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Effects of dietary Jerusalem artichoke (*Helianthus tuberosus*) tuber powder and medium/long-chain fatty acids on production performance and fatty acid profile in laying hens

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ABSTRACT

This study considers the use of Jerusalem artichoke (*Helianthus tuberosus*) tuber powder (HTT) and medium/long-chain fatty acids (MLCFAs) in the nutrition of laying hens. A total of forty; 30-week-old laying hens were randomly distributed into four groups: control (C), 0.1% MLCFAs (T1), 2.0% HTT (T2), and both 0.1% MLCFAs and 2.0% HTT (T3). The fatty acid (FA) profile of eggs was analysed by gas chromatography, cholesterol by HPLC, and egg quality using a multifunctional automatic egg analyzer; production parameters of hens were also analysed. The results revealed that MLCFAs had a significant effect on accumulation of α-linolenic acid (C18:3) and docosahexaenoic acid (C22:6) in egg yolk, but a higher impact on addition of HTT and MLCFAs was observed. Omega-3 FAs and omega-6/omega-3 FA ratio in egg yolk increased significantly on addition of MLCFAs alone or in combination with HTT. There were no significant effects of HTT and/or MLCFAs on body weight, feed intake, feed conversion ratio or egg mass output of laying hens. In addition, for HTT alone or in combination with MLCFAs, egg shell breaking strength was increased significantly (by 2%). A significant increase of Haugh unit was determined in the MLCFA group and in the HTT group. A significant decrease of cholesterol level was observed in all experimental groups. Supplementing laying hens' feed with HTT and MLCFAs facilitated the production of FAs-enriched eggs. Slight effects of HTT and MLCFAs on egg quality, except Haugh unit, yolk cholesterol level and eggshell strength) and production performance of laying hens were observed.

Keywords: Egg quality, Fatty acid profile, Jerusalem artichoke (*Helianthus tuberosus* L.), Laying hens, Medium chain fatty acids

Intensive egg production of laying hens is focused on maximizing productivity parameters such as laying performance, feed conversion and egg quality. Proper nutrition of the birds is the key factor to reach the goal. Since the European Union enforced the ban on antibiotic growth promoters, higher nutritional awareness in consumers and an increasing popularity of healthy life styles have focused scientific interest on natural substances as antibiotic alternatives (Buclaw 2017).

Prebiotics are considered as non-digestible compounds that, through their metabolisation by microorganisms in the gut, modulate the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host (Bindels *et al.* 2015). Health effects of prebiotics include benefits to the gastrointestinal tract (inhibition of pathogens, immune stimulation), cardio metabolism (reduction in blood lipid levels, effects upon insulin resistance), mental health (metabolites that influence brain function, energy and cognition) and bone structure (mineral bioavailability) (Gibson *et al.* 2017).

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Inulin is one of the prebiotics; it occurs naturally in many plants as a storage material (Buclaw 2017). Jerusalem artichoke (*Helianthus tuberosus* L.) tubers (HTT) accumulate inulin, macro-/microelements, vitamin B complex, vitamin C and β-carotene (Radovanovic *et al.* 2015).

Poultry, including laying hens, respond to prebiotics despite having a short large intestine (Tang *et al.* 2017). The consumption of prebiotic inulin-type fructans reduces the activity of some liver enzymes which are involved in the synthesis of fatty acids (Aparecida dos Reis *et al.* 2015); for this reason, the combination of inulin-based prebiotics and medium/long-chain fatty acids (MLCFAs) as a feed supplement can be very promising. Also, FAs (fatty acids) supplements can have an influence on egg yolk FAs composition, and manipulation of FAs in egg yolks may provide a possibility to improve the nutritive value of eggs (Alagawany *et al.* 2018).

There is no published scientific research based on application of both inulin, produced from Jerusalem artichoke (*H. tuberosus*) tubers, and MLCFAs on laying hens' production and egg quality with a focus on FAenriched eggs. The aim of the study was to evaluate the effect of *H. tuberosus* L. and MLCFAs on laying hens' productivity and egg quality characteristics.

MATERIALS AND METHODS

Experimental design: The experiment was performed in 2018 and a total of forty; 30-week-old Lohmann Brown laying hens with similar body weights were selected for this study. The hens were housed for 8 weeks individually in wire cages (40 cm × 45 cm × 50 cm) in a room at 22°C temperature and with a 16 h/8 h dark cycle. The compound feed was provided at 125 g/day individually, and water was provided *ad lib*. The feed was formulated as per NRC (1994) to meet the nutrient requirements of laying hens. The laying hens were randomly distributed into four groups; each group consisted of 10 hens. Diets were supplemented with nothing (control (C)), 0.1% MLCFAs (T_1), 2.0% HTT powder (T_2), or both 0.1% MLCFAs and 2.0% HTT (T_3).

Quality parameters (crude protein 17.25%, crude fat 6.19%, crude fibre 3.61%, Ca 3.44%, total P 0.68%, Na 0.15%) of the compound feed were analysed as described in the *Official Methods of Analysis of AOAC International* (Latimer, 2019).

The inulin content in HTT powder was determined by a spectrophotometric method as described by Saengkanuk *et al.* (2011), and fat, protein, ash, carbohydrates and moisture were determined using the methods described in the *Official Methods of Analysis of AOAC International* (Latimer, 2019). HTT powder consisted of fat (1.12%), protein (9.41%), ash (3.96%), carbohydrates (78.1%, including 51.40% inulin) and moisture (7.41%).

The FAs composition of the MLCFAs was: 0.50% caproic acid (C6:0), 4% caprylic acid (C8:0), 4% capric acid (C10:0), 44% lauric acid (C12:0), 20% myristic acid (C14:0), 10% palmitic acid (C16:0), 0.25% palmitoleic acid (C16:1), 4% stearic acid (C18:0), 10% oleic acid (C18:1), 3% linoleic acid (C18:2) and 0.25% linolenic acid (C18:3). FAs were analysed as described by Keum *et al.* (2018).

All animal procedures were conducted according to the guidelines of Council Directive 2010/63/EU (2010) on the protection of animals used for scientific purposes.

Analysis of fatty acids and cholesterol: The FAs profile in compound feed and egg yolk was determined as described by Keum *et al.* (2018). Extraction of lipids for FA profile analysis of feed was performed with chloroform/methanol (2:1 v/v). Methyl esters of FAs were dissolved in cyclohexane (100 mg in 4 ml) and were prepared by trans-methylation using 8 ml of 1.5% sulphuric acid in methanol by keeping it at 60°C for 12 h. Egg yolk sampling was performed by the direct methylation method. FA methyl esters were analysed with a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) using anRxi-5ms column (length 30 m, ID 0.25 mm, df 0.25 µm). Identified FAs were expressed as a percentage of total FAs.

Total cholesterol content in the egg yolks was determined according to the method adapted by Sasyte *et al.* (2017). Egg yolk was first diluted. Cholesterol was then extracted with ethanol and then saponification conducted with potassium hydroxide. After incubation (1 h at 50°C) and cholesterol separation with hexane and water, the extracts were centrifuged and dried under a nitrogen stream, and the rest of the substances were dissolved with mobile phase (acetonitrile : 2-propanol 55 : 45). Quantification was performed by HPLC using a Supelco Ascentis Ò C18 chromatographic column (length 150 mm, ID 4.6 mm, particle size 5 µm). The sample injection volume was 10 µl.

Production parameters: Body weight (BW) of laying hens was estimated at the beginning of the study (30 weeks of age) and at the end of the experiment (at 38 weeks of age). Laying hens' egg production, egg weight and feed intake were recorded daily. Feed conversion ratio (FCR) was calculated as grams of feed consumed per day per hen divided by grams of egg mass per day per hen. Egg mass output (EMO) was calculated by multiplying egg weight by hen-day egg production (Poeikhampha *et al.* 2013). Egg, yolk, albumen and egg shell weight was measured using a Scaltec SB C22 electronic scale (Goettingen, Germany). Albumen height and Haugh unit were evaluated using a Robotmation Egg Multi-Tester EMT-5200, a multi-functional automatic egg characteristics analyser (Tokyo, Japan); eggshell breaking strength was determined using a Robotmation LTD analyser (Tokyo, Japan).

Statistical analysis: All chemical analytical analysis was carried out in triplicate. In order to evaluate the influence of different supplements on the egg parameters analysed, data were subjected to univariate analysis of variance (ANOVA) and the Tukey HSD test as a post-hoc test (R 3.2.1 statistical program, R Core Team 2014). Results were considered statistically significant at P>0.05.

RESULTS AND DISCUSSION

Analysis of fatty acids and cholesterol: The FAs composition (% of total FAs) of the compound feed was determined. MLCFAs had a significant influence on the content of oleic (C18:1), capric (C10:0), lauric (C12:0), myristoleic (C14:1), palmitoleic (C16:1), linoleic (C18:2), stearic (C18:0), arachidonic (C20:4) and docosadienoic (C22:2) acids, SFAs, MUFAs, PUFAs and omega-6 FAs in compound feed. A significantly higher content of capric (C10:0), linoleic (C18:2), stearic (C18:0), eicosadienoic (C20:2) and docosadienoic (C22:2) acids, SFAs and PUFAs was estimated for addition of HTT. In the feed the content of capric (C10:0), lauric (C12:0), elaidic (C18:1 *trans*), dihomo-γ-linolenic (DGLA; C20:3), eicosadienoic (C20:2) and docosadienoic (C22:2) acids and omega-6/omega-3 FAs was significantly higher for diet supplementation with both MLCFAs and HTT.

The FAs composition (% of total FAs) and cholesterol concentration (mg/g) in egg yolk are shown in Table 1.

In the present study, different treatments did not significantly influence the total sum of yolk SFAs, MUFAs and PUFAs. The feed additives supplied to fatteners' diet were found to contribute to significant changes in omega-

Table 1. Fatty acid (FA) composition (% of total fatty acids) and cholesterol content (mg/g) in egg yolk

FAs profile	Group			
	C	T_1	T_2	T_3
Myristic acid C14:0	1.47±0.12	1.77±0.11	1.79±0.12	1.48±0.12
Palmitoleic acid C16:1	2.33±0.21	2.13±0.12	2.24±0.10	2.24±0.15
Palmitic acid C16:0	25.56±1.13	25.03±2.11	25.29±1.87	25.15±1.72
Margaric acid C17:0	0.22±0.02	0.23±0.03	0.21±0.04	0.08±0.05
α-Linolenic acid (ALA) C18:3	0.48±0.04^{a}	0.64±0.09^{b}	0.40±0.10^{a}	0.81±0.13^{b}
γ-Linolenic acid (GLA) C18:3	0.07±0.01	0.09±0.02	0.07±0.01	0.07±0.02
Linoleic acid C18:2	18.04±1.12	18.05±2.15	17.87±1.16	18.00±1.24
Oleic acid C18:1	36.69±2.15	36.64±1.87	36.83±1.92	36.93±1.32
Elaidic acid C18:1	1.05±0.05	0.95±0.06	0.97±0.07	1.12±0.11
Stearic acid C18:0	7.78±0.12	8.38±0.36	8.47±0.97	8.30±0.45
Arachidic acid (AA) C20:0	2.79±0.41	2.42±0.27	2.38±0.32	2.53±0.21
Eicosapentaenoic acid (EPA) C20:5	0.58±0.04	0.67±0.02	0.63±0.03	0.67±0.04
Dihomo-γ-linolenic acid (DGLA) C20:3	0.31±0.03^{a}	0.15±0.05	0.14±0.04	0.14±0.02
Eicosadienoic acid C20:2	0.64±0.09	0.54±0.08	0.56±0.06	0.51±0.07
Paullinicacid C20:1	0.15±0.03	0.39±0.05	0.21±0.02	0.09±0.03
Docosahexaenoic acid (DHA) C22:6	1.42±0.02^{a}	1.51±0.06^{b}	1.40±0.05^{a}	1.48±0.03^{b}
Docosapentaenoic acid (DPA) C22:5	0.06±0.01	0.05±0.01	0.06±0.02	0.05±0.01
Adrenicacid C22:4	0.12±0.01	0.11±0.01	0.18±0.01	0.16±0.03
Docosadienoic acid C22:2	0.12±0.02	0.12±0.02	0.13±0.02	0.11±0.02
Behenic acid C22:0	0.12±0.02	0.13±0.02	0.17±0.02	0.08±0.01
Σ SFA	35.15±1.15	35.54±1.17	35.93±1.28	35.09±1.34
Σ MUFA	40.22±1.54	40.11±1.26	40.25±1.17	40.38±1.55
Σ PUFA	24.63±1.62	24.35±1.13	23.82±1.25	24.53±1.27
Σ omega 6 FAs	21.45±1.09	20.94±1.88	20.77±1.13	21.01±1.01
Σ omega 3 FAs	2.54±0.23^{a}	2.87±0.21^{b}	2.49±0.16^{a}	3.01±0.18^{b}
Σ omega 6/Σ omega 3 FAs	8.44±0.42^{a}	7.30±0.36^{b}	8.34±0.34^{a}	6.98±0.27^{b}
Cholesterol (mg/g)	9.90±0.09^{a}	9.28±0.17^{ab}	8.32±0.10^{b}	8.57±0.15^{b}

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

6/omega-3 FAs ratio. The FAs composition of eggs is dependent on the FAs composition of the feed of the laying hens, and FAs can be incorporated into the eggs (Hammershoj and Johansen 2016; Nyberg 2017).

In our study significantly (P<0.05) lower egg yolks cholesterol content among the experimental groups was found, compared with the control. SCFAs, the end-products of prebiotic fermentation, are involved in lipid metabolism. They enter the portal blood stream where they are utilized by the liver. Acetate is converted to acetyl CoA in the liver and acts as a lipogenic substrate for de novo lipogenesis, whereas propionate inhibits lipid synthesis. Prebiotics decrease low-density lipoprotein (LDL) cholesterol, triglycerides and total cholesterol by decreasing the activity of all lipogenic enzymes like acetyl-CoA carboxylase, FAs synthase, malic enzyme, ATP citrate lyase and glucose-6-phosphate dehydrogenase. The cholesterol content of yolks is reduced in prebiotic-fed laying hens (Samal *et al.* 2015; Haq and Khan 2018). The key factors for cholesterol absorption include animal species, dietary factors, pharmacological influence, bile acid factors, genetic factors and intestinal lumen factors. Intestinal absorption efficiency can also be affected by the factors that influence cholesterol transport from the small intestine to the lymphatic system. Changes in these factors can help to explain the differences in the absorption efficiency of cholesterol in the small intestine within individuals and species (Kuang *et al.* 2018).

Production parameters: There were no significant effects of HTT and/or MLCFAs on BW, feed intake, FCR or EMO of laying hens, compared with the control group (Table 2).

In laying hens, little research has been conducted on dietary inulin supplementation. Swiatkiewicz *et al.* (2010) reported no significant effect of dietary supplemental inulin on egg production performance, EMO and FCR. Park and Park (2012) reported that laying production, egg weight and feed consumption increased in groups treated with inulin. The use of inulin as a possible alternative to antimicrobial growth promoters has given contradictory results, while its use is mostly based on benefits to the gastrointestinal tract (inhibition of pathogens, immune stimulation). This also demands repetitive researches with different levels of inulin or other prebiotic (FOS, GOS) supplementation in the diets of poultry (Adhikari *et al.* 2017). Application of MLCFAs in the diet of laying hens is focused on the modification of FA profiles, with a slight effect on the improvement of production parameters. Addition of FAs to the feed of laying hens has proven to be a viable tool for producing enhanced eggs for health-conscious consumers (Keum *et al.* 2018).

Feed additives were not significant factors on egg, yolk

Table 2. Effect of HTT and MLCFAs on laying hen's production performance and egg quality parameters

FAs profile	Group			
	C	T_1	T_2	T_3
BW at 30 weeks, g	1970.15±4.13	1974.17±5.01	1966.28±4.61	1968.78±3.17
BW at 38 weeks, g	1995.17±8.17	2001.14±7.10	1992.68±9.14	1987.61±6.39
Feed intake, g/hen/d	118.91±1.16	116.69±1.12	119.72±0.98	119.72±2.17
Laying rate, %	88.37±2.20^{a}	91.11±3.0^{ab}	92.49±4.81^{ab}	91.58±3.14^{ab}
FCR, g/g egg	2.09±0.20	1.97±0.21	2.04±0.14	1.99±0.13
EMO, g/hen/d	57.65±2.16	60.31±1.15	58.74±2.01	60.21±2.18
Egg weight, g	65.24±3.96	66.20±4.83	63.51±5.15	65.75±5.61
Egg yolk weight, g	15.62±1.78	16.21±1.76	16.02±1.33	15.79±2.40
Egg albumen weight, g	41.26±1.10	41.64±1.15	39.07±2.15	41.43±1.15
Egg yolk ratio, %	23.94±0.19	24.49±0.94	25.22±0.84	24.02±0.75
Egg albumen ratio, %	63.24±2.13	62.9±1.17	61.52±2.14	63.01±1.15
Egg shell ratio, %	12.81±0.45	12.61±0.61	13.26±0.54	12.97±0.61
Egg yolk/albumen ratio, %	0.38±0.06	0.39±0.12	0.41±0.09	0.38±0.16
Egg albumen height (mm)	6.53±1.43	7.06±2.03	6.84±1.21	6.54±1.55
Haugh unit	77.63±6.36^{a}	79.37±7.12^{ac}	81.25±8.78^{c}	77.64±11.83^{a}
Egg shell breaking strength, N	33.65±7.54^{a}	32.06±7.01^{a}	34.39±6.44^{b}	35.36±7.27^{b}

BW, body weight; FCR, feed conversion ratio; EMO, egg mass output.

or albumen weight and ratio. Poeikhampha *et al.* (2013) and Sritiawthai *et al.* (2013) reported that Jerusalem artichoke in the diet significantly increased albumen ratio, yolk: albumen ratio and albumen height of eggs. A significant effect of MLCFAs on Haugh unit was found (by 2%). On addition of HTT, Haugh unit increased by 5% ($P>0.05$), while addition of both inulin and MLCFAs did not significantly influence this parameter. However, in agreement with the present results, an increase in Haugh unit has been reported at different levels of inulin supplementation, and this effect was attributed to increased nutrient absorption and fermentation of beneficial bacteria as evidenced by the increased production of SCFAs (Chávez-Mora *et al.* 2019). HTT was significant factor influencing egg shell breaking strength (it increased by 2%), and by adding a combination of HTT and MLCFAs, this parameter was increased by 5%. These results may be due to the positive effects of Jerusalem artichoke on the intestinal mucosa which results in an increase in the absorption of nutrients and minerals in the gastrointestinal tract that increases absorption of water and minerals to eggs (Park and Park 2012). The positive effect of prebiotics on egg shell strength can be attributed to enhanced intestinal absorption of calcium, magnesium and iron, and this was dependent on SCFAs, especially butyrate. Prebiotics promote the production of SCFAs and mineral absorption by regulating intestinal microbiota (Zeng *et al.* 2017). Hanafy (2010) indicated that supplementing layers with inulin significantly improves egg shell weight percentages and shell thickness.

Decreased egg weight of S (small) grade eggs was observed in all experimental groups. Feeding diet supplemented with HTT powder tended to increase egg weight of M (medium) grade eggs by 3% ($P= 0.210$). Feeding diets supplemented with HTT and MLCFAs resulted in an increase of L (large) and XL (extra-large) grade eggs (16% ($P=0.000$) and 19% ($P=0.000$), respectively).

The results of the study indicate that Jerusalem artichoke tuber powder has a positive effect on eggshell quality (shell breaking strength increased by 2%) and Haugh unit, and it reduces yolk cholesterol level. It is concluded that the use of Jerusalem artichoke tuber powder and medium/long-chain fatty acids could improve the total omega-3 fatty acids and omega-6/omega-3 fatty acid ratio in the laying hens' egg yolks. Hence, Jerusalem artichoke (*H. tuberosus* L.) can be used as antibiotic-free feed additive for laying hens' nutrition, with a positive effect on egg quality. Further studies could be focused on more detailed explanation of intestinal microbiota balance, when Jerusalem artichoke (*H. tuberosus* L.) tuber powder and fatty acids are applied in the nutrition of laying hens.

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