The effect of sea buckthorn (Hippophae rhamnoides L.) fruit residues on performance and egg quality of laying hens

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ABSTRACT
The experiment was conducted on Isa Brown laying hens to study the effects of sea buckthorn fruit residues on hen performance and quality of eggs. The total number of 1440 eighteen-week-old pullets Isa Brown was divided into two groups (720 each) including 24 subgroups (30 hens). The experimental group was fed diets in which 5% of the wheat was replaced by sea buckthorn fruit residues. The control group was fed diets without any colour additives. Egg production and intensity of laying hens were evaluated in seven periods, from 127 to 322 days of age. In our study, a significant effect (P ≤ 0.05) of sea buckthorn fruit residues on total number of laid eggs and egg yolk colour was detected. However, a non-significant effect of sea buckthorn was found on hen performance or egg quality including egg weight, yolk weight, eggshell strength, shape index of egg, eggshell thickness, Haugh units, eggshell colour and blood spot, albumen weight, proportion and Haugh units. Results of our study showed the beneficial effect of feeding sea buckthorn fruit residues on total number of laid eggs and egg yolk colour which is a great concern for egg consumers.

1. Introduction

Sea buckthorn (Hippophae rhamnoides L.) is a deciduous species, widely found all over the world. It is a hardy, deciduous shrub bearing small yellow to orange-red berries and grows widely in Europe, central Asia and the temperate regions of South Asia, India and China. The sea buckthorn berry flour is like a fruit; its seeds and leaves are rich in nutrients and bioactive components such as vitamins, amino acids, lipids, carotenoids, xanthophyll, phenolic and flavonoids and have a higher content of essential oils (Repyakh et al. 1990; Bekker & Gluschenkova 1997; Ranjith et al. 2006; Singh et al. 2006; Yang 2009), making it a very beneficial feed supplement for laying hens. Dumbrava et al. (2006) reported that feeding poultry with a meal made from sea buckthorn fruit has been observed to increase the pigmentation of the egg yolk. The most accumulated in yolks are: zeaxanthin and lutein, pigments with a very high biological value (Dumbrava et al. 2006). Moreover, the sea buckthorn plays an important role in improving the efficiency of feed and has been used as an alternative feed, particularly in poultry, to maintain their production, performance and high-quality yield. For the content of bioactive substances, the sea buckthorn was used for many years in different forms in human nutrition as a health supplement. The sea buckthorn and its by-products are examined in recent years as a supplement in animal nutrition. In the Czech Republic there are some companies that produce sea buckthorn products for human nutrition, or supply their products to pharmaceutical companies and sell for the same price as wheat and therefore can be used as an ingredient of compound feed mixtures for poultry.

For the poultry breeder, farmers and food, egg sorting and marketing companies, the main priorities are to deliver a safe product which is accepted by the consumers. Therefore, eggs with good eggshell and internal egg quality are important for the economic viability of the poultry industry (Solomon 1991; Johnson 2000). Mainly the major economic losses for egg producers are the consequences of lower eggshell strength which results in eggshell breakage. Egg quality consists of different quality aspects, each of which can be related to interior egg quality or external egg quality. The internal egg quality relates in general to the quality of the albumen and yolk (Bamelis 2003). Carotenoids are natural compounds present in animals and plants; with yellow, orange and red typically associated with birds. The quality of eggs including their weight, shell characteristics and internal components are affected by a wide range of factors of which the genotype, nutrition and age of hens are the most important factors. Moreover, nutrition is important for controlling egg internal components and eggshell quality and can successfully enrich the egg in some minor components of interest for human nutrition. Plant extracts were further discussed in many studies to investigate their effect on egg quality of the laying hens. In a more recent study on Boris Brown layers, Lokaewmanee et al. (2014) cited that dietary plant extracts improved significantly the eggshell quality of strength against breaking, and this positive effect could be attributed to an observed improvement in...
small-intestine histological parameters. Sharma et al. (2009) observed increased eggshell thickness (by 10.0%) and strength against breaking (by 15.2%), as well as decreased number of eggs with shell defects (by 2.5 percentage points) in hens fed diet supplemented with herbal products. In contrast, several authors did not find any beneficial effect of dietary plant extracts in terms of improvement in eggshell quality (Bozkurt et al. 2012; Siwatkiewicz et al. 2013). On the other hand Wang (1997) reported that in poultry, sea buckthorn positively affected the egg production and body weight of laying hens.

The aim of this study was to investigate the possibility of using sea buckthorn (H. rhamnoides L.) fruit residues in compound feeds for laying hens and their effect on the colouration of egg yolk, performance and health.

2. Material and methods

This experiment was conducted at the ITP (International Testing of Poultry) in Ustrašice, the Czech Republic. The total number of 1440 eighteen-week-old pullets Isa Brown was divided into two groups of 720 hens and each of them had 24 subgroups (30 hens each). The hens were housed in an air-conditioned hall with an enriched cage system (756 cm² area of the cage floor per hen). All the hen groups had identical environmental conditions. Feed and water were provided ad libitum. Automatic nipple drinkers and trough feeders were used. In each cage the roosts, nest, roosting ash place and a trading of the overgrown claws were attached. The removing of excrements was done with a belt conveyer. The eggs were collected by hand.

The lighting programme for hens was as follows: (19th week: time of lighting from 5:00 to 19:00 h (14 h), 20th week: time of lighting from 5:00 to 20:00 h (15 h), 21st week: time of lighting from 5:00 to 20:30 h (15.5 h), 22nd week until the end of the test: time of lighting from 5:00 to 21:00 h (16 h).

Temperature in the poultry house was kept between 18°C and 20°C and relative air humidity between 60% and 70%. In 5% of the experimental diets, wheat was replaced by sea buckthorn fruit residues. The sea buckthorn fruit residues were berries from which the juice had been extracted by pressing and then dried. The residues were made by Sevak Star Company in the Czech Republic. The chemical analyses results of sea buckthorn fruit residues are given in Table 1. The composition of the layer diets is shown in Table 2. The content of crude protein (CP) was 0.4% higher and metabolizable energy (ME) level of 0.15 MJ lower in the experimental diet than the control diet. The contents of lysine and methionine were also slightly higher in the experimental diet compared with the control diet.

Live weight of layers was recorded at 322 days of age. Feed consumption was recorded at the end of the experiment and calculated as: per one hen (cumulative), per one egg, per 1 kg of egg mass, per one hen/day.

Monitoring of egg production was performed daily. Eggs were collected at the same time manually for each subgroup separately. Egg production and intensity of laying were evaluated in the periods, from 127 to 322 days of age: per one hen initial number, per one hen average number, per one hen initial number for each period.

The average egg weight was determined in each period; moreover the egg mass production and the share of non-standard eggs were determined. The quality of eggs was evaluated by the following characteristics: egg weight, yolk weight, strength of eggshell, egg shape index, thickness of eggshell, Haugh units, yolk colour and shell colour.

The egg quality was determined with ‘FUTURA’ equipment that includes: albumen height measuring systems; eggshell tester; special type of micrometer crew for measuring shell thickness (shell thickness shown on LCD display); electronic precision scales; working vessel; technical supplies; FUTURA data processors link individual measuring devices to a PC for completely integrated measuring and data recording system. Haugh units were calculated from egg weight and albumen height. The yolk colour was assessed by the DSM Yolk Fan expressed on a 1–15 scale. The eggs have been sampled so that 3 consecutive days were in number of 30 pieces of each group of hens in 3, 5 and 7 period used for the analysis.

The chemical analysis of the feeds (dry matter (DM), ether extract (EE), ash – gravimetric; CP – Kjeldahl method; CF–CSN EN ISO 6865; starch and sugars – polarimetry, Ca and P – gas chromatography-flame ionization detector (GC-FID), lysine and methionine) was carried out in line with Act No 356/2008

| Table 1. Chemical analysis of sea buckthorn fruit residue (g/kg) and MEₙ (calculated) MJ/kg. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Ingredient                      | Control group   | Experimental group |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| DM                              | 934.30          | 934.30          | 35.80           | 35.80           |
| CP                              | 208.70          | 208.70          | 7.85            | 7.85            |
| EE                              | 71.40           | 71.40           | 2.82            | 2.82            |
| Crude fibre                     | 181.30          | 181.30          | 0.40            | 0.40            |
| Ash                             | 20.20           | 20.20           | 3.21            | 3.21            |
| Starch                          | 17.90           | 17.90           | 9.9             | 9.9             |
| Carotenoid                      | 10.81 mg/100g   | 10.81 mg/100g   |                 |                 |

Notes: The carotenoid levels (zeaxanthin and lutein) are given in Table 1. The highest carotenoid content was found in the mixture consisting of four sea buckthorn cultivars (10.81 mg/100 g).

* Lipowski et al. (2009).
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ME was calculated according to the equation recommended by the Subcommittee Energy of Working Group No. 2 Nutrition of the European Federation of Branches of the World’s Poultry Association (Zelenka et al. 1999). The data were analysed by ANOVA using the GLM procedure of SAS (SAS Institute 1985). Means were compared by Duncan’s multiple range test at an overall significance level of $P = .05$ (Duncan 1955).

### 3. Results and discussion

The results obtained in this experiment have shown that replacing wheat with 5% of sea buckthorn fruit residues significantly ($P \leq .05$) increased the total number of laid eggs (Table 3). The results are in agreement with the findings of Englmaierova et al. (2013) who reported that feeding diets supplemented with synthetic carotenoids such as lutein significantly increased the egg production of Isa Brown hens. Moreover, Hasanuzzaman et al. (2012) reported that mortality (%) compared with the control group. Moreover, Hasanuzzaman et al. (2012) reported that the mortality of birds was not affected by different levels of sea buckthorn.

Evaluation of total number of laid eggs and the share of non-standard eggs are given in Table 6. All parameters (cracked eggs, broken eggs, double yolk, membrane and total non-standard) did not differ significantly among groups.

The percentage of non-standard eggs did not exceed their usual occurrence. This was explained by King’ori (2012) who did not find any effect of fed diets supplemented with synthetic carotenoids such as lutein on feed intake (g/day/hen), feed intake (g/egg) and feed conversion ratio.

Mortality of hens (Table 5) did not significantly differ between the control group and experimental group of layers. Also, the mortality reasons (diseases of the genitals, metabolic disorders and cannibalism) of the hens were not affected by diet with 5% sea buckthorn fruit residues. These results are in concordance with the study of Englmaierova et al. (2013) who indicated that Isa Brown hens that were fed diets supplemented with synthetic carotenoids such as lutein did not differ in terms of mortality (%) compared with the control group. Moreover, Hasanuzzaman et al. (2012) reported that the mortality of birds was not affected by different levels of sea buckthorn.

### Table 3. Results of egg production for all periods.

| Group | Total number of laid eggs | Egg production per hen of initial number | Egg mass per hen of average number | Egg mass per hen of initial number | Egg production per hen of average number |
|-------|---------------------------|----------------------------------------|-----------------------------------|-----------------------------------|----------------------------------------|
|       | Mean ± SD | P-value | Mean ± SD | P-value | Mean ± SD | P-value | Mean ± SD | P-value | Mean ± SD | P-value |
| Control | 116,788 $^a$ ± 174.86 | .05 | 162.21 ± 15.81 | NS | 10.1 ± 0.79 | NS | 9.7 ± 0.74 | NS | 168.80 ± 13.71 | NS |
| Exp. | 118,481 $^b$ ± 186.88 | | 164.56 ± 17.70 | | 10.3 ± 0.84 | | 9.9 ± 0.77 | | 171.10 ± 14.74 | |

Notes: Results are expressed as mean ± standard error of the mean. NS, not significant.

### Table 4. Feed consumption and average live weight at the end of egg production.

| Group | Feed consumption | Per egg | Per 1 kg of egg mass | Per day of per hen | Average of live weight |
|-------|------------------|---------|---------------------|-------------------|-----------------------|
|       |                   | (g) ± SD | P-value | (g) ± SD | P-value | (g) ± SD | P-value | (g) ± SD | P-value |
| Control | 149.52 ± 15.20 | NS | 2.50 ± 0.03 | NS | 127.97 ± 7.03 | NS | 1979.1 ± 255 | NS |
| Exp. | 147.44 ± 17.72 | | 2.49 ± 0.04 | | 127.73 ± 8.59 | | 1951.5 ± 225 | |

Note: NS, not significant.

### Table 5. Mortality of layers.

| Indicators | Control | Exp. | P-value |
|------------|---------|------|---------|
| Start (n) | 720 | 720 | NS |
| Final (n) | 650 | 670 | NS |
| Mortality (n) | 70 | 50 | NS |
| Mortality (%) | 9.7 | 6.9 | NS |

Mortality reasons

| Diseases of the genitals (n) | 42 | 28 | NS |
| Diseases of the genitals (%) | 5.8 | 3.9 | NS |
| Metabolic disorders (n) | 14 | 8 | NS |
| Metabolic disorders (%) | 1.9 | 1.1 | NS |
| Cannibalism (n) | 14 | 14 | NS |
| Cannibalism (%) | 1.9 | 1.9 | NS |

Note: NS, not significant.

### Table 6. The percentage of non-standard eggs for all periods of laying.

| Group | Cracked eggs | Broke eggs | Double yolk | Membrane | Total non-standard |
|-------|-------------|-----------|------------|----------|-------------------|
| Control | 3120 ± 122.22 | 1969 ± 132.29 | 389 ± 25.50 | 1219 ± 217 | 6697 ± 44.7 |
| Exp. | 3185 ± 125.28 | 1990 ± 135.40 | 393 ± 27.27 | 1210 ± 215 | 6778 ± 46.4 |

Note: NS, not significant.
Table 7. The classification of egg weights.

| Group    | Average egg weight (g) ± SD | XL (≥73 g) % | L (63–73 g) % | M (53–63 g) % | S (≥53 g) % | P-value |
|----------|-----------------------------|--------------|---------------|---------------|-------------|---------|
| Control  | 60.03 ± 1.12                | 1.0 ± 0.04   | 30.2 ± 2.41   | 65.1 ± 8.39   | 3.7 ± 0.64  | NS      |
| Exp.     | 60.01 ± 0.73                | 1.2 ± 0.04   | 29.5 ± 2.34   | 65.4 ± 8.41   | 3.9 ± 0.67  |         |

Note: Non-significant differences between the averages at P < .05.

Table 8. Means and standard deviation of qualitative evaluations of eggs in three periods.

| 3rd period (182–210 days of age) | P-value | 5th period (238–266 days of age) | P-value | 7th period (294–322 days of age) | P-value |
|----------------------------------|---------|----------------------------------|---------|----------------------------------|---------|
| Egg weight (g)                   |         |                                  |         |                                  |         |
| Control                          | 60.59 ± 4.01 | NS                                 | 62.83 ± 3.93 | NS                                 | 63.39 ± 4.77 | NS      |
| Exp.                             | 59.97 ± 4.75 | NS                                 | 62.09 ± 4.27 | NS                                 | 61.27 ± 4.75 |         |
| Yolk weight (g)                  | 16.13 ± 1.47 | NS                                 | 16.65 ± 1.49 | NS                                 | 16.76 ± 1.46 | NS      |
| Control                          | 15.77 ± 1.20 | NS                                 | 16.42 ± 1.41 | NS                                 | 16.10 ± 1.52 |         |
| Exp.                             | 44.80 ± 9.39 | NS                                 | 46.03 ± 10.70 | NS                                 | 42.00 ± 08.38 | NS      |
| Eggshell strength (N)            | 43.17 ± 8.33 | NS                                 | 48.37 ± 11.62 | NS                                 | 40.43 ± 10.66 |         |
| Shape Index of egg               | 1.28 ± 0.05 | NS                                 | 1.29 ± 0.05 | NS                                 | 1.29 ± 0.05 | NS      |
| Control                          | 1.28 ± 0.04 | NS                                 | 1.29 ± 0.05 | NS                                 | 1.30 ± 0.04 |         |
| Exp.                             | 0.34 ± 0.04 | NS                                 | 0.36 ± 0.04 | NS                                 | 0.35 ± 0.03 | NS      |
| Haugh unit                       | 0.33 ± 0.03 | NS                                 | 0.35 ± 0.02 | NS                                 | 0.35 ± 0.02 |         |
| Control                          | 89.10 ± 8.60 | NS                                 | 92.50 ± 4.82 | NS                                 | 93.87 ± 7.11 | NS      |
| Exp.                             | 88.53 ± 7.67 | NS                                 | 91.97 ± 9.12 | NS                                 | 90.83 ± 9.11 |         |
| Colour of egg yolk               | 8.27 ± 0.69 | 0.05                               | 8.83 ± 0.87 | 0.05                               | 0.97 ± 1.10 | 0.05    |
| Control                          | 9.40 ± 0.77 | 0.05                               | 10.93 ± 1.34 | 0.05                               | 11.97 ± 0.96 |         |
| Exp.                             | 4.00 ± 0.74 | NS                                 | 4.07 ± 0.83 | NS                                 | 4.13 ± 0.73 | NS      |
| Eggshell colour                  | 4.27 ± 0.74 | NS                                 | 4.03 ± 0.72 | NS                                 | 4.27 ± 0.74 |         |
| Blood spot (egg)                 | 0.10 ± 0.31 | NS                                 | 0.13 ± 0.35 | NS                                 | 0.07 ± 0.25 | NS      |
| Control                          | 0.17 ± 0.38 | NS                                 | 0.03 ± 0.18 | NS                                 | 0.07 ± 0.25 |         |

Note: NS, not significant.

Means within the same column with different superscripts differ (P < .05).

reported that double yolks appear when ovulation occurs too rapidly, or when one yolk somehow gets stuck before shell- ing and is joined by the next yolk. Double yolk eggs may be laid by a pullet whose productive cycle is not yet well synchronized. Double shelled eggs are so rare and it might be related to very active shell glands.

The classification of eggs weights (Table 7) laid over the whole laying period, the average egg weight 60 g was similar in both groups of hens with a small difference. On the contrary, Englmaierova et al. (2013) reported that Isa Brown hens fed diets supplemented with synthetic carotenoids such as lutein had significantly increased egg weight. However, the results obtained in the present study confirm the earlier finding by Hasanuzzaman et al. (2012) who reported that egg weight, yolk weight, shell strength, Haugh units, eggshell and blood spots. The observations recorded in the present study are in agreement with those reported by Hasanuzzaman et al. (2012) who did not find any significant differences in values of external and internal quality traits for: the egg weight, yolk weight, eggshell strength, shape index of egg, shell thickness, Haugh unit and shell colour in control group compared with experimental group which was fed diets with 6.5% of sea buckthorn. Similar results were also observed in another experiment (Hasanuzzaman et al. 2011) in which no significant effect was found on the qualitative evaluation of eggs value for: the weight of eggs, yolk weight, shell strength, Haugh units, eggshell and blood spots. The observations recorded in the present study are in agreement with those reported by Hasanuzzaman et al. (2012) who did not find any significant differences in values of external and internal quality traits for: the egg weight, yolk weight, eggshell strength, shape index of egg, shell thickness, Haugh unit and shell colour in control group compared with experimental group which was fed diets with 6.5% of sea buckthorn. Similar results were also observed in another experiment (Hasanuzzaman et al. 2011) in which no significant effect was found on the qualitative evaluation of eggs value for: the egg weight, yolk weight, eggshell strength, shape index of egg, shell thickness, Haugh unit and shell colour in layers fed diets with 10% CP of control feed replaced with CP of sea buckthorn.

Egg yolk colour of the experimental group was significantly (P ≤ .05) darker compared to the control group. The enhanced colour of egg yolk is associated with the increased level of plant pigments in the sea buckthorn fruit residues diets which represents a rich source of carotenoids in feed. Our assumption is in agreement with Beardsworth and Hernandez (2004) and
Hamelin and Altemueller (2012) who reported that yolk colour is mainly determined in the laying hens by the content of carotenoids presented in the feed. Moreover, the obtained results also correspond with Dumbrava et al. (2006) who indicated that adding sea buckthorn berry flour in the hen’s feed in a relatively small proportion had a favourable effect on the carotenoids’ accumulation in yolk, especially zeaxanthin and lutein. However, these results are very important as the attractiveness of eggs increases in respect to consumer demands of dark yolk colour (Beardsworth & Hernandez 2004).

4. Conclusion

The results of our study concluded that the replacement of 5% of the feed wheat with sea buckthorn fruit residues significantly affected the total number of laid eggs as well as the egg yolk colour being darker which is more favoured by consumers.

The other qualitative characteristics of eggs and performance did not differ. However, further studies on the effect of sea buckthorn are needed.

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