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The effects of Silymarin on ovarian activity and productivity of laying hens

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ABSTRACT - In a previous work we evaluated the effects of Silymarin, a natural antioxidant and hepatoprotective polyphenolic compound, on laying hens performances and health status. The aim of the present work was to further confirm the previous results and to investigate the effects of Silymarin on ovarian endocrine activity and productivity of laying hens as well as on egg traits. Eighteen laying hens were randomly allotted into 3 groups and observed for 20 weeks: control hens were fed control diet, while treated groups received supplementations of 200 (S200) and 400 (S400) ppm of Silymarin. S200 group showed improved eggs laying rate (+2.91%), feed conversion rate (-4.52%) and a significant ($P<0.05$) increase of dry matter content (+0.54%), total lipids (+0.72%) and total sterols (0.02%) of the eggs. Any significant difference was shown for Silymarin at the highest dose (400 mg/kg of feed). At the end of the trial the hens were sacrificed and hierarchical follicles were removed and cultured for 48h. Media were assayed for progesterone (P4) and estradiol-17 beta (E2). Average E2 production increased ($P<0.05$) from F5 (follicles with initial development) to F3 (follicles with intermediate development), then decreased ($P<0.05$) from F3 to F1 (pre-ovulatory follicles). Basal P4 production augmented ($P<0.05$) throughout all follicle growth. Both Silymarin treatment inhibited ($P<0.05$) steroidogenesis. Silymarin may act as an endocrine-modulating chemical affecting hen performances.

Key words: Laying hens, Silymarin, Health status, Performance.

Introduction – Silymarin is a vegetal preparation obtained from milk thistle and Cynara cardunculus and is composed of three flavonolignans: silybin, silydianin and silycristin. Silymarin is known for its hepatoprotective properties (Valenzuela and Garrido, 1994; Dehmlow *et al.,* 1996a; Leng-Peschlow, 1996). Moreover it has demonstrated to have antioxidant activity, to inhibit lipid peroxidation (Bosisio *et al.,* 1992), to stimulate liver detoxification (Baer-Dubowska et al., 1998; Halim *et al*., 1997) to prevent hepatocytes glutathione depletion (Campos *et al*., 1989). Silymarin shows anti-inflammatory action (Dehmlow *et al*., 1996a; De La Puerta *et al*., 1996) and promotes the regeneration of liver tissue increasing hepatocytes protein synthesis (Sonnenbichler and Zeti, 1984). Clinical investigation and trials conducted on livestock animals have demonstrated that Silymarin diet supplementation can improve productive performances and health status with particular regard to hepatic function (Tedesco, 2001; Quarantelli *et al*., 2004). In a previous experimental trial (Righi *et al*., 2005) we demonstrated the positive effect of low doses of Silymarin on health status, productive performances and egg quality of laying hens producing eggs for industry egg products. These animals are characterized by low body weight and high productivity. This lead to a considerable metabolic effort that inevitably determines variations of the physical condition often associated with degenerative phenomena of hepatic parenchyma and steatosis. The aim of the present work was to further confirm our previous results and to investigate the effects of Silymarin on ovarian steroidogenesis, in the attempt to contribute to the comprehension of Silymarin pharmacodynamics.

Material and methods - Eighteen laying hens Hy-line® W 36 White Leghorn pullets, 20 weeks old, were employed. Birds were reared in wire cage of three animals each one and were randomly allotted into 3 groups; control hens were fed with basal diet, while a supplementation with 200 and 400 ppm of Silymarin was carried out in group S200 and S400 during the first 20 weeks of productive cycle. During the trial the health status, egg production and hens feed consumption were registered daily for feed conversion rate (FCR) calculation. Egg quality traits were determined monthly on a sample of 6 eggs per group. After 5 months, the hens of each group were sacrificed and hierarchical follicles, classified according to their increasing size from F5/6 to F1, were removed, slit and carefully inverted. Individual follicles were placed into a single well of a 6-well plate and incubated for 48h. At the end, media were collected and assayed for progesteron (P4) and estradiol-17 beta (E2) content by RIA. Statistical analysis were performed using ANOVA (SPSS 15.0).

Results and conclusions - Table 1 and Table 2 report some productive parameters of control and treated groups. Silymarin at the dose of 200 mg/kg improved, even not significantly, egg laying rate (+2.91%), feed conversion rate (-4.52%) and dry matter content (+0.54%). Moreover, it induced a significant increase (P<0.05) in total lipids (+0.72%) and total sterols levels (+0.02%) of the eggs. Any significant difference in eggs quality was found for Silymarin at the highest dose (400 mg/kg of feed). These results are substantially consistent with findings reported by Righi *et al.* (2005).

Table 1. Productive parameters (between 20 e 40 weeks of age – mean values).

Parameters		Control group	S 200	S 400
Egg laying rate	%	89.15 ± 13.57	92.06 ± 9.06	86.37 ± 13.31
Average egg weight	g	51.25±4.52	52.01±4.81	48.14±4.27
FCR (feed/kg egg)	kg	1.99 ± 0.42	1.90 ± 0.24	2.03 ± 0.37
Daily feed consumption	g/hen	99.78	98.24	95.50
Feed/Egg	g	115.23 ± 28.77	107.68 ± 17.41	112.12 ± 19.77
Final Body weight	g	1485.83 ± 96.46	1470.00 ± 125.54	1492.50 ± 88.75
Mortality rate	%	0	0	0

Table 2. Egg quality traits (between 20 e 36 weeks of age – mean values).

Parameters		Control group	S 200	S 400
Dry Matter	%	23.90 ± 1.78	24.44±1.50	23.98 ± 1.90
Crude Protein	%DM	13.33 ± 0.47	13.21 ± 0.70	13.26 ± 0.53
Total lipids	%DM	9.58 ± 1.12b	10.30 ± 0.84a	9.64±1.23b
Sterols	%DM	0.37 ± 0.06b	0.39 ± 0.05a	0.38±0.06b
Yolk colour	ppm	37.81 ± 18.40	38.85 ± 19.94	41.20 ± 17.72
Shell thickness	mm	0.347 ± 0.040	0.351 ± 0.038	0.346 ± 0.040

a, b: P<0.05.

In general, basal E2 production significantly increased (P<0.05) from F5 to F3, then significantly decreased (P<0.05) from F3 to F1. Basal P4 production significantly augmented (P<0.05) throughout the entire follicle growth (Table 3). On average, both Silymarin concentration were able to inhibit steroidogenesis (Table 4). Nevertheless, as summarised in Table 4, estradiol-17 beta (E2) secretion was more depressed at the lower dose of Silymarin supplementation but the difference did not appear significant. This behaviour led to an higher ratio between P4 and E2 in medium of S 200 explanted

Table 3. *In vitro* ovary explants hormonal secretion.

Parameter/Follicle	Control	S 200	S 400
Progesteron P4 (ng/ml)			
F5	3.23 ± 3.50[c]	3.21 ± 3.32[c]	3.30 ± 0.81[c]
F3	8.06 ± 3.93[b]	7.13 ± 3.48[b]	7.04 ± 3.11[b]
F1	10.46 ± 4.71[a]	8.76 ± 4.21[a]	8.80 ± 4.09[a]
Estradiol-17 beta E2 (ng/ml)			
F5	0.15 ± 0.07[b]	0.18 ± 0.06[b]	0.23 ± 0.28[b]
F3	0.51 ± 0.36[a]	0.37 ± 0.16[a]	0.41 ± 0.24[a]
F1	0.26 ± 0.19[b]	0.17 ± 0.14[b]	0.34 ± 0.32[b]

[a], [b], [c]: P<0.05.

Table 4. Overall P4 and E2 means and relative concentration ratio.

Parameter		Control	S 200	S 400
Progesteron (P4) F1÷F5	ng/ml	7.79	6.79	6.74
Estradiol-17 beta (E2) F1÷F5	ng/ml	0.36	0.26	0.29
Ratio P4 / E2		21.66	26.14	23.02

ovary. *In vivo*, this effect could be responsible for the higher ovary activity of S200 group observed in the present study (Proudman, 1995). Taken together our results suggest that Silymarin can effectively act as an endocrine-modulating chemical.

More studies are necessary to further explain the pharmacodynamic of Silymarin in relation with the reproductive system.

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