Synergistic effect of feeding *Aspergillus awamori* and lactic acid bacteria on performance, egg traits, egg yolk cholesterol and fatty acid profile in laying hens

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**ABSTRACT**

This study was conducted to examine the effects of a combined supplementation of *Aspergillus awamori* (AA) and lactic acid bacteria (LAB) in feed on growth and egg quality. Hens (28-week old) were fed on a basal diet as control group; diets supplemented with 0.05% AA, 0.10% LAB, or a combination of AA and LAB (6 birds/group) for 6 weeks. The growth performance of the birds was improved by all the treatments. Synergistic effects of AA and LAB were observed on feed intake, egg production, total egg weight and feed conversion (p < .05). Weights and heights of yolk and albumin was not affected by treatment while, yolk fat, shell weight and thickness were increased (p < .05). On the other hand, egg yolk total cholesterol was decreased and synergistically by the combination of AA and LAB (p < .05). Serum glucose, total cholesterol, ALT and triglyceride were reduced by all the treatment groups. Conversely, serum superoxide dismutase (SOD) was synergistically increased by the combination. Ca, P and Zn concentration in yolk was increased by AA and LAB and synergistically increased by the combination (p < .05). Interestingly, saturated fatty acids (SFA) were decreased while; unsaturated fatty acids (USFA) were increased in egg yolk in all groups. In conclusion, the combined supplementation of AA and LAB synergistically had no effect on the growth of laying hens. In addition, AA and LAB modify the egg yolk fatty acid profile by increasing unsaturated fatty acid and reducing saturated fatty acid.

**ARTICLE HISTORY**

Received 15 May 2016
Revised 21 October 2016
Accepted 9 November 2016

**KEYWORDS**

*Aspergillus awamori*; hens; fatty acids; growth; lactic acid bacteria

**Introduction**

According to FAO and WHO probiotics are ‘live micro-organisms’ (Anonymous 2002). The use of these organisms in order to sustain appropriate homeostasis of the digestive tract and protect it against pathogenic micro-flora is a common practice in poultry production worldwide, according to Yu et al. (2008). The feeding of probiotics to laying hens has been found to improve: nutrient digestibilities and therefore, bird performance such as feed efficiency and egg production (Mohan et al. 1995; Nahashon et al. 1996; Jin et al. 1997; Balevi et al. 2001; Davis & Anderson 2002). Also, probiotics was shown to modulate the intestinal micro-flora by controlling the growth of the pathogenic bacteria (Jin et al. 1997; Yu et al. 2008). Moreover, feeding probiotics may also play good role in altering the lipid metabolism of broilers and hens as various studies have shown that probiotics could reduce total cholesterol and triglyceride contents of egg yolk (Mohan et al. 1995) and serum (Kalavathy et al. 2009; Saleh et al. 2012a).

Currently many species are being used in probiotic preparations. These are mostly lactic acid bacteria (LAB), *Aspergillus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus thermophilus*, *Enterococcus faecalis*, *Bifidobacterium* spp. and *Escherichia coli*. All the previous strains are intestinal strains except *Lactobacillus bulgaricus* and *Streptococcus thermophilus* which are considered as yoghurt starter organisms (Fuller 1989).

*Aspergillus awamori* (AA) and LAB have been used as probiotics by many studies (Jin et al. 1997;
Saleh et al. 2012a, 2012b). Until recently, information on an AA based probiotic has been lacking, although its application in poultry production is increasing (Saleh 2014). AA is a fungus that has long been used for food processing in Japan. The products processed by AA are considered Generally Recognized as Safe by the U.S. Food and Drug Administration (Bigelis & Lasure 1987). LAB live symbiotically in the digestive tract of birds were found to enhance the immunity of the host by controlling the pathogenic bacteria (Zulkifli et al. 2000). LAB have adhesive properties and can colonise different parts of the avian digestive tract (Jin et al. 1997). LAB can produce lactic acid from metabolising glucose, which acidizes the digestive milieu, and bactericides which inhibit pathogenic bacteria such as E. coli, and Listeria (Patterson & Burkholder 2003).

In the present study we aimed to explore AA and LAB as probiotic strains and study the synergistic effects of feeding AA and LAB on growth, egg quality and fatty acid profiles in egg yolk were examined in laying hens. In addition, the serum lipids and antioxidative activities of AA and LAB were shown.

**Materials and methods**

The experiment was carried out at the experimental poultry farm of the Department of Animal Nutrition Faculty of Agro-biology and Food Resources Slovak University of Agriculture in Nitra (Slovak Republic). All the procedures used in this experiment were approved by Animal Ethics Committee at the University of Agriculture in Nitra City (Slovak Republic).

**Animals and diets**

A total of twenty four 28-week-old Hy-Line Brown pullets with almost the same body weight (BW) were randomly divided to four treatment groups. Each treatment had six laying hens that were individually caged in Big Dutchman double-sided divided into three-tier battery cages (40 × 35 × 60 cm, with a floor slope of 12°; 740 cm2 per hen). The birds at 22 week of age were obtained from local commercial flocks and were previously vaccinated for infectious bronchitis, Newcastle disease virus, also for egg drop syndrome disease. All hens were housed in a windowless and environmentally controlled room, with room temperature kept at 24 to 26 °C. Light cycle was 14h/d. Each cage was equipped with an individual nipple drinker. A continuous, metal small trough was divided by replicate to ensure that the hens were not able to consume feed assigned to the adjoining replicates.

The trial started at 28 weeks of age, and it lasted for 6 weeks (34 weeks of age). Control group hens received a corn-soybean basal diet with no additives. The basal diet was formulated to either meet or exceed the commercial layer requirements (CP: 17% and ME; 2750 kcal/kg). In the other three groups, depending on probiotic type addition, they were labelled as follows: basal diet supplemented with AA 0.05%, LAB 0.10% or AA 0.05% + LAB 0.10% for treatments two, three and four, respectively. The basal diet was in mash form and was formulated to meet 28- to 34-week-old brown layer nutritional requirements, as proposed by Hy-Line Breeder Company (Hy-Line International, 2010). Experimental diets and water were provided ad libitum throughout the study. The fungi and bacteria were mixed in the basal diet. The number of Aspergillus spores was about 25 × 10^4/g diets. The LAB used in the study contained different strains of bacteria such as Lactobacillus paracasei (KKP 824), L. rhamnosus (KKP 825) and L. rhamnosus (KKP 826) and the concentration was (6.7 × 10^8 cfu/g) at 1:2:2 ratios from the Institute of Agricultural and Food Biotechnology, Warsaw. The inclusion levels of AA (0.05%) and LAB (0.10%) were decided according to our previous study (Saleh et al. 2013) and preliminary experiments respectively.

**Traits recorded and methods**

**Applied: laying performance**

The birds were weighed at the commencement (28 weeks of age) and the end (34 weeks of age) of the experiment. Eggs were collected daily and egg production was expressed on a hen-day basis (% hens-day). Individual egg weights were recorded then used to calculate mean egg weight for all experimental period. The total egg mass was calculated by multiplying egg weights by egg production. Feed intake was measured on a cage basis (hen) every week. Daily feed intake per bird was calculated on a cage total feed intake basis for the entire experimental period and for the number of days in all the period. Feed conversion ratio (kg of feed/kg of eggs) for the all period was calculated on a cage basis from egg production, egg weight, and feed consumption.

**Quality of eggs and yolk minerals**

Egg and eggshell quality examinations (shell weight, egg shell thickness, yolk and albumin weights, yolk colour, albumen and yolk heights) were undertaken at 28 weeks of age and then at the end of experiment. For this purpose, five eggs laid between 0800 and
1200 h were randomly picked from each group on the first day of 28 and 34 weeks of age (a total of 10 eggs per group during the experiment). Eggs were weighed individually and a specific gravity of eggs, as an index of shell thickness, was measured. After the eggs had been broken on the plate measurement stand EQM, the height of albumen and yolk was measured. Yolk colour intensity was evaluated and recorded according to the Roche yolk colour fan method. Albumen weight was calculated by subtracting the weights of yolk and shell from the whole egg weight. To measured shell weight, eggshells were cleaned of any adhering albumen then the membrane was removed; eggshells were then dried at room temperature and expressed as a percentage of the whole egg. An egg quality evaluation was performed on individual eggs, similarly to egg weight measured. Egg yolk minerals concentration (Ca, P, Mg, Na, K, Cu, Fe, Mn and Zn) were measured by the digestion method. Egg yolk samples were analysed for dry matter content (DM) by drying samples at 105 °C for 24 h in forced air oven. The crude protein was determined by Kjeldahl method (AOAC 1995) and crude fat was determined by Soxhlet method (AOAC 1995).

Total cholesterol and fatty acids profile were determined in fat separated via extraction from the egg yolk with a mix of chloroform and methanol (2:1 vol: vol; according to Folch et al. 1957). Cholesterol was separated from fat after saponification with KOH according to the modified method of the International Dairy Federation (1992). The samples were subjected to chromatographic analysis in a PU-4600 (PyeUnican, Cambridge, UK) chromatograph with flame ionisation detectors, using the following conditions: the length of a glass column, 1 m; internal diameter, 4 mm; temperature: in detector, 300 °C; at injector, 290 °C; in column, 260 °C; carrier gas, argon; flow rate, 50 cm 3/min and internal standard Dotriacontane (Sigma, St. Louis, MO). Egg yolk cholesterol was calculated and expressed as milligrams/gram of yolk. Fatty acids profile was identified based on retention times and expressed as a percentage (wt/wt) of total FA.

**Serum biochemistry**

Blood samples for serum parameters were collected into tubes and for antioxidant parameters into heparinised tubes. The serum was separated from whole blood by centrifugation at 3000g for 30 min. The concentrations of serum parameters [glucose, total cholesterol (TC), total proteins (TP), triglycerides, also, the minerals content in serum as like calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chlorides (Cl), alanine amino-transferase (ALT), alkaline-phosphatase (ALP) and gamma glutamyl transferase (GGT)] were analysed. Ecoline kits on automatic analyser RX Monza (Randox Labs., Crumlin, UK), spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc., Waltham, MA) were used according to manufacturer condition.

The activities of an antioxidant enzyme superoxide dismutase (SOD) and albumins content were assayed by spectrophotometer Genesis 10 (using antioxidant RANDOX kits (Randox Labs., Crumlin, UK) according to the manufacturer’s instructions. Content of bilirubin was analysed spectrofotometrically by RX Monza device (Randox Labs., Crumlin, UK) using DiaSys kit (Diagnostic Systems GmbH, Holzheim, Germany).

**Statistical analysis**

The differences among treatments were statistically analysed with two-way analysis of variance (ANOVA) test in a completely randomised design using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). Repeated measures ANOVA (six replicates in one treatment group) were applied to show main effects of AA and LAB and their interaction. Tukey’s multiple comparison test was used to identify which treatments conditions were significantly different from each other at a significance level of $p < .05$.

**Results and discussion**

**Laying performance**

Laying hens’ growth performance is reported in Table 1. There were no differences in the average body weight gain of hens between experimental treatments. However, feed intake was decreased ($p < .05$), by the combination of AA and LAB synergistically decreased feed intake. Feeding AA and LAB significantly increased egg weight, egg mass and egg production. Thus, the feed conversion ratio was improved in all the treatment groups and synergistically improved by the combination of AA and LAB. The improvement of growth performance by feeding AA appears to result from an increase in the feed efficiency of laying hens and metabolisable energy from the diet. The AA possesses the ability to digest raw starches according to (Amsal et al. 1999) and to produce cellulase and xylanase, which are required for the digestion of soluble non-starch polysaccharides (Bhat & Hazlewood 2001). In addition, Aspergillus could improve the nutritional quality of soybean meal.
because the high level of trypsin inhibitor contained in unprocessed soybean is degraded by *Aspergillus* spp. (Hong et al. 2004). Similarly to the results obtained in this experiment, Tortuero and Fernandez (1995) found a significant improvement in performance, egg production in hens supplemented with a product containing a mixture of probiotic, including *Lactobacillus* and *Bacillus*. Conversely, Davis and Anderson (2002) found that no improvement in egg production in laying hens supplemented with probiotic bacteria, including *Lactobacillus* and *Bacillus*. This is also in agreement with Kalavathy et al. (2009) who reported that the supplementation of probiotic had no significant effect on egg production in hens. Egg production in hens fed with probiotic content of bacteria and fungi was not significantly different from control hens aged from 40 to 52 week (Balevi et al. 2001), but it was significantly higher during the late laying period, from 54 to 65 week of age (Yoruk et al. 2004).

**Egg components and egg quality**

The effects of dietary supplementation with AA and LAB on egg quality and egg components in laying hens are presented in Table 2. There were no differences found between the treatment groups with respect to egg and yolk weights, albumin height and yolk crude protein. However, shell thickness, yolk colour, yolk height and yolk crude fat was synergistically increased by the combination of AA and LAB. On the other hand, yolk cholesterol content was decreased (p < .05). The improvement in egg quality due to the probiotic supplementation as reported in the present study was primarily reflected by an increase in egg weight, including eggshell proportion. Similar significant results in egg weight and eggshell quality were also obtained in hens fed diets with a mixture of probiotic content *Lactobacillus* cultures (Davis & Anderson 2002; Kalavathy et al. 2009). Also, Miles et al. (1981) showed that feeding live *Lactobacillus acidophilus* culture resulted significant increase in egg production probiotic inclusion did not influence the egg weight significantly, which has already been reported by Mohan et al. (1995); Haddadin et al. (1996) and Chen and Chen (2003). But there are also some reports which have different opinions (Tortuero & Fernandez 1995), this difference might be related to the type and strain of probiotic used.

### Table 1. Effects of feeding *Aspergillus awamori* and/or lactic acid bacteria on growth performance in laying hens.

| Treatments | Initial body weight, g |
|------------|------------------------|
| Control    | 1695                   |
| AA         | 1745                   |
| LAB        | 1797                   |
| AA + LAB   | 1668                   |
| SEM        | 65                     |
| NS         | NS                     |
| NS         | NS                     |
| NS         | NS                     |

| Treatments | Body weight gain, g/30 day |
|------------|----------------------------|
| Control    | 103                       |
| AA         | 125                       |
| LAB        | 73                        |
| AA + LAB   | 110                       |
| SEM        | 24                      |
| NS         | NS                     |
| NS         | NS                     |
| NS         | NS                     |

| Treatments | Feed intake, g/day |
|------------|-------------------|
| Control    | 152               |
| AA         | 140               |
| LAB        | 141              |
| AA + LAB   | 131              |
| SEM        | 1.58             |
| NS         | NS                |
| NS         | NS                |
| NS         | NS                |

| Treatments | Egg production HD, % |
|------------|----------------------|
| Control    | 78.90                |
| AA         | 81.10                |
| LAB        | 77.20                |
| AA + LAB   | 83.30                |
| SEM        | 0.57                |
| NS         | *                   |
| NS         | NS                   |
| NS         | NS                   |

| Treatments | Total egg weight, g |
|------------|---------------------|
| Control    | 6523                |
| AA         | 7224                |
| LAB        | 6547                |
| AA + LAB   | 6621                |
| SEM        | 2.80                |
| **          | **                  |
| **          | **                  |
| **          | **                  |

| Treatments | Egg mass, g of egg/hen |
|------------|------------------------|
| Control    | 5147                  |
| AA         | 5859                  |
| LAB        | 5055                  |
| AA + LAB   | 5516                  |
| SEM        | 37                   |
| **          | **                  |
| NS         | NS                   |

| Treatments | FCR, kg of feed consumed/kg of egg produced |
|------------|--------------------------------------------|
| Control    | 2.95                                     |
| AA         | 2.39                                     |
| LAB        | 2.79                                     |
| AA + LAB   | 2.38                                     |
| SEM        | 0.02                                     |
| NS         | NS                                       |
| NS         | NS                                       |

### Table 2. Effects of feeding *Aspergillus awamori* and/or lactic acid bacteria on egg quality in laying hens.

| Treatments | Egg weight, g |
|------------|---------------|
| Control    | 51.03         |
| AA         | 52.44         |
| LAB        | 52.73         |
| AA + LAB   | 55.26         |
| SEM        | 0.80          |
| NS         | NS            |
| NS         | NS            |
| NS         | NS            |

| Treatments | Yolk weight, g |
|------------|---------------|
| Control    | 11.20         |
| AA         | 12.81         |
| LAB        | 12.06         |
| AA + LAB   | 14.99         |
| SEM        | 0.30          |
| NS         | NS            |
| NS         | NS            |
| NS         | NS            |

| Treatments | Albumen weight, g |
|------------|------------------|
| Control    | 34.49            |
| AA         | 34.95            |
| LAB        | 35.43            |
| AA + LAB   | 36.34            |
| SEM        | 0.73             |
| NS         | NS               |
| NS         | NS               |
| NS         | NS               |

| Treatments | Shell weight, g |
|------------|----------------|
| Control    | 4.94            |
| AA         | 5.27            |
| LAB        | 5.43            |
| AA + LAB   | 5.13            |
| SEM        | 0.20            |
| **          | **              |
| **          | **              |
| NS         | NS               |

| Treatments | Yolk colour |
|------------|------------|
| Control    | 7.14       |
| AA         | 7.50       |
| LAB        | 7.50       |
| AA + LAB   | 7.70       |
| SEM        | 0.20       |
| NS         | NS         |
| NS         | NS         |

| Treatments | Shell thickness, mm |
|------------|---------------------|
| Control    | 0.33                |
| AA         | 0.35                |
| LAB        | 0.35                |
| AA + LAB   | 0.36                |
| SEM        | 0.01                |
| **          | **                  |
| **          | **                  |
| NS         | NS                   |

| Treatments | Yolk height, mm |
|------------|----------------|
| Control    | 17.92           |
| AA         | 18.20           |
| LAB        | 18.30           |
| AA + LAB   | 18.70           |
| SEM        | 0.30            |
| NS         | NS               |
| NS         | NS               |
| NS         | NS               |

| Treatments | Albumen height, mm |
|------------|--------------------|
| Control    | 7.14               |
| AA         | 7.47               |
| LAB        | 7.28               |
| AA + LAB   | 7.26               |
| SEM        | 0.20               |
| NS         | NS                 |
| NS         | NS                 |
| NS         | NS                 |

| Treatments | Yolk crude protein, % of DM |
|------------|----------------------------|
| Control    | 30.90                      |
| AA         | 30.90                      |
| LAB        | 30.80                      |
| AA + LAB   | 31.20                      |
| SEM        | 0.20                       |
| NS         | NS                         |
| NS         | NS                         |
| NS         | NS                         |

| Treatments | Yolk cholesterol, mg/g⁻¹ |
|------------|-------------------------|
| Control    | 28.60                    |
| AA         | 24.30                    |
| LAB        | 24.10                    |
| AA + LAB   | 23.00                    |
| SEM        | 0.53                     |
| **          | **                       |
| **          | **                       |
| NS         | NS                       |

AA and LAB were added to the basal diet at levels of 0.05% and 0.10%, respectively. Data were analysed by two-way analysis of variance and Tukey’s multiple comparison test was used to identify which treatments conditions were significantly different from each other at a significance level of p < .05. Means within a row not sharing a common superscript significantly differ from each other.

NS: not significant (p > .05).

*p < .05.
**p < .01.
probiotics used, or the concentration and the form of bacteria used (viability, dryness or their products). Tortuero and Fernandez (1995) showed that using vital biomass of probiotic supplements affects the egg weight significantly. Complementary reports by the Nahashon et al. (1996) and Haddadin et al. (1996) suggested that addition of biological additives did not influence the egg weight significantly. These controversial results might be related to the dosages of probiotic and concentration of bacteria used in the diet.

On the other hand, addition of probiotic had no significant effect (p > .05) on shell hardness and shell thickness and these were expected which have already been reported (Haddadin et al. 1996; Chen & Chen 2003). Although, the increase of albumen quality was not significant (p > .05), no reasonable explanation can be offered for the improvement in albumen quality in the microbial additive groups. Jensen et al. (1978) found significant improvements in interior egg quality which measured by Hough Units in hens fed distillers feeds and corn fermentation soluble. Subsequent studies indicated that trace elements had been involved (Jensen & Maurice 1978). But Tortuero and Fernandez (1995) described that the difference in plasma mineral concentration were not sufficient to implicate supporting the hypothesis that trace elements were improved albumen quality with microbial supplementation. Addition of probiotic had significant effect on egg yolk cholesterol (mg/g of yolk). Haddadin et al. (1996) observed similar results. They reported that inclusion of probiotic Lactobacillus acidophilus in different ages (40, 44 and 48 weeks) affects egg cholesterol in 40 weeks of production not 44 and 48. These results have already been confirmed by Marks and Washburn (1991) reports.

**Blood parameters**

The effects of dietary AA and LAB supplementation on serum parameters are summarised in Table 3. Serum glucose, ALT and triglyceride were decreased by feeding AA or LAB but not significant by the combination of AA and LAB. However, serum total cholesterol was decreased in all supplementation groups and synergistically by the combination of AA and LAB. Serum AST and ALP were not influenced by probiotics feeding. Serum SOD was synergistically increased by the combination of AA and LAB. Several studies have concluded that diets containing probiotic had a negative effect on plasma total cholesterol and triglycerides in chickens (Mohan et al. 1995). Mohan et al. (1995) reported that the supplementation of probiotic bacteria resulted in decreasing the serum total cholesterol level in white leghorn layers from 176.5 to 114.3 mg/dl. Hajjaj et al. (2005) and Saleh et al. (2012b) reported that the mechanism underlying the cholesterol-lowering effect of AA could be due to the inhibition of 3-hydroxyl-3-methylglutaryl-coenzyme (HMG-CoA) reductase. Statin is a well-known HMG-CoA reductase inhibitor which extracted from a fungus, Penicillium citrinum (Endo 1985). It has been recognised as safe and has been long used to treat patients with hypercholesterolaemia (Serruys et al. 2002). A HMG-CoA reductase inhibitor produced by AA might be responsible for the decrease in abdominal fat deposition. In addition, AA might affect fat deposition by influencing the activity of hormone-sensitive lipase and malate dehydrogenase enzyme in adipose tissues (Mersmann 1998). Also, one of the purported mechanism through which probiotic culture may exert its hypocholesterolemic action is via bile acids as reported by (Klaver & Van-Der-Meer 1993). Cholic and deoxycholic bile acids are produced from cholesterol by hepatocytes and are conjugated with taurine, respectively. These acids enter the small intestine, where they are absorbed and directed to the bird liver, and a decrease in bile acid recycling would ultimately result in lowering serum cholesterol concentrations because cholesterol is used for bile acid synthesis (Stonge et al. 2000).

### Table 3. Effects of feeding Aspergillus awamori and/or lactic acid bacteria on blood parameters (mg/ml) in laying hens.

| Blood parameters | Control AA | LAB AA | AA + LAB | SEM | AA LAB | AA X LAB |
|------------------|------------|--------|----------|-----|--------|----------|
| Serum glucose    | 15.20a     | 13.32b | 13.28b   | 13.50b | 0.39   | * * NS   |
| Serum AST        | 3.25       | 3.31   | 3.63     | 3.30 | 0.17   | NS NS NS |
| Serum ALT        | 0.22a      | 0.14b  | 0.13b    | 0.12b | 0.03   | * * NS   |
| Serum ALP        | 2.44       | 2.90   | 3.58     | 1.63 | 0.62   | NS NS NS |
| Serum cholesterol| 5.39a      | 3.77b  | 3.05b    | 2.64b | 0.49   | * * **   |
| Serum BILI       | 7.60a      | 5.40b  | 10.60b   | 12.60b | 1.23   | * NS *   |
| Serum TG         | 23.30      | 13.90b | 13.50b   | 15.60b | 2.32   | * * NS   |
| Serum SOD        | 55.50c     | 69.30b | 76.30a   | 79.60a | 3.52   | * ** NS  |

AA and LAB were added to the basal diet at levels of 0.05% and 0.10%, respectively. Tukey’s multiple comparison test was used to identify which treatments conditions were significantly different from each other at a significance level of p < .05. Means within a row not sharing a common superscript significantly differ from each other.

NS: not significant (p > .05).

* p < .05.

** p < .01.
Lipid peroxidation is a process of vital importance to the egg processing industry as it adversely affects the nutritional value, taste and the sensory quality of food (Botsoglou et al. 1997). Furthermore, Saleh et al. (2012b) reported that muscle thiobarbituric acid reactive substances (TBARS) decreased and muscle \( \alpha \)-tocopherol content increased by feeding AA (0.05%), indicating that the fungus has antioxidative properties. These results indicate that AA produces antioxidative substances. In addition, feeding diets containing probiotics increased the mRNA expressions of antioxidant enzymes (i.e. GPX; catalase and superoxide dismutase). These results suggest that probiotics can act as an antioxidant when fed to broiler chickens (Saleh et al. 2013).

Egg yolk fatty acid profile and minerals content

Effect of feeding AA or LAB and their combination on yolk fatty acids profile in laying hens were presented in Table 4. Palmitic acid and stearic acid were decreased by feeding AA or LAB. On the other hand, oleic acid and arachidonic acid were all increased by feeding AA and LAB and synergistically increased by the combination of AA and LAB. Linoleic acid was increased by feeding LAB and combination of AA and LAB while, \( \gamma \)-linolenic acid were not influenced by supplemented groups. So that the poly-unsaturated fatty acids (PUSFA) were increased and saturated fatty acids (SFA) were decreased. Several studies have shown that oleic acid (n-9) and linoleic acid (n-6) are the most common unsaturated fatty acids produced by different types of probiotics and linoleic acid is a major constituent of fungal and bacteria lipid (Mazur et al. 1991; Calvo et al. 2001; Richard et al. 2004; Tsitsigiannis et al. 2004). Thus, it is probable that the increase in oleic, linoleic and alpha-linolenic acids in the egg yolk may be due to the intestinal activities of AA and LAB. This may also refer to that AA produces desaturase which may change saturated fatty acid to unsaturated fatty acids (Richard et al. 2004; Saleh et al. 2013).

Effects of feeding AA, LAB, and the combination AA and LAB on egg yolk minerals content were presented in Table 5. Egg yolk Ca, P and Zn content were increased by feeding AA and LAB however, Mg, Na, K, Cu, Fe and Mn were not affected by feeding AA and LAB. The increase in Ca and Zn contents in yolk may be due to the hypothesis that an acidic environment created in the digestive tract by AA and LAB facilitates the ionisation of minerals and improves their absorption (Haddadin et al. 1996). Mikulski et al. (2012) found that increasing in Ca retention in hens fed probiotic-supplemented diets.

Conclusions

Diets supplemented with AA and LAB showed a potential for improving egg weight, feed efficiency...
Table 5. Effects of feeding *Aspergillus awamori* and/or lactic acid bacteria on egg yolk minerals content (mg/Kg \(^{-1}\)) in laying hens.

| Treatments | Main Effects |
|-------------|--------------|
|             | Control | AA | LAB | AA + LAB | SEM | AA | LAB | AA X LAB |
| Ca          | 2.17\(^\text{a}\) | 2.38\(^\text{a}\) | 2.39\(^\text{a}\) | 2.54\(^\text{a}\) | 0.13 | *   | *   | *        |
| P           | 8.31\(^\text{b}\) | 8.56\(^\text{b}\) | 8.89\(^\text{b}\) | 9.74\(^\text{a}\) | 0.29 | NS  | NS  | *        |
| Mg          | 242      | 221 | 213 | 221      | 30  | NS  | NS  | NS       |
| Na          | 1.03     | 1.07 | 0.91 | 1.00     | 0.23 | NS  | NS  | NS       |
| K           | 3.16     | 3.16 | 2.85 | 2.84     | 0.66 | NS  | NS  | NS       |
| Cu          | 7.60\(^\text{a}\) | 9.40\(^\text{a}\) | 8.30\(^\text{b}\) | 8.20\(^\text{b}\) | 1.35 | *   | NS  | NS       |
| Fe          | 174      | 175 | 170 | 176      | 60  | NS  | NS  | NS       |
| Mn          | 2.20     | 2.30 | 2   | 2.10     | 0.50 | NS  | NS  | NS       |
| Zn          | 72\(^\text{b}\) | 77\(^\text{b}\) | 80\(^b\) | 85\(^\text{b}\) | 15  | *   | *   | *        |

AA and LAB were added to the basal diet at levels of 0.05% and 0.10%, respectively. Tukey’s multiple comparison test was used to identify which treatments conditions were significantly different from each other at a significance level of \(p < .05\). Means within a row not sharing a common superscript significantly differ from each other.

NS: not significant (\(p > .05\)).

\(^{a}\)\(^{p} < .05\).

\(^{b}\)\(^{p} < .01\).

and eggshell quality during the early laying period. Furthermore, the dietary supplementation of these types of probiotic resulted in a decrease in yolk cholesterol levels and increase in unsaturated fatty acids by the collective addition of AA and LAB to laying hen diets.

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through Research Group Project No. RGP-273.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding

This work was supported by the Deanship of Scientific Research at King Saud University, Riyadh, Saudi Arabia and Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and Slovak Academy of Sciences (project n. 1/0723/15).

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