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EFFECT OF USING A WINERY BY-PRODUCT WITH ANTIOXIDANT PROPERTIES IN LATER DIETS ENRICHED IN POLYUNSATURATED FATTY ACIDS, ON EGG QUALITY

Vasile BUNDUC[1], Margareta OLTEANU[2], Rodica Diana CRISTE[2], Tatiana Dumitra PANAITE[2], Mariana ROPOTA[2], Monica MITOI[3]

[1]University of Agricultural Sciences and Veterinary Medicine,
3 Aleea Mihail Sadoveanu, Iasi, Romania;
[2]National Research Development Institute for Animal Biology and Nutrition,
1 Calea Bucuresti, Balotesti, 077015, Ilfov, Romania;
[3]Institute of Biology Bucharest, 296 Splaiul Independentei, 060031, Romania.

Corresponding author email: margaretaolteanu@yahoo.com

Abstract

Due to their multiple positive, acknowledged effects of the omega-3 unsaturated fatty acids, present day consumers are increasing interested in ways of incorporating these fatty acids in their daily diet. The production of eggs enriched in polyunsaturated fatty acids via the feeds given to layers is one way, but solutions have to found to preserve for a longer time the organoleptic properties of these enriched eggs. It appeared thus necessary to use antioxidants, natural mainly, in layer diets. Due to their high content of polyphenols, the natural antioxidants block oxidation by their reaction with the free radicals. The purpose of our study was to evaluate the effect of a winery by-product, grape seeds meal, displaying antioxidant properties, given to layers, on egg quality. The experiment was conducted at a commercial poultry farm owned by Avicola Lumina SA, for 4 weeks, on a total of 64.000 layers aged 27 weeks, Tetra SL LL hybrid. The layers were assigned to two groups, control (C) and experimental (E), of 32.000 layers each. The diets were based on corn, soybean meal and sunflower meal, and had the same protein and energy content. The diet of the experimental group differed by the inclusion of flax meal and camelina meal, as ingredients rich in unsaturated fatty acids, and of 1% grape seed meal, as natural antioxidant. Egg samples were collected in the end of the experiment and assayed for their quality. The higher concentration of omega-3 unsaturated fatty acids in the diet for group E (12.26 ± 0.15 g/100 g fat) compared to group C (7.28 ± 0.89 g/100 g fat) was also found in the yolk of the sampled eggs, which increased from 3.33 ± 0.20 g/100 g fat in group C, to 5.76 ± 0.30 g/100 g fat in group E. The higher antioxidant capacity, 7.157 ± 0.662 mM Trolox equivalent / g of the diet for group E, compared to 6.507 ± 0.401 mM Trolox equivalent / g of the diet for group C, also increased the antioxidant capacity of the eggs. Thus, the eggs harvested from group E had an antioxidant capacity of 126.353 ± 4.523 mM Trolox equivalent / g, compared to 115.300 ± 7.269 mM Trolox equivalent / g in the eggs collected from group C. These results that the winey by-product, grape seed meal, can be used as antioxidant feed additive in layer diets enriched in unsaturated fatty acids, thus improving the properties of the eggs.

Key words: *antioxidants, grape seeds meal, eggs, quality.*

INTRODUCTION

Recent researches in human diets show that a high level of omega-3 polyunsaturated fatty acids in the animal foods for human consumption have beneficial effects on human health (Riediger et al., 2009, Huang, 2010, Shapiro et al., 2010, Turner et al., 2011). Considerable research efforts are directed towards the nutritional manipulation of the fatty acids profile from the hen eggs (Criste et al., 2009). Fraeye et al. (2012) published a review of 26 studies conducted in 1991-2011, regarding the possibilities of enriching the hen eggs in omega-3 polyunsaturated fatty acids, which used flax seeds, fish oil and/or micro-algae as sources of omega-3 fatty acids. The changes in egg composition, following the supplementation, are quite varied.

The rather high concentration of polyunsaturated fatty acids in the yolk, make the egg susceptible to oxidative degradation during heat preparation and during storage. The Lipid oxidation produces free radicals, which cause several oxidative degradations and start the reactions of secondary oxidation (Ren et al., 2013). It appeared thus necessary to use antioxidants in layer diets enriched in polyunsaturated fatty acids, particularly natural antioxidants which, by their high concentration

of polyphenols, block oxidation by reacting with the free radicals. Many researches have been conducted during the past decade, which have shown that winery by-products can be used in poultry feeding, particularly the grape marc. The wine-making process produces large amounts of this waste, which can be used efficiently in corn-soybean diets for poultry (Su et al., 2008; Hu et al., 2013; Juśkiewicz et al., 2015).

The paper reports on the experiments aiming to evaluate the effect on egg quality of a winery by-product, grape seeds meal resulting from oil extraction, used as antioxidant in layer diets high in polyunsaturated fatty acids.

MATERIALS AND METHODS

The experiment was conducted in a commercial company, Avicola Lumina SA, for four weeks, on a stock of 64.000 TETRA SL LL layers aged 27 weeks. The layers were assigned to two groups, a control group (C) and an experimental (E) group, each with 32.000 layers. The two experimental halls (Photo 1) belong to module 5 of SC Avicola Lumina SA, where the layers are reared in enriched, EUROVENT 1500-type cages. These cages, manufactured by Big DUTCHMAN (Germany) are stacked on 2, 3 or 4 tiers. The area of each hall is of 2000 sq.m, with 6 rows × 33 cages each × 2 tiers each × 60 layers / cage. These cages for layers are fitted with nipple drinkers. Each hall is fitted with 8, 1.1 kW fans, with a capacity of 32.000 cubic meters per hour. Each hall is also fitted with a Big Dutchman computer which regulates the feeding, watering, air admission, ventilation and heating.

The diet formulations were based on corn, soybean meal and sunflower meal. The formulation for the experimental group also included flax meal and camelina meal as ingredients rich in polyunsaturated fatty acids, plus 1% grape seeds meal, as natural antioxidant. After 2 and 4 weeks of feeding (end of the experiment), egg samples (10 eggs per group) were collected and assayed for the fatty acids concentration in the yolk; for the concentration of total polyphenols; for the content of flavonoids and for the antioxidant capacity in the methanol extracts of yolk.

Standardized methods were used to assay the concentration of main nutrients in the feeds, as follows:

- The dry matter (DM) was determined using the gravimetric method, whose working principle involves the determination of sample mass by drying at 103^{o}C, according to *Regulation (CE) 152/2009 and standard SR ISO 6496:2001*. We used a Sartorius (Gottingen, Germany) scale and BMT drying closet, ECOCELL Blueline Comfort (Neuremberg, Germany);
- The crude protein (CP) was determined using the Kjeldahl method, whose working principle involves sample digestion by heating with sulphuric acid in the presence of a catalyst, for the conversion of the protein nitrogen into ammonium sulphate. The reaction products are alkalinized with sodium hydroxide, for the release of the trapped ammonia, by distillation in a solution of boric acid in excess, followed by titration in a solution of hydrochloric acid. The method complies with *Regulation (CE) 152/2009 and standard SR ISO 5983-2:2009*. We used a semiautomatic KJELTEC auto 2300 system – Tecator (Sweden);
- The ether extractives (EE) were determined by extraction is organic solvents, which involves fat extraction by petrol ether, removal of the solvent by distillation, drying and weighing the residue. The method complies with *Regulation (CE) 152/2009 and standard SR ISO 6492:2001*. We used a SOXTEC-2055 FOSS system – Tecator (Sweden);
- The fatty acids were determined using the chromatographic method, which involved the transformation of the fatty acids from the sample in methyl esters, followed by the separation of the compounds in a chromatographic column and their identification by comparison with standard chromatograms. The method complies with *Regulation (CE) 152/2009 and standard SR CEN ISO/TS 17764 -2: 2008*. We used a Perkin Elmer-Clarus 500 chromatograph, fitted with a system for injection into the capillary column, with high polarity stationary phase (BPX70: 60m x 0.25mm inner diameters and 0.25µm thick film); or high polarity cyanopril phases, which have similar resolution for different geometric isomers (THERMO TR-Fame: 120m x 0.25mm ID x 0.25µm film).

- The crude fibre (CF) was determined with the method with intermediary filtration, whose working principle involves weighing the sample boiled successively with solutions of sulphuric acid and sodium hydroxide. The resulting residue was filtered, dried, burnt and weighed. The method complies with *Regulation (CE) 152/2009 and standard SR EN ISO 6865:2002*. We used a FIBERTEC 2010 system – Tecator (Sweden);
- The ash (Ash) was determined by the gravimetric method, which involves sample decomposition by burning and weighing of the resulting ash. The method complies with *Regulation (CE) 152/2009 and standard SR EN ISO 2171:2010*. We used a Caloris CL 1206 furnace.
- The gross energy was determined by calculation using the gross chemical analysis (dry matter, protein, fibre, fat, nitrogen-free extractives and ash) using the equations of Burlacu et al. (2002).

To determine the concentration of polyphenols and the antioxidant capacity of the samples, we first extracted the phenol compounds in acidified methanol (methanol:HCl=80:20). To 1g sample we added 10 ml acidified methanol and stirred at room temperature for 48 h. The homogenate has been centrifuged twice at 10,000 RCF, for 15 min, at room temperature, and the final supernatant (methanol extract) has been preserved at 4^{o}C, until analysed. Instruments: orbital stirrer Heidolph Unimax 1010, Microstirrer Vepl Scientific, Centrifuge Eppendorf 5810R, RADWAG AS220/C/2 (10-220 mg) and PS600/C/2 (0.01-600 g) scales, pH metre WTW Senix-HW

- The polyphenol content of the methanol extracts has been determined according to the method described by Mihailovic et al. (2013), modified. The reaction mixture consisted of: the methanol extract in proper dilution according to the analysed sample, Folin-Ciocâlteu reagent and a solution of 7.5% de Na_2CO_3. The reaction mixture was maintained for 30 min. at room temperature, thereafter absorbance was read at 765 nm. Three replicates have been done for the same sample, and the average value of the readings, representing the total concentration of phenols, was expressed in equivalents gallic acid/g fresh matter (mg EAG/g sample). We used a UV-VIS Thermo Scientific spectrophotometer.
- The flavonoids content of the methanol extracts has been determined according to the method described by Zhishen et al. (1999).
- The determination of the antioxidant capacity of the methanol extracts has been done using the DPPH method proposed by Marxen et al. (2007). The antioxidant capacity has been estimated by calculating the difference between the control and the sample, compared to a standard curve which used Trolox (synthetic antioxidant analogue to α-tocopherol), as standard antioxidant. Three replicates have been done for the same sample, and the average value of the readings, representing the antioxidant capacity has been expressed in Trolox equivalents/g fresh matter (mM Trolox/g sample). We used a UV-VIS Analytik Jena Specord 250 Plus spectrophotometer with thermostatic carousel.

The results of the experiment as presented as mean values with standard deviations, the statistic processing being done with Origin 5 software, using the t-Test (2 populations). The differences were considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSIONS

The grape seeds meal, resulting from oil extraction by a Romanian company, Chimia Bistrita SCM, was characterized by 11.59 ± 0.534 % protein, 5.29 ± 0.303 % fat, 42.83 ± 1.635 % fibre and 2.87 ± 0.136 % ash, values comparable with literature data (Olteanu et al., 2014; 2015).

Table 1. Data on the antioxidant properties of the grape seeds meal

Item	Total polyphenols (mgEAG/g)	Flavonoids (mg ERU/ g)	Antioxidant capacity (mM ET/g)
Sample 1	12.022	16.367	157.603
Sample 2	11.093	15.988	160.120
Sample 3	10.913	14.912	145.755
Sample 4	10.926	16.715	145.163
Average	*11.239*	*15.996*	*152.160*
Standard deviation	±0.529	±0.781	±7.810
Coefficient of variation	0.047	0.049	0.051

EAG – gallic acid equivalents; ERU –Rutin equivalents; ET – Trolox equivalents.

Table 1 shows the concentration of polyphenols and flavonoids, as well as the antioxidant capacity of the grape seeds meal determined on 4 samples.

The concentration of total polyphenols in the grape seeds meal, 11.239 ± 0.529 mg gallic acid equivalents / g sample, resembles to other plants such as coriander - 8.80 mg gallic acid equivalents / g sample; dill – 9.80 mg gallic acid equivalents / g sample, chilli – 8.60 mg gallic acid equivalents / g sample (Bin Shan et al., 2005), birthwort – 11.04 mg gallic acid equivalents / g sample (Papuc et al., 2010). Poudel et al. (2008) cited by En-Qin Xian et al. (2010) has shown that the antioxidant capacity of the grape seeds ranges between 16.8-92 mM Trolox equivalents /g sample, while it ranges between 157-113.3 mM Trolox equivalents /g sample in the grape peels.

The nutrients from the feed samples, collected after manufacturing, have shown that nutritionally, they meet the quality parameters, being isoprotein (about 19.5 % protein) and isoenergy (about 16.5 MJ /kg gross energy).

The compound feed for group E has been enriched in omega 3 polyunsaturated fatty acids using two unconventional forages (flax and camelina), as meal. The analytical results from CF analyses show a significantly ($P \leq 0.05$) higher concentration of omega 3 fatty acids in group E (12.26 ± 0.15 g / 100 g fat), given mainly by the α-linolenic acid, compared to just 7.28 ± 0.89 g / 100 g fat in group C.

As expected, the use of 1% grape seeds meal as natural antioxidant, in the feeds for group E, increased by 2.36 % the polyphenols concentration, by 3.18% the flavonoids concentration, and by 9.99% of the antioxidant capacity, compared to the corresponding values for group C (Figures 1, 2 and 3).

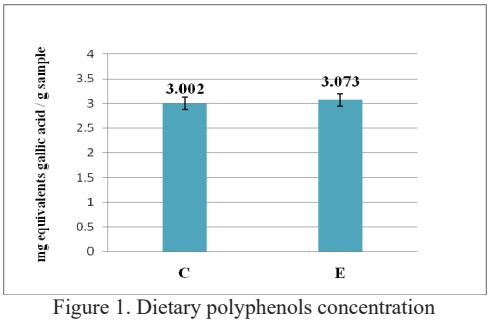

Figure 1. Dietary polyphenols concentration

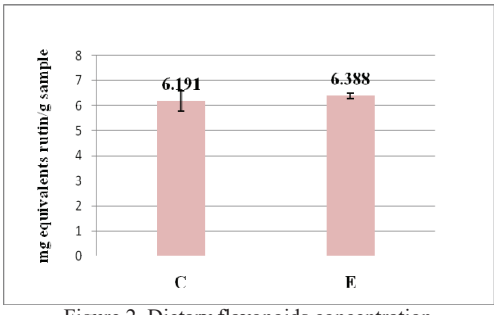

Figure 2. Dietary flavonoids concentration

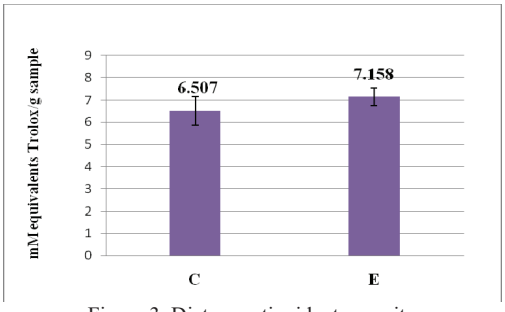

Figure 3. Dietary antioxidant capacity

At the same time, as shown in Figure 4, there was a good correlation between the antioxidant capacity and the polyphenols concentration in the feeds.

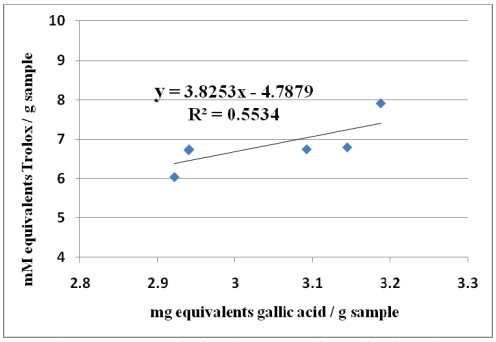

Figure 4. Correlation between the polyphenols concentration and the antioxidant capacity of the feeds

The quality evaluation of the eggs collected at the end of the trial has shown that the concentration of omega 3 fatty acids in the yolk from group E was of 5.76 ± 0.30 g / g fat, significantly ($P \leq 0.05$) higher compared to 3.33 ± 0.20 g / g in the yolk from group C. this is due to the higher concentration of omega 3 fatty acids in the feed for group E compared to group C.

Regarding the oxidative status of the eggs (Figures 4 and 5), one can notice that the polyphenols concentration in the yolk of eggs from group E was 0.466 ± 0.039 mg gallic acid equivalents / g sample, while in the yolk of the eggs from group C, it was 0.491 ± 0.044 mg gallic acid equivalents / g sample, with no significant differences.

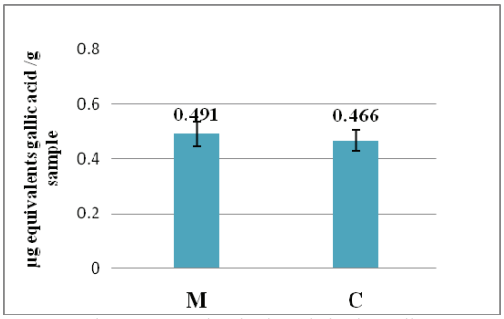

Figure 5. Total polyphenols in the yolk

On the other hand, the antioxidant capacity of the yolk from group E eggs was 126.353 ± 4.524 mM Trolox equivalents / g sample, significantly (P≤0.05) higher, by 9.59%, compared to group C, 115.300 ± 7.270 mM Trolox equivalents / g sample (Figure 6).

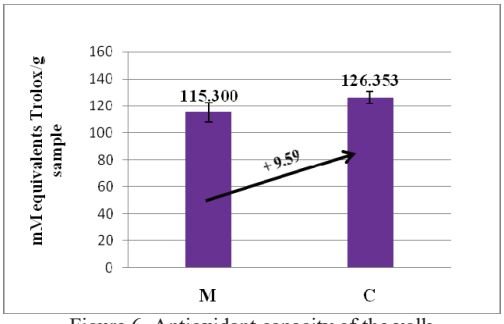

Figure 6. Antioxidant capacity of the yolk

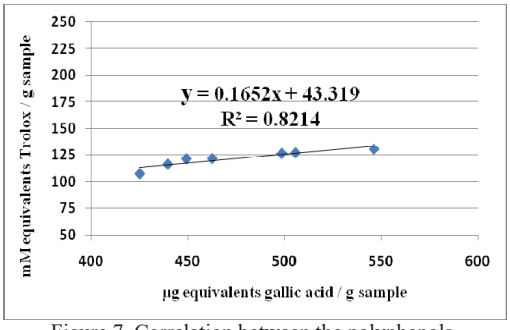

Figure 7. Correlation between the polyphenols concentration and the antioxidant capacity of the yolk

As shown, there has been a rather close correlation between the polyphenols concentration and the antioxidant capacity of the eggs, particularly of the yolk ($R^2 = 0.8214$), as shown in Figure 7.

CONCLUSIONS

Although the concentration of polyunsaturated fatty acids was higher in the feeds for the experimental group, the addition of 1% grape seeds meal, balanced the oxidative status of the compound feed, allowing a good correlation between the polyphenols concentration and the antioxidant capacity of the feeds.

The determinations of polyphenols concentration and antioxidant capacity performed on yolk samples, revealed a higher antioxidant capacity (by 9.59%) compared to the control group, confirming the existence of a close correlation between the antioxidant capacity and the polyphenols concentration ($R^2 = 0.8214$).

The experimental results show that this winery by-product, grape seeds meal, has antioxidant properties and can be recommended as feed additive for layer diet formulations enriched in polyunsaturated fatty acids, enhancing thus egg quality.

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REFERENCES

Bin S., Yizhong Z. Cai, Mei S., Harold C., 2005. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. J. Agric. Food Chem., 53(20): 7749–7759; DOI: 10.1021/jf051513y.

Burlacu G.H., Cavache A., Burlacu R., 2002. Potentialul productiv al nutreturilor si utilizarea lor. Ed. Ceres, ISBN: 973-40-0541-3.

Criste R.D., Panaite T.D., Ciurescu C., Ropota M., Rachieru D., 2009. Effects of moderate (5%) levels of linseed in layer diets. Archiva Zootechnica, 12(3): 11-21.

En-Qin X., Gui-Fang D., Ya-Jun G., Hua-Bin L., 2010. Biological Activities of Polyphenols from Grapes. Int. J. Mol. Sci., 11: 622-646; doi: 10.3390/ijms11020622.

Fraeye I., Bruneel C., Lemahieu C., Buyse J., Muylaert K., Foubert I., 2012. Dietary enrichment of eggs with omega-3 fatty acids: A review. Food Research International, 48: 961–969.

Hu R., Wang Y., Wang X., Yang T., Chen S., Yang X., Yao J., 2013. Grape Seed Extract: Effects on Performance and Yolk Cholesterol Content in Laying Hens. Chinese Journal of Animal Nutrition, 9.

Huang T.L., 2010. Omega-3 fatty acids, cognitive decline, and Alzheimer's disease: a critical review and evaluation of the literature. J. Alzheimer's Dis. 21: 673–90.

Juśkiewicz J., Jankowski J., Zduńczyk Z., Kołodziejczyk K., Mikulski D., Zduńczyk P., 2015. The chemical composition of selected dried fruit pomaces and their effects on the growth performance and post-slaughter parameters of young turkeys. Journal of Animal and Feed Sciences, 24(1): 53-60.

Marxen K., Vanselow K.H., Lippemeier S., Hintze R., Ruser A., Hansen, U.P., 2007. Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. Sensors 2007, 7: 2080-2095.

Mihailović V., Matić S., Mišić D., Solujić S., Stanić S., Katanić J., Stanković N., 2013. Chemical composition, antioxidant and antigenotoxic activities of different fractions of Gentiana asclepiadea L. roots extract. EXCLI Journal, 12: 807-823.

Olteanu M., Bunduc V., Panaite C., Cornescu M., Panaite T.D., Ropota M., Mitoi M., 2015. Study on the efficiency of grape seed cakes used as antioxidants, compared to vitamin E, in layer diets enriched in polyunsaturated fatty acids, XVI European Symposium on the "Quality of Eggs and Egg Products" Nantes, 10-13 May.

Olteanu M., Criste R.D., Panaite T.D., Ropota M., Mitoi M., Varzaru I., Untea A.E., 2014. Study of the feeding value and antioxidant capacity of winery by-products, potential natural antioxidants for farm animal diet formulations. Archiva Zootechnica, 17(2) :55–69.

Papuc C., Crivineanu M., Goran G., Nicorescu V., Durdun N., 2010. Free radicals scavenging and antioxidant activity of European mistletoe (Viscum album) and European birthwort (Aristolochia clematitis). Revista de Chimie, 61(7): 619-622.

Poudel P.R., Tamura H., Kataoka I., Mochioka R., 2008. Phenolic compounds and antioxidant activities of skins and seeds of five wild grapes and two hybrids native to Japan. J. Food Comp. Anal., 21: 622–625.

Ren Y., Perez T. I., Zuidhof M.J., Renema R. A., Wu J., 2013. Oxidative stability of omega-3 polyunsaturated fatty acids enriched eggs. Journal of Agricultural and Food Chemistry, 61(47): 11595-11602.

Riediger N.D., Othman R.A, Suh M., Moghadasian M.H., 2009. A systemic review of the roles of n-3 fatty acids in health and disease. J. Am. Diet. Assoc., 109:668–79.

Shapiro H., Theilla M., Attal-Singer J., Singer P., 2010. Effects of polyunsaturated fatty acid consumption in diabetic nephropathy. Nat. Rev. Nephrol., 7:110–121.

Su X., Liu H., Yu W., Pang Z., Zhuang Y., 2008. Study of Effect of Grape Seed on Growth Performance in Pigs and Hens. China Animal Husbandry & Veterinary Medicine, 35(2): 9-13.

Turner D., Shah P.S., Steinhart A.H., Zlotkin S., Griffiths A.M., 2011. Maintenance of remission in inflammatory bowel disease using omega-3 fatty acids (fish oil): a systematic review and meta-analyses. Inflamm. Bowel Dis., 17:336–45.

Zhishen J., Mengcheng T., Jianming W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64:555-559.

**** Hibrizii Bábolna tetra TETRA-SL LL, Harco Blanca, GHID DE CRESTERE SI DE PRODUCŢIE PENTRU HIBRIZII OUĂTORI COMERCIALI, Ed Bábolna Tetra Kft. H-2943 Bábolna, Radnóti u. 16, Hungary.