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Effects of dried *Portulaca oleracea* supplementation to the laying hen diet on productive performance, egg physical traits, fatty acid composition, and cholesterol content

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Abstract: *Portulaca oleracea* is a widespread herbaceous plant particularly rich in polyunsaturated fatty acids (PUFA), antioxidant compounds and characterised by a healthy omega-6/omega-3 ratio. The focus of this research was to evaluate the effects of *Portulaca oleracea* supplementation to the diet of laying hens on productive performance, egg physical traits, fatty acid composition and cholesterol content. Twenty-six 24-week-old Warren strain layers were randomly assigned to two different groups of 18 and eight birds, respectively: one group received a commercial diet (C) whereas the other group was given the same control diet supplemented with 20% of dried *Portulaca oleracea* (PO). Hens were fed for 21 days. Feed and water were provided *ad libitum*. The poor palatability of the PO diet compromised the optimisation of the productive performance, with a significant reduction of the oviposition efficiency (0.69 vs 0.88 for PO and C, respectively; $P < 0.05$) and egg physical traits. Considering the egg nutritional traits, dietary PO significantly decreased the yolk proportion of saturated fatty acid (43.0% vs 44.1%, $P < 0.05$), while it increased the content of PUFA (19.4% vs 17.8%, $P < 0.001$), and within the latter, both omega-6 and omega-3 proportion significantly increased in comparison with C group (16.4% vs 17.6%, $P < 0.001$ and 1.46% vs 1.80%, $P < 0.001$, for n-6 and n-3, respectively). This resulted in an improvement of the omega-6/omega-3 ratio (10.4 vs 11.3 for PO and C, respectively; $P < 0.05$). Egg yolk cholesterol content did not vary between dietary treatments. It could be concluded that the use of *Portulaca oleracea* for producing omega-3-enriched eggs is feasible once the problem of palatability has been overcome and the energy requirements of the hens have been covered.

Keywords: egg quality; omega-3 fatty acids; poultry; purslane; yolk

The human population already surpassed 7 billion people in 2013 and the forecasting data suggest that it will reach 10 billion by the end of the 21st century. This scenario related with the increasing welfare of people living in emergent economies entails a growing demand for high quality food, which means animal products with high nutritional value and valuable protein and fatty acid (FA) profile.

Over the last decades the outcome of this situation has been intensive breeding and production that are not sustainable anymore because of the lowering amount of available resources and all

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the environment-related issues (Yan et al. 2021). A clear example of a limited resource that has been exploited in the last years is the ocean fish stock, especially because of its amino acid composition, vitamins and high content of long-chain polyunsaturated fatty acids (PUFA) that have a lot of beneficial effects on human health, such as preventing coronary and heart diseases in adults and promoting the correct brain development of children amongst all (Ashraf et al. 2020). Besides that, fish by-products such as fish oil and fish meal are widely used for farmed animal feeding to increase the quality of animal products for human consumption as it happens in the egg laying poultry farming.

Given this scenario, the main concern is to find alternative, sustainable and valuable feeding sources in order to partly avoid the consumption of Earth's finite resources without affecting the food quality and properties. Throughout the last years in the egg laying poultry farming, different attempts have been made to modify egg chemical composition, especially regarding omega-3 (n-3) PUFA content, exploiting the hen ability to impart the diet characteristics to the eggs they produce.

Omega-3 PUFA are essential for human growth, health and disease prevention, nevertheless they cannot be synthesised by the human organism and their consumption through the diet is generally low among people, thus it is necessary to find different ways of increasing their level in animal food products. Not enough, it is broadly acknowledged that the omega-6/omega-3 (n-6/n-3) PUFA ratio should be lower than four (Simopoulos 2002) and for this reason a source rich in n-3 is even more essential. Several authors fed to hens diets supplemented with different n-3 PUFA sources, such as linseed oil (Dalle Zotte et al. 2015) and extruded *Camelina sativa* (Kakani et al. 2012), successfully improving fatty acid composition and n-6/n-3 PUFA ratio of the eggs without affecting egg production performance. Besides that, a higher n-3 PUFA content in the diet has beneficial effects on the health status of hens as well. Indeed, according to Ebeid et al. (2008) and Ebeid (2011), the increase of dietary n-3 PUFA improves the antioxidant status of the tissues, decreases the peroxidative reactions with a consequent beneficial effect on the hen immune system, both speeding up the antibody response and reducing inflammatory processes. In addition, it has also been proved that it hardly ever affects either the productive performance of hens or reproductive morphology parameters such as ovarian follicle, ovary, and oviduct size.

Portulaca oleracea has recently risen as one of the candidates to be a suitable source as an alternative feed in poultry farming. Commonly known as purslane, it is a green leaf plant widespread in warmer countries and it has a lot of beneficial effects due to its composition. Indeed, it is one of the richest plants in n-3 FA, in particular alpha-linolenic FA (18:3 n-3), the precursor of docosahexaenoic acid (22:6 n-3) and eicosapentaenoic acid (20:5 n-3). Furthermore, *Portulaca oleracea* provides antioxidant compounds like alpha-tocopherol, beta-carotene and ascorbic acid, which are key factors to prevent cancer, cardiovascular and infective diseases. In addition, it has a lower cholesterol level compared to fish oils. Therefore, *Portulaca oleracea* could represent a valuable source as an alternative dietary supplement for laying hens by relying on the hen's ability to transfer the FA profile of their diet to the eggs they lay.

Few studies have been conducted to investigate *Portulaca oleracea* effectiveness on hen productive performance and egg quality traits providing it through different dietary formulations. Nobakht (2014) fed 65-week-old layers including up to 2% of *Portulaca oleracea* powder in the diet, instead Evaris et al. (2015) added 10% and 20% of ground *Portulaca oleracea* to the commercial meal of 30-week-old laying hens, whereas Moazedian and Saemi (2018) provided the hens with four different inclusion levels of *Portulaca oleracea* seeds in the diet. All these studies highlighted the positive impact that *Portulaca oleracea* may have on both egg production and egg physicochemical parameters, shedding light on the potential that this plant could have on replacing conventional n-3 PUFA sources, improving the egg nutritional value and at the same time without affecting either the production or the layers' health status.

Thanks to years of balanced breeding, rusticity and resilience, together with a high egg nutritional value, have become the main qualities of the Warren strain hen, which presents an average live weight of 2 100 g, daily feed intake of 125 g, and lays eggs of about 62 g.

However, since the scarcity and heterogeneity of the data in the literature do not allow to clearly define the effectiveness of the use of *Portulaca oleracea* in layers, it is worth investigating. Therefore,

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the aim of this study was to evaluate the productive performance and some egg physicochemical traits, in particular fatty acid class composition and cholesterol content, of laying hens fed a diet supplemented with 20% of dried *Portulaca oleracea*.

MATERIAL AND METHODS

Animals and diets

The study was performed at the experimental farming facility of the University of Padova in Legnaro, Italy. Twenty-six Warren strain 24-week-old laying hens, coming from a single poultry house, were randomly distributed into two different groups of 18 and eight animals. Each treatment consisted of nine and four replicates for each group, respectively (two hens per cage). The larger group acted as the control batch and received a commercial diet (C), whereas the smaller one was fed the C diet supplemented with 20% of dried *Portulaca oleracea* (PO). The diets and water were provided *ad libitum* for 21 days, the light regime was 16 h of light followed by 8 h of dark throughout the entire experiment and the temperature ranged from 15 °C to 22 °C.

Due to the limited amount of available dried *Portulaca oleracea*, the authors preferred investigating the effects of such a high dietary inclusion rather than reducing the inclusion level with more hens, as the study aimed at increasing the omega-3 FA content in eggs. The experimental diets were offered for three weeks, which was considered a sufficient time to transfer dietary nutrients to the egg and to reach the equilibrium (Dalle Zotte et al. 2015).

All the layers were handled according to the principles set down by the European Commission Directive 86/609/EC with regard to the protection of animals used for experimental and other scientific purposes. For the present experiment, the approval of the Ethical Committee was not requested.

Data collection and analyses

The two diets were analysed for dry matter, crude protein, ether extract, N-free extract, fibre fractions (AOAC 1984) contents (Table 1), and for fatty acid profile (Table 2). The metabolisable energy content was calculated (Sibbald 1984).

Table 1. Chemical composition of the experimental diets (as fed)

	Diets	
	control	*Portulaca oleracea*
Inclusion level (%)	–	20.0
Moisture (%)	9.00	8.30
Crude protein	17.5	18.8
Ether extract	5.60	3.40
Ash	12.8	16.6
N-free extracts	51.2	46.8
NDF	14.0	16.2
ADF	3.50	6.50
ADL	0.34	1.89
Calculated ME[1] (MJ/kg)	12.87	11.04

ADF = acid detergent fibre; ADL = acid detergent lignin; ME = metabolisable energy; NDF = neutral detergent fibre
[1]Sibbald 1984

Fatty acids were extracted using a chloroform/methanol mixture (2 : 1 vol/vol) as developed by Folch et al. (1957). For each sample 20 mg of

Table 2. Fatty acid profile of experimental diets (%total fatty acid methyl esters)

	Diets	
	control	*Portulaca oleracea*
Saturated fatty acids (SFA)		
C12:0	0.00	0.13
C14:0	1.57	1.26
C16:0	21.8	21.1
C18:0	10.1	5.70
Monounsaturated fatty acids (MUFA)		
C16:1	1.74	0.91
C18:1 n-9	32.6	24.3
Polyunsaturated fatty acids (PUFA)		
C18:2 n-6	30.4	41.7
C18:3 n-3	1.87	4.77
Total SFA	33.4	28.2
Total MUFA	34.4	25.2
Total PUFA	32.2	46.5
Total UFA	66.6	71.7
UFA/SFA	1.99	2.54
PUFA/SFA	0.97	1.65
n-6	30.4	41.7
n-3	1.87	4.77
n-6/n-3	16.2	8.80

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fat were exposed to acid derivatisation with 2 ml of methanolic H_2SO_4, 10% vol/vol (Sigma-Aldrich, St. Louis, MO, USA) at 65 °C for 1 h and mixed. After dilution with deionised water, fatty acid methyl esters (FAME) were extracted using hexane and then separated and quantified using gas chromatography (Shimadzu GC17A, with a FID detector and an Omegawax 250 column 30 m × 0.25 mm × 0.25 μm; Shimadzu, Kyoto, Japan).

The eggs laid daily by hens on cage basis were collected, counted, and individually weighed to compute egg production. Egg production efficiency (oviposition) was calculated by dividing the number of eggs collected per cage by the number of days of collection. The hens of each experimental group were weighed at the beginning and at the end of the trial. Feed intake was calculated on cage basis weighing back uneaten feed before each feed supply. Feed conversion ratio was calculated dividing the overall feed intake by the overall egg production (g/g).

The eggs collected during the last week of the trial were used for both shell, yolk, albumen weights, and yolk and albumen pH and colour, the former measured in duplicate with a portable pH-meter (Piccolo, Hanna Instruments, Villafranca Padovana, Italy), the latter expressed as L* (lightness), a* (redness) and b* (yellowness) according to the CIELab system (CIE 1976) by using a Minolta CR300 chroma meter (Minolta, Osaka, Japan). The illuminant was D65, and an incidence angle of 0 was used. The values corresponded to the mean of two measurements per sample. Then, samples of yolks (of two eggs each) were used for the FA profile and cholesterol content determination.

The yolk FA profile was analysed as described above for the diet. Yolk cholesterol content was determined through high performance liquid chromatography (HPLC) and the method described by Casiraghi et al. (1994). Samples of 500 mg were weighed and added 1 ml of mobile phase solution (7% isopropyl alcohol in n-hexane) and injected in the HPLC system (LC-10 ADVP, System Controller SCL 10VP; Shimadzu, Kyoto, Japan). The instrument was equipped with auto-injector SIL-10 ADVP, column mode Bondclone 10 μm Silica 300 × 3.9 mm (Phenomenex, Torrance, CA, USA) and spectrophotometric detector LC 90 UV (Perkin Elmer, Waltham, MA, USA) at a 208 nm wavelength. Samples were injected at a volume of 20 μl at a speed of 0.7 ml/minute.

Statistical analysis

Data on hen performance and production and egg quality traits were subjected to one-way analysis of variance using the ANOVA procedure (SAS Institute 1990), considering the effect of the two experimental diets. The least-square means were obtained using the Bonferroni test, and the significance was calculated at a 5% confidence level.

RESULTS AND DISCUSSION

Daily feed intake (FI) was significantly (P < 0.001) lower in the PO group in comparison with the C one during the entire experimental period (Table 3). This parameter was found to be controversial in previous studies. Evaris et al. (2015) and Nobakht (2014) recorded an increase of the daily FI in hens fed a diet supplemented with 2% and 20% of *Portulaca oleracea*, respectively, whereas Moazedian and Saemi (2018) and Aydin and Dogan (2010) did not find any change of FI when *Portulaca oleracea* was supplemented up to 25% and 2%, respectively. However, Aydin and Dogan (2010) noticed a significant decrease of the final body weight in the group of laying hens fed the diet with 2% of *Portulaca oleracea*, affirming that it should be considered a favourable aspect because it was correlated with an increase of the egg production.

Evaris et al. (2015) clarified the difference in FI observed as related to the self-regulation in birds upon the diet energy level: the higher the energy, the lower the ingestion. Nevertheless, these affirmations did not match what was found out in the present study, as hens belonging to the PO treatment received a diet with lower metabolisable energy than the C group without increasing their daily feed intake. Even though some studies have already proved that increasing dietary n-3 PUFA content has no or little effect on feed intake (Zhang et al. 2020), other research testing different dietary sources rich in n-3 PUFA to hens led to a decrease in feed intake and consequently of egg production efficiency (Ebeid et al. 2008). A plausible explanation may be represented by the lower palatability of the diet due to PUFA oxidation. The high PUFA content indeed might have led to faster degradation of unsaturated fatty ac-

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Table 3. Performance and egg production of laying hens during 21 days of dietary treatment

	Diets		Significance	RMSE
	control	*Portulaca oleracea*		
Hens (*n*)	18	8		
Initial body weight (g)	1 866	1 849	ns	181
Final body weight (g)	1 964	1 838	*	144
Feed intake (g/hen/day)				
First week	116.9	87.8	***	7.0
Second week	118.8	94.6	**	9.5
Third week	117.6	106.0	*	7.2
Total	117.8	96.1	***	6.4
Egg output/cage (2 hens/cage)				
First week (*n*)	13.6	10.5	ns	2.8
Second week (*n*)	12.6	9.8	ns	2.7
Third week (*n*)	12.6	10.0	ns	2.1
Total (21 days) (*n*)	38.7	30.3	*	6.0
Oviposition efficiency	0.88	0.69	*	0.14
Egg weight (g)	61.8	56.0	**	2.2
Overall egg weight/cage (g)	2 388	1 682	**	332
Egg mass/hen/day (g)	54.3	38.2	**	7.5
Feed intake (g)	4 947	4 038	***	269
Feed conversion ratio (kg feed/kg egg)	2.09	2.50	ns	0.36

ns = not significant; RMSE = root mean squared error
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

ids, producing off-flavour and rancid odours, thus reducing the hen diet acceptability (Ahmad et al. 2012). This hypothesis supports the behavioural observations that have been registered throughout the current study. As a matter of fact, hens were observed to peck trying to select portions of the meal that did not contain dried *Portulaca oleracea*. This selective behaviour might indicate the poor palatability of *Portulaca oleracea* and therefore explain why the feed intake dropped, and consequently the body weight of the hens of the PO group.

Aydin and Dogan (2010) also enriched the diet with dried *Portulaca oleracea*, and found no difference in FI, even if the final body weight was significantly lower; a possible explanation could lie in the low inclusion level (2%) of dried *Portulaca oleracea*, which may not have altered the taste, and therefore not caused the selective pecking behaviour in birds, observed instead in the present study.

These findings may suggest that the inclusion level and physical form of *Portulaca oleracea* are key factors for palatability and therefore need to be considered for practical applications.

As regards egg production, all the traits considered (overall egg output and weight, oviposition efficiency, egg mass/hen/day, Table 3; egg constituent weights, Table 4) were found to be significantly lower in the PO group.

These results largely depend on the reduced FI of the hens receiving the PO diet, whereas the lower metabolisable energy (ME) concentration of the PO diet would have only minimally and indirectly affected egg production, since the ME range was within the limits of the thermostatic appetite control. Results in the literature on the dietary inclusion of *Portulaca oleracea* (Aydin and Dogan 2010; Nobakht 2014; Evaris et al. 2015) or other n-3 PUFA sources (Ahmad et al. 2012) have shown that when the FI was not modified or was even increased, all the egg production parameters were not affected or increased, as the birds had a positive energy balance that allowed them to maximise their egg production performance. However,

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Table 4. Egg measurements and pH of yolk and albumen

Diet	Diets		Significance	RMSE
	control	*Portulaca oleracea*		
Eggs (*n*)	108	48		
Egg weight (g)	59.9	56.0	***	5.1
Shell weight (g)	7.53	7.05	**	0.87
Yolk weight (g)	14.0	12.8	**	2.1
Albumen weight (g)	38.3	36.1	***	3.3
pH yolk	6.30	6.29	ns	0.18
pH albumen	8.97	9.09	***	0.13

ns = not significant; RMSE = root mean squared error
*P < 0.05; **P < 0.01; ***P < 0.001

other studies showed that an increased amount of dietary PUFA sources such as linseed oil or sunflower seeds might lead to a decrease of egg production performance in comparison with laying hens fed a commercial diet (Aguillon-Paez et al. 2020). Ahmad et al. 2012 suggested that a different explanation from the lower diet palatability due to PUFA oxidation may be represented by antinutritional factors which are contained in the employed n-3 PUFA sources and could affect hen digestion and consequently nutrient absorption, being responsible for the decrease of yolk and consequently egg weight. As a matter of fact, they reported that these factors could alter protein metabolism and synthesis ultimately ending in a significant reduction of egg weight on a dose-dependent basis. In addition, they suggested that the egg and yolk weight reduction may have also been provoked by a decrease of triglycerides due to the increase of dietary n-3 PUFA that may have reduced the amount of lipids available for yolk formation.

Plenty of factors linked to increased dietary n-3 PUFA sources could affect either positively or negatively the hen production performance, thus it is worth investigating not only the most palatable shape of *Portulaca oleracea* but also both the part of the plant that contains the lowest amount of antinutritional factors (Petropoulos et al. 2020) and the maximum amount of dietary n-3 PUFA sources to minimize their impact on animals' performance.

Yolk pH showed no differences between dietary groups whereas albumen pH significantly increased in the PO group (8.97 vs 9.09 for C and PO, respectively; P < 0.001; Table 4). The increase of albumen pH recorded in this study may indicate that the natural antioxidants provided to the hens through the diet did not play their role against lipid oxidation products in eggs (Vlaicu et al. 2021), possibly because they were quantitatively insufficient, again due to the reduced FI. This could be the plausible reason, since in the study of Kartikasari et al. (2017) no differences were observed in albumen pH between the control diet and diets containing *Portulaca oleracea*.

Yolk lightness and yellowness, as well as albumen yellowness significantly (P < 0.001) decreased in the PO group in comparison with the C group (Table 5). However, a recent study of Kartikasari et al. (2020) found an increase of yolk colour intensity when 8% of *Portulaca oleracea* powder was supplemented in the hen diet. Indeed, *Portulaca oleracea* is a plant rich in beta-carotene and xanthophylls that could enhance the yolk lightness and yellowness, nevertheless its inclusion did not enhance yolk colour, and the reason remains to be clarified. Considering that the absorption and further deposition of oxycarotenoids responsible for the egg yolk pigmentation are positively correlated with the lipid content in the diet, a possible

Table 5. L*a*b* colour values of yolk and albumen

Diet	Diets		Significance	RMSE
	control	*Portulaca oleracea*		
Eggs (*n*)	54	48		
Yolk				
L*	59.7	58.2	***	2.3
a*	–1.80	–1.59	ns	1.12
b*	49.6	46.9	***	3.2
C*	49.6	47.0	***	3.1
H°	89.9	89.9	ns	0.1
Albumen				
L*	78.8	79.7	ns	2.9
a*	–5.35	–5.06	***	0.43
b*	17.8	16.6	***	1.6
C*	18.6	17.4	***	1.6
H°	84.6	84.6	ns	0.6

C* = square root of $a^2 + b^2$; H° = ratio of arctan (b/a); L*a*b* = lightness, redness, yellowness; ns = not significant; RMSE = root mean squared error
***P < 0.001

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explanation could lie either on the lower lipid ingestion by the PO hens or on the hypotriglyceridaemic effect due to the increase of dietary n-3 PUFA in the experimental diet as mentioned above (Ahmad et al. 2012).

The proportion of saturated fatty acids (SFA) and PUFA classes in the yolk lipids (Figure 1) reflected that of the experimental diets (Table 2). Thus, yolks belonging to the PO group had a lower proportion of SFA (44.1% vs 43.0% total FAME for C and PO, respectively; $P < 0.05$), and a higher proportion of PUFA (17.8% vs 19.4% total FAME for C and PO, respectively; $P < 0.001$). Within the PUFA, PO yolks showed a significant increase of both n-6 (16.4% vs 17.6% total FAME for C and PO, respectively; $P < 0.001$) and n-3 fatty acids (1.46% vs 1.80% total FAME for C and PO, respectively; $P < 0.001$), which led to an improvement of the n-6/n-3 ratio (11.3 vs 10.4 for C and PO, respectively; $P < 0.05$) (Figure 2). The results are supported by studies in the literature (Aydin and Dogan 2010; Evaris et al. 2015; Moazedian and Saemi 2018) on the dietary use of *Portulaca oleracea* in laying hens.

When compared to other vegetable raw materials rich in n-3 PUFA, such as linseed (Dalle Zotte et al. 2015), included in laying hen diet, similar results were obtained for the proportion of the three FA classes in the yolk, although the magnitude of the proportion of n-3 FA was in favour of linseed compared to *Portulaca oleracea*. Similarly, Timova et al. (2020) replaced extracted soybean meal with lupin meal as a protein source in the diet of laying hens, improving the quality of the yolk FA profile decreasing saturated and increasing n-3 PUFA proportion.

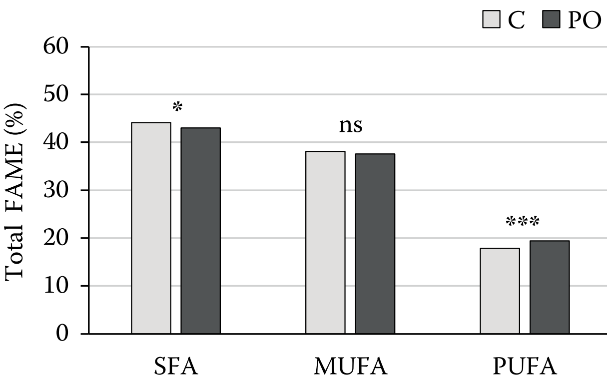

Figure 1. Fatty acid classes of yolk lipids from laying hens fed experimental diets

C = control; FAME = fatty acid methyl esters; MUFA = monounsaturated fatty acids; ns = not significant; PO = *Portulaca oleracea*; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids

$^{*}P < 0.05$; $^{***}P < 0.001$

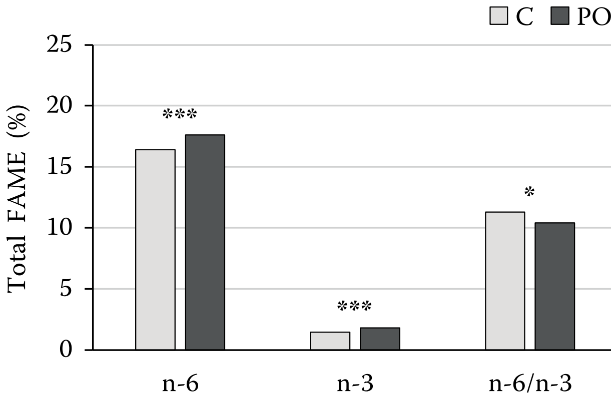

Figure 2. Total n-6 and n-3 fatty acids (FAME) and n-6/n-3 ratio of yolk lipids from laying hens fed experimental diets

C = control; ns = not significant; PO = *Portulaca oleracea*

$^{*}P < 0.05$; $^{***}P < 0.001$

The present study confirmed the great ability of hens to transfer dietary FA to the eggs they lay. Although the FI was lower in the PO group than in the C group, and presumably dried *Portulaca oleracea* was not the preferred food for hens, the effect of *Portulaca oleracea* added to the diet on the yolk FA classes profile was visible and significant even after only 21 days of ingestion, considered the shorter period to obtain diet-induced changes in eggs (Ehr et al. 2017).

Therefore, by exploiting the ability of hens to transfer the nutrients of their diet, in particular FA, into the eggs they produce, it is possible to improve them and make them an even healthier food for humans. It is known that the n-6/n-3 ratio lower than four has beneficial effects in the prevention of many diseases such as cancer, inflammation and cardiovascular disease, and *Portulaca oleracea* could represent a further dietary supplement to be studied to achieve this goal.

Moreover, increasing dietary n-3 PUFA has beneficial effects not only on egg composition and nutritional value but also on the welfare of birds and their meat. Ebeid et al. (2011) proved that meat obtained from quails fed diets enriched with different sources of n-3 PUFA had an improved antioxidant capacity and lower lipid peroxidation in the constituting tissues, without affecting the live weight and feed intake of animals. In addition, quails fed

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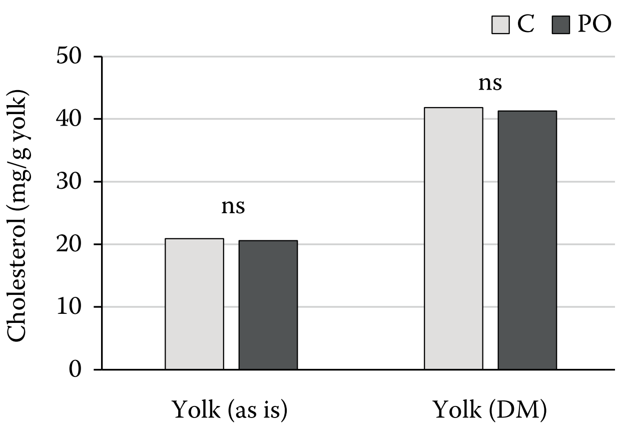

Figure 3. Cholesterol content of yolk from laying hens fed experimental diets

C = control; DM = dry matter; ns = not significant; PO = *Portulaca oleracea*

n-3 PUFA-rich diets showed beneficial effects on their immune system and on bone morphological characteristics.

Therefore, these results show a potential for using *Portulaca oleracea* as a PUFA food supplement, n-3 PUFA in particular, for laying hens, but more research is needed to study formulations and processes more suitable for overcoming the barrier of poor palatability and the presence of antinutritional factors.

Cholesterol content in the yolk was similar in the two feeding groups, either expressed as is (20.9 vs 20.6 mg/g yolk) or as dry matter basis (41.8 vs 41.3 mg/g yolk for C and PO, respectively; Figure 3). In this regard, the results are conflicting. Our results support those of Aydin and Dogan (2010) (2% *Portulaca oleracea*), whereas Moazedian and Saemi (2018) obtained a significant decrease of the yolk cholesterol concentration in hens fed a 25% supplementation of *Portulaca oleracea* seeds.

Even when considering other dietary sources of PUFA, results are variable. Some authors found a significant reduction in yolk cholesterol content feeding animals diets enriched with linseed or olive oil (Zhang and Kim 2014) whereas other researchers did not register any effects on yolk cholesterol modifying the dietary FA profile (Mattioli et al. 2017). Yalcyn et al. (2007), who did not find a cholesterol drop when laying hens received linseed enriched diets, suggested that yolk cholesterol content may be affected by a higher amount of dietary crude fibre in the meal rather than by an increased PUFA content. Theoretically, it has been demonstrated that the higher the PUFA proportion in the blood stream, the higher the plasma high-density lipoproteins and, on the other hand, the lower the plasma low-density ones, which are generated by saturated fatty acids, thus reducing the bloodstream cholesterol level. Therefore, being cholesterol mainly taken up from the bloodstream into the yolk, it should be inferred that increasing the dietary proportion of PUFA at the expense of saturated fatty acids may have a beneficial effect reducing the egg cholesterol content. Nevertheless, the plasma lipid regulation has not been cleared yet, thus more studies are needed to completely understand the role and hypothetical effect of PUFA on egg cholesterol content (Ahmad et al. 2012).

However, in the present study, it might be supposed that both the limited number of eggs tested and the length of the study itself were not enough to observe a significant decrease of the yolk cholesterol concentration. These results suggest that *Portulaca oleracea* at least does not negatively affect the egg cholesterol concentration, indeed it has a good potential to decrease it.

CONCLUSION

In conclusion, this study showed that *Portulaca oleracea* supplementation in the diet of laying hens has multiple beneficial effects on eggs: it increases the proportion of PUFA, reduces the n-6/n-3 ratio, and maintains/reduces the cholesterol content. Nevertheless, it is essential to provide it to hens in a more palatable food formulation in order to achieve significant improvements in hen performance and egg production as well.

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Conflict of interest

The authors declare no conflict of interest.

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