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The effects of kale (*Brassica oleracea* ssp. *acephala*), basil (*Ocimum basilicum*) and thyme (*Thymus vulgaris*) as forage material in organic egg production on egg quality

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Abstract 1. In organic egg production, forage material as part of the diet for laying hens is mandatory. The purpose of the present study was to examine the effect of feeding with forage materials including maize silage, herbs or kale on egg production and various egg quality parameters of the shell, yolk colour, egg albumen, sensory properties, fatty acid and carotenoid composition of the egg yolk.

2. A total of 5 dietary treatments were tested for 5 weeks, consisting of a basal organic feed plus 120 g/hen.d of the following forage materials: 1) maize silage (control), 2) maize silage incl. 15 g/kg basil, 3) maize silage incl. 30 g/kg basil, 4) maize silage incl. 15 g/kg thyme, or 5) fresh kale leaves. Each was supplied to three replicates of 20 hens. A total of 300 hens was used.

3. Feed intake, forage intake and laying rate did not differ with treatment, but egg weight and egg mass produced increased significantly with the kale treatment.

4. The egg shell strength tended to be higher with the kale treatment, and egg yolk colour was significantly more red with the kale treatment and more yellow with basil and kale treatments. The albumen DM content and albumen gel strength were lowest with the thyme treatment. By sensory evaluation, the kale treatment resulted in eggs with less sulphur aroma, higher yolk colour score, and more sweet and less watery albumen taste. Furthermore, the eggs of the kale treatment had significantly higher lutein and β-carotene content. Also, violaxanthin, an orange xanthophyll, tended to be higher in kale and eggs from hens receiving kale.

5. In conclusion, forage material, especially basil and kale, resulted in increased egg production and eggs of high and differentiable quality.

INTRODUCTION

Organic egg production requires access to forage material besides the basal diet (The Council of the European Union, 2007). Forage material can either be available for the hens as a crop/pasture in the hen yard or supplied as a roughage as silages or vegetables. Few studies have focused on the impact of forage material types on the quality properties of eggs (Horsted *et al.*, 2006; Hammershoj and Steenfeldt, 2009). However, due to the relatively high amount of forage material that laying hens are able to consume, it is expected to be a potential contributor in affecting the different quality parameters of eggs as human food, *e.g.* carotenoids (Karadas *et al.*, 2006; Hammershoj *et al.*, 2010) and supply of essential amino acids, vitamins and minerals (Penz and Jensen, 1991; Surai and Sparks, 2001; Kovacs-Nolan *et al.*, 2005; Seuss-Baum, 2005). Also, the transfer of specific fatty acids from oil-rich forage material to the egg yolk (Lopez-Bote *et al.*, 1998; Woods and Fearon, 2009) are areas of interest for the egg quality and areas where

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various types of forage material may impose a difference. Furthermore, flavours from the diet/forage material can be transferred to the egg either directly (Tserveni-Gousi, 2001; Narahari, 2003) or as a consequence of changed microflora composition of the gut upon change in forage material, thereby introducing new flavours to the egg (Richter *et al.*, 2002).

Based on the hypothesis that feeding hens with different aromatic herbs and vegetables significantly affect the egg properties, it was the objective of the present study to examine the short-term effect of using either fresh kale or dried herbs mixed into maize silage as forage material in organic egg production on egg quality parameters.

MATERIALS AND METHODS

Hens, housing and feed

A total of 300 Lohmann Silver hens at the age of 44 weeks were distributed into 5 groups with three replicate flocks of 20 hens each. Each replicate flock was housed in a wooden house of 6 m^2, equipped with perches, 5 nests, 1 nipple drinker, 1 feed silo and 1 wooden box for forage material. The hens had 24 h access to an outdoor yard of 105 m^2. Throughout the experimental period, the birds were fed on a commercial organic basal diet (Danish Agro, Copenhagen, Denmark), which was given *ad libitum*. No synthetic essential amino acids were added to the diet since these ingredients are prohibited in organic egg-production. The calculated contents of crude protein and metabolisable energy in the basal diet were 185 g/kg and 11·4 MJ ME/kg. The composition of the basal diet and the analysed contents of diet and foraging material are shown in Table 1. The analysed amino acid content is given in Table 2.

Experimental design and measurements

The experimental period lasted 5 weeks and started at a hen age of 44 weeks and consisted of the following 5 experimental forage material treatments as:

C: (control) 1000 g/kg maize silage,
BL: 985 g/kg maize silage mixed with 15 g/kg dried basil (*Ocimum basilicum*),
BH: 970 g/kg maize silage mixed with 30 g/kg dried basil (*Ocimum basilicum*),
T: 985 g/kg maize silage mixed with 15 g/kg dried thyme (*Thymus vulgaris*), and
K: 1000 g/kg fresh kale (*Brassica oleracea ssp. Acephala*) leaves.

The dried herbs of basil and thyme were obtained from Askhim's Horse Herbs (Malling, Denmark). The reason for two concentration levels of basil herbs and only one of thyme was both due to practical limitations, in order to study an eventual dose response and based on the expectation that the high (30 g/kg)

Table 1. *Composition and chemical analysis of the organic basal diet and maize silage and kale*

Ingredients	Basal diet	Maize silage	Kale
Composition (g/kg)			
Wheat	449·9		
Oats	100·0		
Barley	50·0		
Maize	50·0		
Soybean, toasted, whole	43·0		
Fishmeal	20·0		
Soybean cake	25·0		
Sunflower seed cake	80·0		
Maize gluten	65·0		
Potato protein concentrate	20·0		
Calcium carbonate	53·8		
Oyster shells	30·0		
Monocalcium phosphate	5·8		
Sodium bicarbonate	2·8		
Sodium chloride	1·7		
Vitamins and mineral mixture[a]	2·5		
Belfeed[b]	0·5		
Chemical analysis (g/kg DM)			
Dry matter	897·4	318·3	149·2
Ash	104·9	38·0	108·4
Protein (N x 6·25)	206·3	92·5	226·9
Fat (acid ether extract)	43·7	26·1	39·1
Calcium	31·9	1·8	18·8
Phosphorus	6·0	2·5	4·2
Starch	440·3	281·7	71·6
Glucose	0·6	1·2	58·1
Fructose	0·4	1·3	82·8
Sucrose	19·5	7·8	18·8
Raffinose	2·7	t	3·3
Stachyose	5·6	t	t
Verbascose	1·3	t	t
Cellulose (Cel.)	31·0	172·0	85·0
Non-cellulose polysaccharides (NCP)			
Rhamnose	1·0	1·0	4·0
Fucose	t	t	2·0
Arabinose	21·0	24·0	36·0
Xylose	38·0	118·0	15·0
Mannose	4·0	3·0	8·0
Galactose	9·0	9·0	25·0
Glucose	12·0	11·0	8·0
Uronic acids	9·0	15·0	116·0
Non-starch polysaccharides (NSP = Cel. + NCP)	125·0	353·0	299·0
Soluble NSP	28·0	11·0	158·0
Insoluble NSP	97·0	343·0	141·0
Lignin	30·0	80·0	37·0
Dietary Fibre (NSP + lignin)	155·0	433·0	336·0

t: trace.
[a]The premix provided per kg diet: retinol 3 mg; cholecalciferol 63 µg; (α-tocopherol) 36 mg; menadione 6 mg, thiamine 1 mg; riboflavin 4 mg; pyridoxine 3 mg; cobalamin 0·02 mg; d-pantothenic acid 8 mg; nicotinic acid 30 mg; choline 180 mg; folic acid 0·5 mg; biotin 0·03 mg; iron 25 mg; zinc 60 mg; manganese 75 mg; copper 5 mg; iodine 0·5 mg; selenium 0·3 mg.
[b]Betaxylanase EU 3·2.1·8 (E51), 10 U/kg diet.

Table 2. *Amino acid content (g/kg DM) of organic basal diet, maize silage and kale*

Amino acid	Basal diet	Maize silage	Kale
Alanine	9·82	7·86	10·56
Arginine	11·50	2·06	10·66
Asparagine	17·02	6·15	18·90
Cystine	3·56	1·23	2·36
Glutamine	41·27	11·43	21·48
Glycine	8·86	3·74	9·60
Histidine	4·89	1·95	4·40
Isoleucine	8·67	3·45	8·05
Leucine	16·73	8·54	14·54
Lysine	9·34	3·25	12·12
Methionine	3·56	1·60	3·58
Phenylalanine	9·84	3·92	9·25
Proline	13·86	6·36	20·41
Serine	10·28	4·40	9·63
Threonine	7·52	3·37	8·91
Tyrosine	6·97	1·92	6·25
Valine	9·38	4·90	11·37

concentration of herbs might cause excessively strong flavour effects. The forage material was distributed every second d to the hens at an amount of 120 g/bird.d and was not processed in any way. Once a week, leftovers were removed from the forage material boxes in each group and weighed in order to calculate the actual consumption.

During the experimental period of 35 d, eggs were collected 3 times per week and the number of eggs and egg weight were recorded. From the laying rate and egg weight, the daily egg mass output (g/hen.d) was calculated. Feed consumption was registered weekly per pen. The feed conversion ratio was calculated as g feed/g egg.

Three times during the experimental period, *i.e.* at 0, 14 and 28 d, a total of 45 eggs (5 treatments * 3 eggs * 3 replicate flocks) were collected. The eggs were stored for 1 d at room temperature before analysis of different egg quality parameters.

The hens were not beak-trimmed and the experiment complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of birds under study. No hens died during the 5 week period.

Chemical analysis of diet and forage material

The DM content was determined by drying at 105°C for 8 h. Protein (N × 6·25) was determined by the Kjeldahl method (Association of Official Analytical Chemists, 1990*a*) using a Kjell-Foss 16 200 autoanalyser and energy by a LECO AC 300 automated calorimeter system 789-500 (LECO, St. Joseph, Michigan). Ash was analysed according to method 923·03 (Association of Official Analytical Chemists, 1990*b*), and fat (hydrochloric acid-fat) was extracted with diethyl ether after acid-hydrolysis (Stoldt, 1952). Amino acids were analysed as previously described (Mason *et al.*, 1980). The sugars (glucose, fructose and sucrose), and the oligosaccharides (raffinose, stachyose and verbascose) were extracted with 50% (v/v) ethanol at 60°C and quantified by gas-liquid chromatography (GLC) (Bach-Knudsen and Li, 1991). Starch was analysed by the enzymatic-colorimetric method (Bach-Knudsen, 1997). Total NSP and their constituent sugars were determined as alditol acetates by GLC for neutral sugars and by colorimetric method for uronic acids using a modification of the Uppsala procedure (Theander *et al.*, 1994; Bach-Knudsen, 1997). Cellulose was determined as the difference in glucose content of non-starch polysaccharides (NSP) when the swelling step with 12 M sulphuric acid was included and omitted, respectively, and the content of cellulose, non-cellulosic (NCP) and soluble NSP was calculated as previously described (Bach-Knudsen, 1997). Klason lignin was measured gravimetrically as the residue obtained of the treatment with 12 M sulfuric acid (Theander *et al.*, 1994). All analyses were performed in duplicate.

Shell strength

The shell strength was analysed by uni-axial compression at the equator of the egg on a TA-Hd*i* Texture Analyser (Stable Micro Systems Ltd., Surrey, England) with a 100 kg load cell, 0·001 N detection range, 75 mm diameter flat plate probe and a compression speed of 0·1 mm/s. Recordings of force (N) and displacement (m) were obtained until fracture of the shell. The maximum force recorded was used as shell strength value (N). The position (mm) of maximum force detection was used as a measure of shell elasticity. The compression modulus (N/mm) was calculated as the slope of the initial part (0·01–0·03 mm) of the force-displacement curve.

Yolk colour

Individual eggs were weighed, broken, and the yolk was separated from albumen. The yolk colour was analysed by a Minolta Chroma Meter CR-300 (Minolta Co. Ltd., Osaka, Japan) using the CIE (Commission Internationale de L'Enclairage) Lab scale with standardised daylight (D65). The L*, *a** and *b** values reflect lightness (0 = black, 100 = white), redness (−100 = green, 100 = red) and yellowness (−100 = blue, 100 = yellow), respectively. The instrument was calibrated daily against a white standard plate.

Albumen dry matter, pH and gel textural analysis

Dry matter (DM) content (g kg^{-1}) of egg albumen was determined by heating ~2 g of homogenised albumen to 198°C until equilibration in a HR73 halogen moisture analyser (Mettler Toledo, Schwerzenbach, Switzerland).

Yolk carotenoid analysis

The content of carotenoids in the egg yolk was performed by HPLC-DAD analysis as described previously (Hammershoj *et al.*, 2010).

Sensory evaluation

For sensory evaluation of the aroma, appearance, texture and taste of hardboiled eggs, a total of 120 eggs (8 assessors * 3 replicates * 5 treatments) were collected at d 21 in the experimental period, and stored 4 d at 21°C in a climate cabinet. Eggs (12) were boiled at 100°C for 12 min in 1·2 l water. Thereafter, the eggs were cooled in cold tap water for ~9 min and the shell was removed and the peeled eggs were put into containers of each treatment. Before the sensory evaluation by an 8-member assessor panel, the eggs were cut into halves and each half placed in a closed plastic container. The hard-boiled eggs were served at room temperature (r.t.) to the panel. A training session 3 d prior to the evaluation was conducted in order to establish the descriptors for the specific egg samples (Table 5). Reference materials, *i.e.* a sulphurous egg, a fresh egg, acidic solution, sweet solution, bitter solution, freshly cut cress, and a sample of basal diet, were distributed together with the egg samples in order to ease identification of the descriptors.

Each descriptor was evaluated on a scale with grades ranging from 0–15. Furthermore, a subjective ‘liking’ score was finally given by each assessor for each sample on a scale ranging from 0–9. The evaluation was done in a sensory room, with individual boxes for each assessor and data were obtained through the FIZZ Acquisition Network (Biosystemes, Couternon, France).

Statistical analysis

All data were subjected to statistical analysis by the Generalised Linear Models (GLM) procedure (SAS ver. 9·2, SAS Institute Inc., Cary, NC, USA). For each treatment and each egg collection data were tested for variance homogeneity by a Bartlett test, and outliers (in total 6 data values) were excluded. The normality of the data distribution was checked by a Probit-analysis and resulted in the following data transformation: log (10) of gel stress data.

The model of analysis was:

$$Y_{ijl} = a_i + b_j + c_{ij} + e_{ijl}$$

where a = main effect of treatment i (C, BL, BH, T, K), b = main effect of time j (0, . . . , 35), c = interaction between treatment and day, and e = replicate l (1, . . . , 9) for egg analyses.

For feed intake, the b = day j (7, 14, 21, 28) and e = replicate l (1, . . . , 3). If interactions between class variables were non-significant, the c_{ij} was excluded from the model. The LS-Means were calculated and differences regarded significant at minimum 95%-level ($P \leq 0{\cdot}05$) and classified by the Ryan-Einot-Gabriel-Welsch multiple range test.

For the sensory evaluation the data were analysed by the following model:

$$Y_{ijm} = a_i + b_i + e_{ijm},$$

where a = main effect of treatment i (C, BL, BH, T, K), b = assessor j (1, . . . , 8), m = replicate (1, 2, 3). Data were transformed when criteria of variance homogeneity were not met, *i.e.* by square root function for hardness 8, sweet 11, sulphur 12, cress 13. A total of 40 outlier values were detected and excluded, *i.e.* 1·3% of data.

RESULTS

The hens were fed on a basal diet plus supplement with either maize silage, maize silage + herbs, or kale with the chemical compositions shown in Tables 1 and 2, respectively. It is noticed that the maize silage had relative lower protein content, but was higher in starch than in kale. The content of dietary fibres was highest in maize silage compared to kale, mainly due to the cellulose content being 172 g/kg DM in maize silage and 85 g/kg DM in kale. A main difference between the two types of forage material was the content of soluble and insoluble NSP, where the insoluble NSP in maize silage constituted >90% of total NSP, which was quite different from kale, having a much higher content of soluble NSP constituting >50% of total NSP. The content of the different sugars was highest in kale. The kale appeared to contain high levels of the essential amino acids lysine and methionine (Table 2) that was approximately 3-fold that of the maize silage.

The mean daily intakes of feed and forage material of the five treatments are given in Table 3 together with the egg production parameters. The intake of forage material differed – although insignificantly – where the treatment with kale resulted in the lowest intake of 95 g/hen/d compared with the highest in the control treatment of ~112 g/hen/d. It was observed that even though the kale was offered as only the leaves

Table 3. *Feed consumption and egg production parameters as function of forage material treatment for organic egg laying hens during 5 weeks of experiment,* (n = 18)

	Effect of forage material					SEM	*F*-test
	Maize control	Maize + 15 g/kg Basil	Maize + 30 g/kg Basil	Maize + 15 g/kg Thyme	Kale		
Feed intake, g/hen.d	138·5	134·4	151·3	157·1	162·9	5·40	NS
Forage intake, g/hen.d	111·8	107·7	106·2	108·6	95·3	2·45	NS
Laying rate, eggs/hen.d	0·794	0·782	0·765	0·779	0·840	0·0206	(P = 0·083)
Egg weight, g	62·7^{b}	62·9^{b}	63·2^{ab}	62·7^{b}	64·0^{a}	0·27	**
Egg mass, g/hen.d	49·7^{ab}	49·1^{ab}	48·3^{b}	48·8^{b}	53·7^{a}	1·37	*
FCR, g feed/g egg	2·79	2·80	3·12	3·23	3·02	0·111	NS

^{a,b}Means within one variable not sharing a common superscript are significantly different at *P < 0·05 and **P < 0·01.

Table 4. *Egg quality parameters as function of forage material treatment for organic egg laying hens during 5 weeks of experiment, (n = 18)*

	Effect of forage material					SEM	*F*-test
	Maize control	Maize + 15 g/kg Basil	Maize + 30 g/kg Basil	Maize + 15 g/kg Thyme	Kale		
Egg diameter, mm	43·6	43·8	44·0	44·2	44·3	0·21	NS
Shell							
Fracture strength, N	40·2	43·7	39·4	41·8	43·8	1·39	P = 0·09
Fracture point, µm	234	242	233	240	261	7·3	NS
Modulus, N/m	164	176	166	170	166	5·1	NS
Yolk colour							
Lightness L*	65·0^{ab}	65·1^{ab}	65·7^{a}	64·6^{ab}	63·7^{b}	0·44	*
Redness a*	−2·74^{b}	−2·34^{b}	−2·30^{b}	−2·35^{b}	0·18^{a}	0·287	***
Yellowness b*	53·5^{b}	55·1^{ab}	56·3^{ab}	54·2^{b}	57·6^{a}	0·87	**
Albumen							
DM, g/kg	125·4^{ab}	126·5^{ab}	126·2^{ab}	123·9^{b}	129·9^{a}	137	*
pH	9·10	9·09	9·05	9·08	9·06	0·030	NS
Gel fracture stress, kPa	28·7^{a}	29·1^{a}	29·0^{a}	24·9^{b}	29·3^{a}	0·75	***
Gel fracture strain, (−)	0·959	0·930	0·909	0·922	0·947	0·0177	NS

^{a,b}Means within one variable not sharing a common superscript are significantly different at *P < 0·05, **P < 0·01, and ***P < 0·001, or NS = non-significant.

without the stem, the leaf nerves were left over by the hens and the intake was, therefore, only ~80% by weight of the kale supplied. In contrast, for all other forage treatments in the study the intake was ~90% by weight.

The egg laying rate was not significantly affected by the forage material treatments. A general picture of a decreasing laying rate in the experimental period was ascribed to both the fact that hen age increased from 44 to 49 wks and furthermore, the time of year where light hours at the actual latitude decreased from ~13 h/d to ~10 h/d, and no other supplemental light sources were installed in the hen houses. However, a tendency (P = 0·083, Table 3) towards a higher egg production for hens in the kale treatment was observed, and the egg weight was significantly highest from these hens, with more than 1 g larger eggs compared with the control and the 15 g/kg basil and 15 g/kg thyme treatments. The resultant egg mass produced – as product of the laying rate and the egg weight – was significantly highest also for the kale treatment, where egg mass production in total was more than 4 g/hen.d higher than for the other treatments, which did not differ. Furthermore, it was observed that the hens were very keen on eating the kale, paying immediate attention to this type of forage material at the time of supplying this, which was less pronounced for the other treatments.

The quality of the eggs laid was evaluated by various parameters giving specific measures for the shell, the yolk and the albumen (Table 4). The egg shell quality was evaluated by measures of shell strength by large deformation texture analysis until fracture, and none of the parameters of the analysis showed significant effects on the shell by the forage material treatments, although, there was a tendency (P = 0·09) towards improved shell strength of eggs from the kale treatment.

The yolk colour variables showed a very significant effect of primarily the kale treatment, resulting in significantly darker (L*), more red (a^*) and yellow (b^*) egg yolks (Table 4). The time effect of feeding the different forage materials is shown in the Figure for the colour a^* variable.

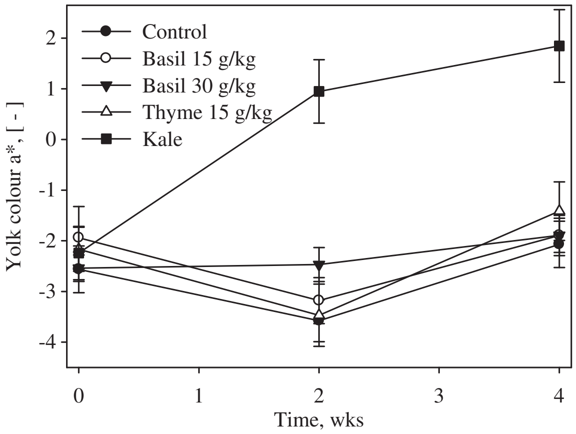

Figure. *Means ± SE of egg yolk colour variable a* (redness) as function of time of feeding different types of forage material to egg laying hens, (n = 9).*

A steep increase was seen after 2 weeks of feeding kale, and after 4 weeks of feeding the yolk redness had increased further, although at a lower rate compared with the first 2 weeks. The herbs had only little effect on the yolk colour variables. There was a tendency towards darker yolks of hens fed on maize silage plus thyme given as a lower mean L* value (Table 4). In addition, the dried basil at both 15 g/kg and 30 g/kg inclusion tended to result in more yellowish (higher b^*) yolks in comparison with the maize silage alone and maize silage plus thyme (Table 4), although insignificant.

For the egg albumen, there were significant effects on the DM content and the gel texture of a heat-denatured egg albumen gel (Table 4). These two parameters correlated in the regard that the highest values of both – and therefore the best quality of albumen – were obtained for the kale treatment and the lowest values were for the 15 g/kg thyme treatment.

As can be seen in Table 5, many of the descriptors from the sensory evaluation of hard

Table 5. *LS-Means of sensory evaluation by 8 assessors of hard boiled eggs analysed by descriptor gradings from 0 (low) to 15 (high) as function of forage material type for laying hens during 5 weeks experimental period, (n = 24)*

No.	Descriptor	Forage material					SEM	F-test
		Maize	Maize + 15 g/kg Basil	Maize + 30 g/kg Basil	Maize + 15 g/kg Thyme	Kale		
Whole egg aroma								
1	Fresh	7·64	8·21	7·34	7·50	8·41	0·536	NS
2	Sulfur	5·81[a]	5·23[ab]	4·99[ab]	6·41[a]	4·15[b]	0·561	*
3	Cress	3·58	2·92	3·02	4·01	3·47	0·485	NS
4	Dirty	3·82	4·33	4·59	4·53	3·79	0·652	NS
5	Cabbage	3·89	4·49	4·17	3·22	2·93	0·583	NS
Yolk appearance								
6	Colour	4·67[b]	4·24[b]	5·17[b]	5·04[b]	11·43[a]	0·375	***
Albumen texture								
8	Hardness at chewing	7·26[ab]	6·23[b]	6·63[ab]	8·37[a]	8·00[ab]	0·490	*
Yolk texture								
23	Dryness	7·36	6·79	7·34	6·96	7·66	0·611	NS
Albumen taste								
10	Fresh	7·31	8·26	7·99	8·29	8·11	0·516	NS
11	Sweet	4·42[b]	4·78[b]	5·03[ab]	4·94[b]	6·31[a]	0·468	*
12	Sulphur	4·94	4·01	4·65	5·11	3·80	0·532	NS
13	Cress	3·44	3·70	2·96	3·79	3·71	0·423	NS
15	Watery	7·49[ab]	8·59[a]	7·12[bc]	7·97[ab]	6·13[c]	0·468	*
Yolk taste								
16	Fresh	8·27	9·30	7·97	8·90	7·60	0·530	NS
17	Sweet	6·39	6·01	6·48	7·17	6·45	0·497	NS
18	Sulfur	2·87	3·08	3·10	3·53	2·66	0·379	NS
19	Cress	4·37	3·41	3·48	4·36	4·39	0·498	NS
20	Dirty	3·36	3·23	4·04	3·06	3·34	0·637	NS
25	Kale	3·02	3·61	3·77	2·77	2·43	0·492	NS
26	Bitter	4·30	3·01	3·23	3·50	4·33	0·567	NS
Preference[X]								
22	Liking	4·83	5·75	5·08	4·96	5·29	0·454	NS

[a,b,c]Means within one variable not sharing a common superscript are significantly different at *$P<0{\cdot}05$, **$P<0{\cdot}01$, and ***$P<0{\cdot}001$, NS = non significant.
[x]The overall preference was evaluated on a scale ranging from 0–9.

Table 6. *Fatty acid composition (mg/g) of egg yolks as affected by forage material treatment for organic egg laying hens during 5 weeks of experiment,* (n = *3*)

	Effect of forage material					SEM	*F*-test
	Maize control	Maize + 15 g/kg Basil	Maize + 30 g/kg Basil	Maize + 15 g/kg Thyme	Kale		
C14	0·94	1·00	0·84	0·82	0·84	0·069	NS
C14:1	0·11	0·16	0·12	0·12	0·14	0·013	NS
C15	0·32	0·30	0·34	0·26	0·32	0·062	NS
C16	75·6	82·6	75·8	73·0	80·2	4·12	NS
C16:1	6·21^{b}	7·56^{ab}	6·39^{b}	6·35^{b}	8·37^{a}	0·891	*
C18	31·4	31·4	30·5	30·8	31·9	0·77	NS
C18:1 cis 9	125	120	124	128	139	8·2	NS
C18:2 n-6	66·7	62·8	68·3	50·7	53·1	8·99	NS
cis	2·99^{ab}	2·91^{ab}	2·94^{ab}	2·30^{b}	3·95^{a}	0·594	*
C18:3 n-3	6·42	6·95	6·81	7·37	6·79	0·352	NS
C20:4	5·71^{ab}	5·42^{b}	6·00^{ab}	5·49^{b}	6·41^{a}	0·303	*
C22:6 n-3	107	114	106	104	112	4·1	NS
SFA	131	128	130	134	148	9·0	NS
MUFA	81·8	78·1	84·0	65·8	70·2	9·77	NS
PUFA n-3	8·7^{ab}	8·3^{ab}	8·9^{ab}	7·8^{b}	10·4^{a}	0·86	*

[a,b]Means within one variable not sharing a common superscript are significantly different at *$P < 0·05$.

boiled eggs did not differ significantly due to the treatments. However, the aroma of the whole egg scored significantly ($P < 0·05$) less on 'sulphur', when hens were fed on kale compared with the maize silage and maize silage + thyme treatments. The treatments that included dried basil regardless of concentration did not differ from either of the other treatments (Table 5). The high impact of kale on the yolk colour, as shown in Table 4, was confirmed by the visual evaluation by the sensory panel resulting in a significantly ($P < 0·001$) higher yolk colour score (Table 5). Furthermore, the treatment with green kale had an influence on the egg albumen taste, which is regarded as positive for the perception of eggs, as it resulted in significantly higher 'sweet' and lower 'watery' scores ($P < 0·05$) in comparison with all other treatments except the treatment with 30 g/kg basil. The descriptors for the evaluation of yolk taste did not differ significantly between treatments. Inexplicably, there appeared to be a difference between the albumen texture evaluated by the sensory panel and analysed by mechanical compression. The treatment with inclusion of thyme in the forage material had the lowest albumen gel textural stress value at fracture (Table 4), but the same treatment obtained the highest score for hardness at chewing (Table 5).

The fatty acid contents (mg/g) of the egg yolks are given in Table 6. Significant ($P < 0·05$) treatment effects were seen on the omega-3 fatty acids C18:3 n-3 (α-linolenic acid) and C22:6n-3 (docosahexaenoic acid) which were highest in eggs from the kale treatment, being significantly higher than in eggs from the 15 g/kg thyme and 15 g/kg basil treatments, respectively, but not significantly different from the control eggs. Also, the monounsaturated palmitoleic acid, C16:1, increased significantly ($P < 0·05$) by the kale treatment compared with the control, the 30 g/kg basil, and the 15 g/kg thyme treatments.

This resulted in the overall highest n-3 content in egg yolks from the kale treatment.

In Table 7, the carotenoid contents of the standard feed, the maize silage and green kale used as forage materials, and the dried herbs; basil and thyme are shown. The results of the carotenoid analysis are given per wet weight of the matter, which resembles the state/condition of diet, forage material and herbs that was used. Naturally, the herbs resemble a higher concentration of the substances due to the usage of dried herbs added to the maize silage.

It is noticeable that the kale and basil are high in violaxanthin, which partly is reflected in the egg yolks (Table 8). Furthermore, only thyme contains neoxanthin although all eggs have a low level of this carotenoid in the yolk, which probably originates from the grass cover in the henyard even though this was a relatively small amount; less than 1 cm height. The lutein content is especially high in the kale with a ~30-fold higher concentration than in maize silage (Table 7), and this results in a significantly ($P < 0·001$) greater egg yolk lutein content than in all other treatments. The zeaxanthin is not significantly altered in the egg yolks as consequence of forage material type. However, the β-carotene concentration was also very high in the kale; ~70-fold that in maize silage. Even though β-carotene is the precursor of retinol, and

Table 7. *Means ± S.D. of carotenoid contents (μg/100 g) of feed, forage material and dried herbs analysed by HPLC* (n = 6)

Carotenoid	Diet	Forage material		Herbs	
	A	Maize silage	Kale	Thyme	Basil
Violaxanthin	24 ± 9	18 ± 11	844 ± 62	13 ± 18	576 ± 212
Neoxanthin	nd	nd	nd	99 ± 14	nd
Lutein	237 ± 18	331 ± 24	9289 ± 279	2566 ± 141	8346 ± 721
Zeaxanthin	79 ± 7	55 ± 7	30 ± 23·5	166 ± 13	238 ± 26
β-cryptoxanthin	9 ± 1	2 ± 1	161 ± 10	8 ± 2	1 ± 2
β-carotene isomers	nd	5 ± 1	459 ± 12	33 ± 3	20 ± 15
α-carotene	1 ± 1	nd	50 ± 4	21 ± 3	nd
β-carotene	4 ± 4	48 ± 2	3514 ± 105	291 ± 17	432 ± 39

nd: not detected.

Table 8. *Egg yolk carotenoid concentration (μg/100 g) as affected by forage material treatment for organic egg laying hens during 5 weeks of experiment analysed by HPLC* (n = 6)

Carotenoid	Effect of forage material					SEM	*F*-test
	Maize control	Maize + 15 g/kg Basil	Maize + 30 g/kg Basil	Maize + 15 g/kg Thyme	Kale		
Violaxanthin	92·0	52·7	81·6	55·4	149·9	29·17	NS
Neoxanthin	45·5	34·0	37·4	22·7	35·1	13·22	NS
Lutein	1835[b]	1938[b]	1760[b]	1877[b]	4531[a]	309·0	***
Zeaxanthin	529	608	543	577	582	39·0	NS
β-cryptoxanthin	14·4	18·1	16·4	16·9	21·6	1·85	$P = 0{\cdot}097$
β-carotene isomers	4·4	4·6	3·6	2·3	12·7	3·03	NS
α-carotene	nd	nd	0·8	1·0	1·5	0·89	NS
β-carotene	25·7[b]	20·2[b]	11·4[b]	13·5[b]	98·1[a]	9·80	***

[a,b]Means within one variable not sharing a common superscript are significantly different at ***$P < 0{\cdot}001$.
nd: not detected.

therefore only found at low concentrations in the egg yolk, the kale treatment resulted in a significantly ($P < 0{\cdot}001$) higher concentration of β-carotene, 4 times higher than in egg yolks from the control treatment. In general, the total content of carotenoids was twice as high in the egg yolks from hens fed on kale as forage material ($P < 0{\cdot}01$) than in the other treatments (Table 8). This was mainly caused by the increased content of lutein, which accounted for 83% of the carotenoid content in the yolks from the kale treatment, whereas it ranged between 71–73% in yolks from the other treatments.

DISCUSSION

Overall, the hens had a relatively high feed intake of 134–163 g/hen.d, which probably can be explained by the time of season for the experimental period (from October to November), where outdoor temperature was around 0–5°C. The hen houses were not heated. Hence, the hens required high energy intake to maintain their body temperature. This further is reflected in a rather high FCR, as similarly the egg production dropped during the period as the light hours decreased. The high content of starch in maize silage could contribute with energy to the hens and further, it has been shown in other studies that easily fermented components as sugars and soluble NSP, as found in kale, contribute some energy to the hens as well (Lazaro *et al.*, 2003; Steenfeldt *et al.*, 2007).

The intake of forage material did not affect the feed intake significantly, although a difference of almost 30 g/hen.d of feed intake were seen between the 15 g/kg basil treatment and the kale treatment. The intake of forage material may in general reduce the feed consumption by up to ~20% (Blair, 2008). Another recent study showed that a forage supplement of 70 g carrots/hen.d resulted in a 7–10% reduction in feed intake, however, the total intake on a dry matter basis remained constant (Hammershoj *et al.*, 2010). The type of forage material may both affect the intake of forage as well as the feed consumption. In the study by Steenfeldt *et al.* (2007), intake of 108 g/hen.d of carrots as forage material resulted in a 12% feed intake reduction, whereas a barley-pea-silage as forage material only were eaten at a level of 58 g/hen.d but resulted in 17% reduction of feed intake, whereas a similar intake of maize silage as forage material only reduced feed intake by 9%. This illustrates that several factors such as dry matter content, fibre content, and maybe also flavour of the forage material may affect the hen's consumption, which could also be the case in the present study, where both dry matter and fibre content differs somewhat between maize silage and kale. In a study where egg laying hens foraged on a pasture containing *e.g.* herbs and chicory the daily DM intake reached ~70 g/hen (Horsted *et al.*, 2006), which illustrates that the forage material may account for more than 50% of the hens' feed intake. The question remains of which amounts of kale the hens would be able to ingest if fed *ad libitum*.

As shown in the Results section, the egg production was also positively affected by the kale treatment. It is well known that the organic egg production yield is lower than cage egg production due to several contributing factors such as hen genotype, energy and nutrient requirement and mortality rate. Data from Danish egg production show that the organic versus the cage egg production is approximately 22% lower and feed intake is approximately 25% higher (Hermansen *et al.*, 2008). This brings into focus factors in organic production that can increase egg production and lower feed consumption. The present results indicate that the supply with kale as forage material is beneficial for the egg production and for the laying hen in comparison with maize silage and maize silage + herbs. Probably, the high content of methionine and lysine may be an explanation, as the kale supplies the hens with more methionine and lysine compared with the maize silage. Methionine and lysine are essential and supply of them has positive effects on egg production and egg albumen quality (Prochaska *et al.*, 1996; Shafer *et al.*, 1996; Shafer *et al.*, 1998; Hammershoj and Steenfeldt, 2005; Sundrum *et al.*, 2005) especially if the hen's fulfillment by the diet is limited. This was not the case as the basal diet supplied sufficient amino acids for egg production and in addition the feed intake was high in the kale treatment (Table 3). The average intake of methionine and lysine from the basal diet was 464 and 1218 mg/hen.d, respectively, fulfilling the recommend levels (National Research Council, 1994). It could be speculated as to whether other nutrients, *e.g.* minerals, in the kale were beneficial for egg production. From Janury 2012, all feed for organic layers will be based on 100% organic grown raw materials (The Council of the European Union, 2007), which challenges the organic production systems since alternative feed ingredients are required to fulfil the requirement of essential amino acids for poultry. The use of fishmeal will be questioned due to its competition with human food, and specific vegetable protein sources as potato protein concentrate and maize gluten are expensive and limited resources. The content of essential amino acids in foraging material as kale are not taken into account in current feed formulations for organic layers, which could be necessary and valuable in the future based on a methionine contribution from the forage material.

The effects on egg quality parameters indicated that there might be a positive effect on the shell strength by feeding kale as forage material. As seen in Table 1, the calcium content of kale is relatively high and even though the DM content and intake of this forage material is lower compared with maize silage, a calculation based on the figures from Tables 1 4 shows that the hens are supplied with 267 mg calcium/d from kale and only 63 mg calcium/d from maize silage, *i.e.* a 4-fold difference. In relation to the requirement for egg layers being approximately 3500 mg/hen.d (National Research Council, 1994), this is still only a minor contribution of ~7% of the requirement, which hardly explains an effect on the shell quality.

The vegetables belonging to the *Brassica spp.* are relatively rich in calcium and other minerals, *e.g.* galega kale leaves contain 18·5 mg calcium/g and oxheart cabbage external leaves contain 13·5 mg calcium/g (Martinez *et al.*, 2011), which is somewhat higher than the presently used kale with 2·8 mg calcium/g. The calcium absorption is decreased by the presence of oxalic acid complexing to low soluble calcium oxalate CaC_2O_4, known to be present in spinach (*Spinacea oleracea*); however in kale this appears not to be an issue as the calcium bioavailability is 10-fold that in spinach (Weaver *et al.*, 1987). In general, kale is regarded a good calcium source for humans (Lucarini *et al.*, 1999), and we suggest that is also the case for organic hens fed on kale.

The feeding of forage material providing ~95 g/hen.d of kale turned out to be especially effective regarding the yolk redness, which increased by 4 units on the a^*-scale. The corresponding lutein content in egg yolks showed a 3-fold rise from ~2·2 to ~6·2 mg/100 g yolk compared with the control treatment of maize silage only. The carotenoid analysis of kale showed very high concentrations of lutein, β-caroten, and violaxanthin, which confirmed previous analysis of kale (de Azevedo and Rodriguez-Amaya, 2005), who also detected neoxanthin to be present in kale. We only identified neoxanthin to be present in thyme, although a low concentration of this substance was present in all eggs. This may be a matter of the analysis method and the detection level. The carotenoid composition of kale depends among other factors on season and maturity of the leaves (de Azevedo and Rodriguez-Amaya, 2005). It is assumed that from kale, the violaxanthin – an orange carotenoid – that was also found in the eggs at a concentration of ~150 µg/100 g contributed significantly to the redness of the yolk. In general, the lutein makes out the major part (72–83%) of the egg yolk carotenoids, and it is in general the predominant xanthophyll in egg yolk (Chung *et al.*, 2004; Schlatterer and Breithaupt, 2006; Hammershoj *et al.*, 2010). Lutein is interesting from a human health perspective, as it has a protective function against Age-Related-Macular-Degeneration (Granado *et al.*, 2003). The egg yolk colour depends very much on the carotenoid type – yellow, orange, orange-red, the actual content of them in the diet of the hen, and also on the deposition efficiency (DE), which previously is found to be high for the xanthophylls lutein and zeaxanthin ~20–25% and low for the carotenes, which are retinol precursors e.g. β-carotene <1% (Hammershoj *et al.*, 2010). The DE is calculated on basis of the actual daily intake from basal diet plus forage material in relation to the daily output of egg mass and the yolk content of the carotenoid. In the present, the DE was for lutein high ~50% regarding the treatments with maize silage with or without herbs, but was only ~5% in the kale treatment, which can be explained due to a heavy oversupply of lutein in the diet. Similarly, the DE of violaxanthin is calculated to be ~35%, except in the kale treatment where it was only 2%, again probably due to excess in the supply, which reflects at limitation in the uptake or saturation in the deposition capacity into the yolk.

Only one other study is reported on the effect of kale on egg production and yolk pigmentation (Rojas and Avila, 1977). Here, the hens were fed on kale meal for 42 d, and in comparison to alfalfa and marigold, the egg laying rate, egg weight and FCR were similar, whereas the yolk colour was significantly higher on a Roche Yolk Color scale for eggs fed on the kale meal (Rojas and Avila, 1977).

In the literature, much attention has been on the yolk colour and content of carotenoids in relation to the egg production system. Here, we find that eggs from organic egg production can obtain significant more red and yellow yolk colours based on the type of forage material the hens are fed. This is a reflection of the carotenoid composition in diet and deposition in the egg yolk. Contradictory findings show that *e.g.* in a Danish study the eggs from organic production contains ~2–3 fold more lutein than conventional eggs (Leth *et al.*, 2000); in an Italian study, the lutein content of egg yolks are significantly higher in free range eggs than in cage eggs (Pignoli *et al.*, 2009), whereas in a British study, the lutein concentration in conventional eggs are ~2–4 fold higher compared with organic eggs (Surai *et al.*, 2000). This shows that differences in carotenoid content of the hen's diet and the forage material are reflected in the yolk carotenoid content rather than being an effect of the production system itself.

Of the many descriptors used in the present study for the sensory evaluation of the eggs, only 5 turned out to be significantly affected by the treatments. Overall, the treatments with 15 g/kg thyme and with kale turned out to result in the most differentiable eggs as both whole egg aroma 'sulphur', yolk colour, albumen taste 'sweet' and 'watery were significantly different. The descriptor 'sulphur' scored less for the kale treatment followed by the 30 g/kg basil treatment, which is believed to be preferred by the consumers. Another study with almost similar descriptors argued that sulphur-containing amino acids; methionine and cysteine, in surplus might cause higher scores for 'sulphur' in the eggs (Horsted *et al.*, 2010). This should then mean that hens fed on maize silage with 15 g/kg thyme, which resulted in sulphurous whole egg aroma, albumen taste and yolk taste should have a surplus of sulphur containing amino acids, which is hardly the case from the figures in Table 2. The fact that eggs from the kale treatment scored low on this sensory descriptor surprised us, as species of the *Brassica* family are reported to produce quantifiable amounts of methyl sulphide and dimethyl disulphide gases with distinct aromas (Wang *et al.*, 2009), which apparently was not detected by sensory evaluation of the eggs. Furthermore, the kale treatment also resulted in a significantly high score in albumen taste of 'sweet' and a low score in 'watery' taste, which again are assumed to be positive regarding the overall perception of the egg sensory properties. Feeding egg laying hens with thyme in the form of 20 g/kg thymus meal has previously proven to significantly affect the sensory properties of eggs positively by lower scores for off-flavour and higher scores for odor, flavour, and overall acceptability compared with diets without thymus meal (Tserveni-Gousi, 2001).

No reports have been found on feeding with the basil variety used here *Ochimum basilicum*. Only very few studies are reported on egg laying hens fed on holy basil (*Ochimum sanctum*), which at levels of 2 g/kg is referred to be positive for the sensory properties of eggs (Narahari, 2003) and at 1–2 g/kg as meal increases the α-linolenic acid level in the yolk (Kirubakaran *et al.*, 2011).

The fatty acid composition of the egg yolks were altered by the kale treatment towards higher n-3 proportion, which is regarded to be positive in a human health context (Samman *et al.*, 2009). Again, the treatment containing 15 g/kg thyme turned out to result in lower levels of n-3 fatty acids, which was also a tendency observed by Tserveni-Gousi (2001) when fed together with 100 g/kg whole flaxseed. For inexplicable reasons, treatment with thymus meal results in reduced n-3 fatty acids in total, and specifically C18:3(n-3) and C22:6(n-3) decreased, while C20:5(n-3) and C22.5(n-3) remained constant or increased slightly (Tserveni-Gousi, 2001).

In conclusion, the present study showed that it is possible to produce organic eggs which have differentiable qualities regarding appearance by yolk colour, content of health important compounds as lutein and n-3 fatty acids, and sensory properties of egg aroma and taste. The supply of kale as forage material has high potential for contributing to these food qualities, and furthermore egg production also increased when this forage material was provided.

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