). Lightness of the yolk received the highest value (65.17, p<0.05) in the control group, followed by 18ppm capsaicin (55.87) which was also a higher value (p<0.05) than 36 ppm capsaicin treated hens (51.58). Our results agree with previous research that indicated that an increase in red xanthophyll in the diet increases the red level detected by chroma meter or visually (Fletcher and Halloran, 1981; Fletcher and Halloran, 1981). In this study, both levels (18 and 36 ppm) of dietary capsaicin from paprika oil significantly increased the deposition of red pigment in the egg yolk. Addition of low doses of paprika oleoresin in a diet with yellow xanthophyll from marigold flowers improves the yolk color. Furthermore, paprika peppers are a good source of red pigment such as trans-capsorrubin, trasn-capsantic and yellow pigments like trans-lutein and trans-zeaxanthin, which can have a significant economic impact in countries that require high level of egg yolk pigmentation. The results of the present investigation suggest that the natural capsaicin, extracted from paprika seeds at 36 ppm in the diet, had a prophylactic effect on experimental SE infection in laying hens and did not affect the feed consumption, egg production, or egg weight. Both low and high levels of capsaicin in the diets also improved red pigmentation in the egg yolk.

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[1]Provided by Productos Deshidratados de Mexico S.A. de C.V. Los Mochis Sinaloa, Mexico

[2]Minolta Camera Corp., Meter Division, Ramsey, NJ, USA