

## Organic diets with alfalfa silage for laying hens: Egg quality

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### Abstract

*Laying hens were fed with organic diets containing chopped, extruded or pelleted alfalfa silage and the quality of the eggs was evaluated. Four groups were conformed: a control group (A) fed with a complete feed mixture (CFM) and three silage groups (B, C and D) fed with a supplementary feed mixture (SFM). The SFM was formulated based on an assumed ingestion of 20 % silage and rapeseed oil was used as energy source. Before ensiling, the alfalfa was chopped (B) and additionally extruded (C). One half of the extruded silage was pelleted together with the SFM to produce the pelleted silage (D). Eggs from hens fed with silage (B, C and D) contained 2.4 times more n-3 fatty acids than A. The thermally treated silage (C and D) produced higher concentrations of saturated fatty acids. B and C (high rapeseed oil intakes) showed the highest monounsaturated fatty acids. In spite of the high fat intake, their cholesterol levels were similar to A (A: 12.4; B: 12.3; C: 12.6 mg/g yolk;  $p > 0.05$ ) due to the anti-cholesterolemic effect of the alfalfa. D consumed the lowest amount of fat but the highest amount of silage, corresponding to the lowest cholesterol level. The fat consumed was essential in the absorption of carotenoids. Thus, yolks from the silage groups showed decreasing values for the intensity of red and yellow colour as the intake in terms of the amount of fat/silage decreased.*

### Introduction

The incorporation of alfalfa in the diets of organic laying hens is a viable practice, because alfalfa contains high quality ingredients (n-3 fatty acids (FA), pigments, vitamins, minerals, saponins, etc.). In the first part of the study, hens fed with chopped, extruded or pelleted silage from early harvested alfalfa (Wüstholtz et al. 2016) showed similar laying yields compared to the control, without detriment to bird performance. But the nutritional quality of processed alfalfa does vary. Some studies have shown that the use of thermal treatments to produce alfalfa meal reduces the content of carotenoids (Fonseca et al. 2008). In addition, Alfaiea et al. (2010) observed that some FA, mainly long chain FA, tend to be modified or become oxidized during the thermal treatment. In the present study high temperatures, high screw speed and mechanical pressure in presence of oxygen were applied to extrude the alfalfa and pelletized the silage. These factors could induce an extensive oxidative degradation of both carotenoids and polyunsaturated fatty acids (PUFA) in the resulting silage. Consequently, the attributes of this new silage could affect the quality of the eggs. The present study was aimed at evaluating the impact of these types of silage on the FA profile, the cholesterol content and the colour of egg yolks.

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## Methods

A total of 440 18 weeks-old pullets (Lohman Brown-Classic) were divided into four groups (110 pullets / box) and housed in a mobile barn. The trial involved two feeding phases and lasted 6.5 months. More details are described in Wüstholtz et al. (2016) Young alfalfa was harvested and pre-treated prior to ensiling. All the material was chopped and two thirds of it was extruded. Half of the extruded material was then mixed with the supplementary feed mixture (SFM) and pelletized. The experiment involved four feeding groups: a control group A received a complete feed mixture (CFM) and the silage groups (B, C and D) SFM (table 1). The SFM was formulated assuming a theoretical ingestion of 20 % of silage (calculated on the basis of dry matter). Rapeseed oil was used to achieve the energy requirements of the hens in the SFM. Group B was fed with chopped silage, group C with extruded silage and group D with the silage pellets.

**Table 1: Composition (g/kg) of the complete feed mixture (CFM), supplementary feed mixture (SFM), silage pellets and silages in Phase 2 (Wüstholtz et al. 2016)**

Ingredients	Phase 2			Silage	
	CFM	SFM	Pellets	Chopped	Extruded
Soybean cake	75.0	100.0	80.0	-	-
Sunflower cake	145.0	119.0	95.2	-	-
Alfalfa meal	40.0	-	-	-	-
Maize	210.0	275.0	220.0	-	-
Wheat	277.0	329.0	263.0	-	-
Barley	150.0	-	-	-	-
Rapeseed oil	-	52.5	42.0	-	-
Feed lime	78.0	95.0	76.0	-	-
Mineral mixture	25.0	30.0	24.0	-	-
Alfalfa silage	-	-	200.0	-	-
<b>Nutrients</b>					
Dry matter	906	906	830	452.0	460.0
Crude fat	69.0	105	77.0	25.0	25.0
Crude fibre	49.0	43.0	68.0	225.0	212.0
Crude protein	179	147	163	226.0	222.0

At the end of the experiment, a representative sample of 12 eggs per group (having the average weight of the group) was collected to measure colour, FA and cholesterol content. The colour of the egg yolk was determined using a Minolta Spectrophotometer (CM 508i) in the CIELAB system (L = lightness, a\* = redness, b\* = yellowness). The profile of the FAs was determined according to the validated method of Firl et al. (2014). The cholesterol was enzymatically determined using a colorimetric method (Böhringer Mannheim 1994). The data were analysed statistically using the GLM procedure with SPSS version 22 (2013, SPSS Inc., Chicago IL).

## Results and discussion

The FA profile of the egg yolk was influenced by the alfalfa silage consumption (table 2). The egg yolks from the silage groups showed lower levels of saturated fatty acids (SFA), but higher levels of all n-3 FA than group A. The major monounsaturated fatty acid (MUFA) in the yolks was oleic acid, which is consistent with the reports of Karsten

et al. (2009). The MUFA content was the lowest in group A (42.8 g/100 g FAME), the second lowest in D (45.0 g/100 g FAME) and the highest in B and C (47.3, 47.6 g/100 g FAME). These differences coincide with the fat consumption calculated per group (A: 9.6; B: 13.2; C: 13.8; D: 9.8 g in DM/d) and the rapeseed oil consumption in the silage groups (B: 6, C: 6.2 and D: 4.5 g/d). Rapeseed oil, which is the principal fat and energy ingredient in the SFM of silage groups (table 1), is a rich source of MUFA (mainly oleic acid: 54 %) and is able to significantly alter the FA content of yolks (Gül et al. 2012).

**Table 2: Fatty acid composition (g/100 g FAME), cholesterol (mg/g yolk) and colour of yolks**

Item	Group				SE	p
	A	B	C	D		
SFA	33.07 <sup>a</sup>	28.92 <sup>c</sup>	29.30 <sup>bc</sup>	30.00 <sup>b</sup>	0.27	***
MUFA	42.80 <sup>c</sup>	47.25 <sup>a</sup>	47.56 <sup>a</sup>	45.00 <sup>b</sup>	0.42	***
PUFA	23.99	23.76	23.08	24.96	0.54	n.s.
n-3	1.37 <sup>b</sup>	3.18 <sup>a</sup>	3.18 <sup>a</sup>	3.42 <sup>a</sup>	0.08	***
n-6	22.61 <sup>a</sup>	20.57 <sup>b</sup>	19.88 <sup>b</sup>	21.52 <sup>ab</sup>	0.50	***
Cholesterol mg/g yolk	12.4 <sup>ab</sup>	12.3 <sup>ab</sup>	12.6 <sup>a</sup>	11.7 <sup>b</sup>	0.23	*
L	54.8 <sup>a</sup>	53.4 <sup>b</sup>	54.7 <sup>a</sup>	52.8 <sup>b</sup>	0.22	***
a*	6.7 <sup>b</sup>	7.5 <sup>a</sup>	6.9 <sup>b</sup>	5.2 <sup>c</sup>	0.11	***
b*	37.6 <sup>ab</sup>	38.6 <sup>a</sup>	36.8 <sup>b</sup>	32.9 <sup>c</sup>	0.32	***

n.s., no significant ( $p > 0.05$ ); \*,  $p < 0.05$ ; \*\*,  $p > 0.01$ ; \*\*\*,  $p > 0.001$

High proportions of n-3 fatty acids were reported in fresh or ensiled alfalfa (40 or 36 %; Whiting et al. 2003). This explains the elevated levels of n-3 in the silage groups: 2.3 times higher in the egg yolks of B and C, and 2.5 times higher in D, compared to that of group A. However, they are lower than those observed by Karsten et al. (2009) (yolks from hens fed with fresh alfalfa or clover showed a 2.9 and 2.8 times higher n-3 than the control group). Among the silage groups there were no differences for n-3 (B and C: 3.2; D: 3.4 g/100 g FAME  $p > 0.05$ ), in spite of differences for the silage intake (B: 19.4; C: 28.0 and D: 35.0 gr/d DM in phase 2;  $p < 0.001$ ; Wüstholtz et al. 2016). The results suggest two cases: on the one hand, the degradation or transformation of some FAs is possible during the ensiling process. It was observed in the study conducted by Whiting et al. (2004) that alfalfa silage contained 10 % less n-3 FAs, 24 % more n-6 FAs and 9 % more C18:0 than fresh alfalfa. On the other hand, high temperatures during the extrusion and palletization could cause some oxidative processes of long chain FA (Alfaiea et al. 2010); this would entail a reduction of the levels of n-3. More studies are necessary to clarify this fact.

The highest level of cholesterol in numbers was found in C, followed by A and B, with the lowest in D. Statistical differences were found only between C and D. Low levels of cholesterol were expected in the silage groups, since alfalfa saponins have been shown to reduce the cholesterol level (Laudadio et al. 2014). The lowest level of cholesterol of D coincided with the highest silage intake of this group. Although there was no silage consumption in A, the cholesterol concentration was statistically similar to those from B, C and D. Calculating the daily fat intake per group, it can be observed that group A has the lowest fat intake, while B and C have the highest (A: 8.7; B: 14.1; C: 14.7 and D: 10.2 g fat/d). Therefore the incorporation of silage in the diets of B and C and the corresponding anticholesterolemic effect of the saponins prevent the incorporation of high levels of cholesterol in the yolks, causing them to exhibit levels

comparable to A. It is worth mentioning that the diet of A contained alfalfa meal (4 %); this could cause a slight reduction of cholesterol in A too.

The inclusion of alfalfa meal in the diets of laying hens was associated with the darkest yolk colour due to its richness in carotenoids (Laudadio et al. 2014). It was expected that silage groups would show the highest carotenoids deposition, and therefore the highest  $a^*$  and  $b^*$  in the yolks. However, the highest silage intake (B: 19.4; C: 28.0 and **D: 35.0** gr/d DM) produced the lowest  $a^*$  and  $b^*$  values (B: 7.5; 38.6; C: 6.9; 36.8; **D: 5.2; 32.9**). Na et al. (2004) found that diets with high concentrations of carotenoids lowered the absorption rates of these substances, especially in the case of low lipid intakes. This conclusion confirms the observed low  $a^*$  and  $b^*$  values (low deposition of pigments) in the yolks of D and C and matches the low-ratio fat/silage intake (B: 6.8; C: 5.0; D: 2.8 fat/10 g silage).

### Conclusions

The use of alfalfa silage in the feeding of laying hens is a recommended alternative. It produces healthy eggs with special characteristics for the human consumption (high levels of n-3, low levels of cholesterol and an intense yellow colour). Extrusion of alfalfa and pelletisation of silage besides did not produce any additional benefits on the egg quality. Both processes produced eggs with relatively high levels of SFA.

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