

NF\_2012pc152\_1

## PERFORMANCE AND SOME EGG CHARACTERISTICS OF LAYING HENS FED THE DIET CONTAINING ALFALFA PROTEIN-XANTHOPHYLL CONCENTRATE\*

E.R. Grela<sup>1</sup>, K. Ognik<sup>2</sup>, S. Knaga<sup>3</sup>, J. Matras<sup>1</sup>, and Sz. Sroka<sup>1</sup>

1 - Institute of Animal Nutrition and Bromatology, University of Life Sciences, Lublin, Poland

2 - Dept. of Biochemistry and Toxicology, University of Life Sciences, Lublin, Poland

3 - Dept. of Biological Basis of Animal Production, University of Life Sciences, Lublin, Poland

\* The paper supported by the project No. 12 0005 06 from the National Center of Research and Development, Poland

### ABSTRACT

The alfalfa protein-xanthophyll concentrate (PX), apart from a high share of protein and xanthophylls, contains a lot of valuable bioactive compounds, such as saponins, flavonoids, PUFA, vitamins and minerals. The occurrence of such a great deal of active substances makes PX concentrate reveal multidirectional pharmacological effects, as well as improve the performance of the birds and egg composition. The aim of the study was to determine the effect of alfalfa protein-xanthophyll (PX) preparation addition to a complete laying hen diet on birds productive performance and some egg traits.

The total of 240 21-week-old laying hens were randomly allocated into 3 treatments, 4 replicates per treatment, 20 hens per pen. Group I, a control group was fed standard mixture. The group II and III hens received mixtures with an addition of 1.5% or 3.0% respectively of alfalfa protein-xanthophyll concentrate (PX) instead of soybean meal. The experimental period lasted to 45 week of birds' life. The eggs' number and daily feed intake per pen were recorded. Once a week the eggs were weighed, afterwards they were used in chemical and physical analysis.

The egg laying oscillated from 75.6 to 76.9%. A positive ( $P \leq 0.05$ ) influence of both PX levels in the diet on yolk and shell color was noted. Its higher level significantly increased also

egg weight and zinc, manganese and copper contents in the egg and improved FCR. Protein-xanthophyll concentrate did not cause any changes in oxide-reduction status of the egg (SOD, CAT, MDA).

**KEYWORDS:** protein-xanthophyll concentrate, hen, egg

### INTRODUCTION

A protein-xanthophyll (PX) concentrate of alfalfa, containing ca. 550 g of total protein and over 1200 mg of xanthophyll per 1 kg preparation, additionally possesses a number of other valuable active compounds, e.g. saponin glycosides (2-3%), polyphenolic compounds, compounds exhibiting estrogenic activity (biochanin A, daidzein) as well as vitamins (A, B<sub>1</sub>, B<sub>6</sub>, B<sub>12</sub>, C, E, K), provitamins ( $\beta$ -carotene) and mineral compounds (Newall *et al.*, 1996; EFSA, 2009). The presence of such a numerous group of active substances determines the multi-directional pharmacological effects displayed by alfalfa and products made of it. Many of those active compounds – polyphenols, vitamins E and C, and  $\beta$ -carotene in particular, apart from immunomodulating properties (Dong *et al.*, 2007; Khaleel *et al.*, 2005) may as well exhibit antioxidative properties (Aziz *et al.*, 2006; Xie *et al.*, 2008). A profile of amino acids and fatty acids in PX preparation of alfalfa (EFSA,

2009) also arouses interest. Supplemental PX concentrate of alfalfa comprising numerous biologically active substances can modify bird metabolism and hence, affect egg-laying production as well as egg physical and chemical parameters.

The aim of the study was to determine the effect of alfalfa PX protein-xanthophyll preparation addition to a complete laying hen diet on birds productive performance and some egg parameters.

## MATERIALS AND METHODS

A total of 240 21-week-old laying hens Lohmann Brown were randomly allocated into 3 treatments, 4 replications per treatment, 20 hens per 4 m<sup>2</sup> pen area in floor-litter system with cut straw. The layers were marked and had free access to water (drinking nipples), feed (pan feeders) and hanging nest boxes. The hen housing facility was equipped with mechanical ventilation system and automatically controlled lighting. During the study period, the lighting time was 16 h daily. Group I, a control group was fed standard mixture, while the hens from group II and III received diets supplemented with respectively 1.5% or 3.0% additive of alfalfa protein-xanthophyll concentrate (PX) instead of soybean meal. The experimental period lasted till 45 week of bird life. The eggs number and daily feed intake per pen were recorded. The eggs were weighed once a week and then underwent the chemical and physical analyses.

The following physical parameters were determine: egg mass, yolk weight, protein weight, yolk color, shell strength, shell mass and thickness of 60 eggs randomly chosen from the group. Mineral content (Ca, Mg, K, Na, Fe, Mn, Cu, Zn) was established by atomic absorption spectroscopy with the Varian SpectrAA 220 FS instrument, while phosphorus using the colorimetric method.

In yolk egg superoxide dismutase (SOD) was assayed spectrophotometrically with the adrenaline method according to Misra, in: Greenwald (1985), at the wavelength of 320 nm. The method was modified to obtain greater

selectivity of transitory reaction products at this wavelength (Bartosz, 2004). The analyses were also carried out for catalase (CAT) level – according to Bartosz, 2004. The egg yolk samples were examined for a level of lipids peroxidation products as well, i.e. peroxides (H<sub>2</sub>O<sub>2</sub>) – as per Gay and Gbicki (2000; 2002), and malondialdehyde (MDA) as the end product of tissue lipids oxidation – according to Salih *et al.*, 1987.

Fatty acid determination was performed using the gas chromatography method on a Varian CP-3800 chromatograph. The chromatograph operating conditions for fatty acid separation were: capillary column CP WAX 52CB DF 0,25 mm of 60 m length, gas carrier - helium, flow rate – 1.4 ml/min, column temperature 120 °C gradually increasing by 2 °C/min., determination time – 127 min., feeder temperature – 160 °C, detector temperature – 160 °C, other gases – hydrogen and oxygen.

All collected data was subjected to General Linear Models procedure for the analysis of variance using SAS (2010) software and verified for statistical significance ( $p < 0.05$ ) at 95% confidence level using Duncan's multiple range test.

## RESULTS AND DISCUSSION

The applied dietary supplement did not have significant impact on average mass of egg, yolk, protein and shell quality as evidenced by genetic basis for morphotic composition of egg. The egg laying oscillated from 75.6 to 76.9%. Positive ( $P \leq 0.05$ ) influence of both PX levels in the diet on yolk and shell color was noted. Higher intensity of yolk color resulted from the dietary inclusion of alfalfa leaf extract and herbs which can be successfully used in laying hen rearing, especially in organic farming (Rizzi and Chiericato, 2005).

Higher PX level has also significantly increased zinc, manganese and copper content in the egg and improved FCR. Protein-xanthophyll concentrate did not cause any changes in the oxide-reduction status of the egg (SOD, CAT, MDA). As this additive indicated a

trend toward an increased n-3 fatty acid group content, that makes the n-6/n3 ratio more beneficial in human nutrition. However, it has been challenging to elucidate the functional mechanism of alfalfa phytobiotics in fat metabolism which is quite complex in poultry (Leskanich and Noble, 1997).

## CONCLUSION

Dietary supplement of protein-xanthophyll concentrate of alfalfa has increased manganese, copper and zinc contents in egg, intensified egg yolk color and elevated the content of the n-3 fatty acid group in egg yolk fat.

## REFERENCES

**AZIZ, A.B., GROSSMAN, S., BUDOWSKI, I., ASCARIELLI, I. and BONDI, A.** (2006) Antioxidant properties of lucerne extracts. *Journal of the Science of Food and Agriculture* 19: 605-608.

**BARTOSZ, G.** (2004) Second face of oxygen. PWN, Warsaw.

**DONG, X.F., GAO, W.W., TONG, J.M., JIA, H.Q., SA R.N. and ZHANG Q.** (2007) Effect of polysavone (alfalfa extract) on abdominal fat deposition and immunity in broiler chickens. *Poultry Science* 86:1955-1959.

**EFSA** (2009) Opinion on the safety of "Alfalfa protein concentrate" as food. *The EFSA Journal* 997: 1-19.

**GAY, C. and GBICKI, J.M.** (2000) A critical evaluation of the effect of sorbitol on the ferric-xylene orange hydroperoxide assay. *Analytical Biochemistry* 284:217-220.

**GAY, C. and GBICKI, J.M.** (2002) Perchloric acid enhances sensitivity and reproducibility of the ferric-xylene orange peroxide assay. *Analytical Biochemistry* 304:42-46.

**GREENWALD, R.A.** (1985) CRC Handbook of methods for oxygen radical research. CRC Press Boca Raton. Polish Academy of Science, Institute of Physiology and Animal Nutrition Jabłonna.

**KHALEEL, A.E., GAD, M.Z., EL-MARAGHY, S.A., HIFNAWY, M.S. and ABDEL-SATTER, E.** (2005) Study of hypocholesterolemic and

antiatherosclerotic properties of *Medicago sativa* L. cultivated in Egypt. *Journal of Food and Drug Analysis* 13:212-218.

**LESKANICH, C.O. and NOBLE, R.C.** (1997). Manipulation of the n-3 polyunsaturated fatty acid composition of avian eggs and meat. *World's Poultry Science Journal* 53:155-183.

**NEWALL, C.A., ANDERSON, L.A. and PHILLOPSON, J.D.** (1996) Herbal medicines. A guide for health-care professionals. The Pharmaceutical Press, London.

**RIZZI, C. and CHIERICATO, G.M.** (2005). Organic farming production. Effect of age on the productive yield and egg quality of hens of two commercial hybrid lines and two local breeds. *Italian Journal of Animal Science* 4: 160-162.

**SALIH, A.M., SMITH, D.M., PRICE, J.F. and DAWSON L.E.** (1987) Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Science* 66: 1483-1488.

**SAS** (2010) SAS/IML Studio 3.2 for SAS/STAT Users, Second Edition. Cary, NC, USA.

**XIE, Z., HUANG, J., XU, X. and JIN, Z.** (2008) Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry* 111:370- 376.