Review

The effect of grass and herbs in organic egg production on egg quality with focus on fatty acid composition, egg yolk color and sensory properties

Græsmarkurtens betydning for ægkvalitet – fokus på fedtsyresammensætning, blommefarve og sensorisk kvalitet

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Abstract

The requirement of access to pasture or forage material in the organic egg production may offer a wide range of plant material for the hens. Mainly grasses, herbs and legumes are cultivated in the pasture for the hens. The effects of different grasses and herbs being eaten by the egg-laying hen on the egg quality is elucidated. In relation to the fatty acids, the carotenoids and the aroma compounds in various plants, the consequences for egg yolk fatty acid composition, yolk color and sensory properties of organic eggs are discussed.

Introduction

In organic egg production, it is regulated within EU and i.e. also Denmark that '*Roughage, fresh or dried fodder or silage must be supplied in the hens daily ration*' and '*Poultry should be reared in open-range conditions with free access to open-air run*' (Blair, 2008), and the hen-yard area should be at least 4 m^2 per hen (The Council of the European Union, 2007). The main reason for the supplement of roughage is to fulfill the behavioral needs of the animals.

The roughage in the Danish organic egg production is typically fresh plant material from the pasture in the hen-yard (grass and herbs), freshly cut or harvested plant material (grass, herbs, vegetables), dried plant material (hay, wrap) or silages of plant materials (maize, alfalfa, barley, pea, etc.). The farmer will choose to seed a specific mixture of grass or grass-herbs in the hen-yard area, to fulfill the requirement of vegetation cover.

Until now, only few studies have estimated the intake of forage material or roughage from the pasture to range from 2-57 g dry matter per hen per day (Horsted, Hammershoj, & Hermansen, 2006; Lorenz, Kany, & Grashorn, 2013). This large variation makes it therefore difficult to ascribe the supply of nutrients to the hen from intake of grass and herbs in the pasture, which depends on both the season and botanical composition of the pasture (Breitsameter, Gauly, & Isselstein, 2014) as well as the birds age, genotype, rearing conditions etc. (Lorenz, et al., 2013). Hence, still the knowledge on the nutritive value of plants in the pasture for egg laying hens is limited, and especially knowledge on, how egg quality is affected by deposition of nutrients or other components from the grass and herbs to the egg, is very limited.

As the various grass and herb types used in the pasture for egg laying hens contains different nutrients and compounds that transfer to the eggs laid by the hen, these may have an impact on the eggs. The roughage intake in organic egg production could be an important factor in differentiating the organic egg quality.

It is hypothesized that

- the usage of roughage, here defined as grasses and herbs, affects the quality of the organic eggs by its content of components,
- the different herbs in the grass-mixture vary in fatty acids, carotenoids and aroma compounds, which results in different egg fatty acid composition, egg yolk color and sensory properties,
- the usage of specific herbs in the grass-mixture can differentiate organic eggs as a high quality food.

The aim of this review is to synthesize the existing knowledge in literature on herbs usable in the Danish organic farming, which may have a potential in affecting the egg fatty acid composition, yolk color and egg organoleptic quality when fed to laying hens. These qualities are expected to be important for the consumer and may potentially be a differentiable parameter affecting the egg market share for organic eggs.

1. Grasses and herbs in temperate climate and contents of fatty acids, carotenoids and aroma compounds

The majority of studies on effect of grassland, herbage, herbs, and forages on animal product quality has been focusing on dairy cows and sheep and the effect on milk production, and on the contents and composition of milk and cheese (Larsen, Frette, Kristensen, Eriksen, Soegaard, & Nielsen, 2012; Lourenco, Vlaeminck, Bruinenberg, Demeyer, & Fievez, 2005; Mariaca, Berger, Gauch, Imhof, Jeangros, & Bosset, 1997; Petersen, Soegaard, & Jensen, 2011). Furthermore, the herbs that can be cultivated in temperate climate, which covers the mid-Europe area, are limited due to stop of plant growth during the winter season, with temperatures <0°C. In the following section, the commonly cultivated herbs and grasses in this climate zone are listed together with some potential alternative herbs.

Grasses cover all members of the Poaceae family, and they are usually herb plants with narrow leaves. In the present context, grasses are those varieties included for farmed grassland, e.g. pastures. One of the most efficient sources to provide pigmenting compounds to the egg yolk is a grass pasture in the hens' outdoor area. Grass contains lutein, zeaxanthin and beta-carotene in amounts, which for lutein and beta-carotene are comparable to alfalfa silage (Skrivan & Englmaierova, 2014). I this study from mid Europe together with own data (unpublished) from Denmark, a young grass plant had lutein contents of 122-128 mg/kg DM, zeaxanthin 4-114 mg/kg DM and beta-carotene of 54-79 mg/kg DM. The carotenoid content varies with the botanical composition of the grass, and the early grass cuts has the highest concentrations, which decreases with the growth season.

Alfalfa (*Medicago sativa)* lucerne is a common silage crop, which can be used for organic laying hens (Hammershoj & Steenfeldt, 2015). Even tough alfalfa is well known as a dried feed compound as alfalfa meal for egg layers, it is also worth mentioning as silage, which besides being widely used as cattle-feed also can be fed to organic egg laying hens (Hammershoj, et al., 2015; Steenfeldt & Hammershoj, 2015). Alfalfa contains mainly lutein together with neoxanthin and violaxanthin, of which the two last ones belong to the red xanthophylls (Nys, 2000). The content of carotenoids increases with increasing protein content, and the content in the leaves is higher than in the stem.

Clover includes red clover (*Trifolium pratense*) rødkløver, which is a nitrogen fixating plant resulting in a high protein content, and is commonly used as a feed plant, and white clover (*Trifolium repens*) hvidkløver, which grows wild in Denmark and is often used in grass-herb mixtures for farmed animals' pastures. Especially, the content of phytoestrogens - being secondary plant metabolites - has obtained much attention due to the estrogen-like effect in humans health, and red clover has a substantially high content hereof both in comparison to white clover, as well as to alfalfa and chicory (Horsted, Søegaard, & Kristensen, 2011).

Chicory (*Cichorium intybus)* cikorie is a herb, which has been in focus for grass-herb mixtures, and it grows well in temperate climate. Especially, the leaves are of interest in egg production (Horsted, et al., 2006; Horsted, et al., 2011).

Stinging nettle (*Urtica dioica L.)* brændenælde is a commonly found plant in the temperate climate, and is known as an old medical plant due to its various healing properties (Grevsen, Frette, & Christensen, 2005). Nettle is also used as human food, and the herb is cooked in soups either fresh or as dried during the winter season. The fatty acids composition of stinging nettle is both in mature and young leaves dominated by linolenic acid (C18:3 n-3) with 29.6-40.7%, palmitic acid (C16:0) with 17.9-20.1% and linoleic acid (C18:2 n-6) with 11.6-18.1% (Guil-Guerrero, Rebolloso-Fuentes, & Isasa, 2003). The stinging nettle also contains carotenoids of mainly lutein 366-525 mg/kg, □-carotene of 100-111 mg/kg and zeaxanthin 23-60 mg/kg plant (Loetscher, Kreuzer, & Messikommer, 2013). According to another study the total content of carotenoids is ~50-75 mg/kg DM, of which lutein is the main component, followed by violaxanthin, neoxanthin and β-carotene (Guil-Guerrero, et al., 2003), which means the content of carotenoids resembles the level in grass and alfalfa. Often the stinging nettle grows in the hen-yard, as it is an indicator of nitrogen-rich soil, however often the hens avoid eating them in the hen yard, because the stinging/burning effect exists in the fresh plant, but is lost after harvest and a few hours of drying of the plant.

Industrial hemp (*Cannabis sativa L.*) hamp is an oil-seed plant, which primarily is used for fiber production. It can easily grow in temperate climate, and studies on feeding both cattle and laying hens as hemp seed in the diet as well as silage (Silversides & Lefrancois, 2005), (own data, not published).

The plant burnet (*Sanguisorba minor*) bibernelle also known as salad burnet, is well known as herb for cows, and is rich in minerals and vitamins, however low in protein (Horsted, et al., 2011). It can grow in temperate climate in mixtures with other herbs, however with a reported low yield. No studies on using this herb for egg laying hens are reported in literature.

English plantain (*Plantago lanceolata*) lancet vejbred is a herb, which can be cultivated in temperate climate, and it is a common weed in cultivated land. Only few reports exist on poultry fed this herb, and all on broilers (Chacrabati, Chowdhury, Yesmin, Sano, & Al-Mamun, 2013; Tamura, et al., 2010). There are no reported studies on egg laying hens.

Common sainfoin *(Onobrychis viciifolia)* esparsette is a legume, which until the mid 1950'es was an important forage crop in temperate climate, hereafter it has been replaced by alfalfa and clover in agricultural cultivation. It is reported to grow well in cultures together with grasses and other legumes (Horsted, et al., 2011), although in Danish climate and soil conditions it has shown less competitive. Nevertheless, it has a high content of minerals and protein, but also tannins, which may be anti-nutritional.

Birdsfoot trefoil (*Lotus corniculatus*) kællingetand is a legume, which has been used in feeding of cattle, both for dairy and meat production. It has a high content of beta-carotene and vitamin E, and has an impact on food products such as cheese and beef produced from animals foraging on the legume (Horsted, et al., 2011; MacAdam & Villalba, 2015). Furthermore, the birdsfoot trefoil has a very high fatty acid content of the plant containing 27 g/kg DM of which linolenic acid makes out 54% of the total fatty acid composition (Elgersma, Soegaard, & Jensen, 2013).

Basil (*Ocimum basilicum*) basilikum is one of a wide range of varieties within the family. The plant contains volatile aroma compounds and is popular in human foods. However, the herb is not easy to grow under temperate climate conditions.

Thyme (*Thymus vulgaris*) thimian is a herb with wide use in foods. It is best cultivated in hot climate with well-drained soil, although it can grow in temperate climate and can take deep freezes, it has no widespread cultivation here. There are studies with feeding laying hens with either thymus meal incorporated in the diet (Tserveni-Gousi, 2001), or as dried herb inclusion in a silage (Hammershoj & Steenfeldt, 2012).

Oregano (*Origanum vulgare*) is a herb, which can grow in temperate climate, and are found in open habitats. The herb has strongly aromatic leaves and is widely used in food dishes. Only little is known about its co-existence in grass-herb mixes.

Pot marigold (*Claendula officinalis*) morgenfrue and marigold (*Tagetes erecta L.*) tagetes both belong to the family of Asteraceae and are short-lived garden flowers with aromatic smell, which grows easily in hot temperate climate as well in summer season of Denmark. It has been used in dry form and as extract of the petals in egg layers diet due to its content of pigments (Panaite, Bunduc, Criste, & Cornescu, 2015), however no scientific reports exist on its use as fresh herb in the pasture for organic hens. The flower petals of pot marigold and marigold contains pigmenting carotenoids of mainly lutein, lutein esters, zeaxanthin, lycopene and beta-carotene (Galobart, Sala, Rincon-Carruyo, Manzanilla, Vila, & Gasa, 2004; Hadden, et al., 1999; Karadas, Grammenidis, Surai, Acamovic, & Sparks, 2006; Panaite, et al., 2015).

2. Organic egg production and effect of grass and herbs on egg yolk fatty acids, pigments and sensory quality parameters

2.1 Effect of grass and herbs for laying hens on egg quality

The comparison of egg quality parameters between egg productions, where hens have access to pasture with grass and without outdoor access, has been performed for both sensory quality in grass-clover pasture (Horsted, Hammershoj, & Allesen-Holm, 2010), yolk fatty acid composition (Karsten, Patterson, Stout, & Crews, 2010; Lopez-Bote, Arias, Rey, Castano, Isabel, & Thos, 1998; Mugnai, Sossidou, Dal Bosco, Ruggeri, Mattioli, & Castellini, 2013), vitamins A and E (Karsten, et al., 2010; Lopez-Bote, et al., 1998), and egg yolk carotenoids (Mugnai, et al., 2013; Skrivan, et al., 2014).

2.2 Overall fatty acid composition of egg yolks, egg yolk color and sensory properties

A large number of studies during time have concluded that the fatty acid composition of egg yolks depends highly on the fatty acid composition of the feed for the laying hen (Bruneel, et al., 2013; Halle, 1996; Hammershoj, 1995; Surai & Sparks, 2001). The most typical fatty acid pattern of egg yolks is given in Table 1 from different control diets or commercial diets in various studies, i.e. giving the normal range of contents, where fatty acid manipulation has not been the aim.

Table 1. Fatty acid composition of the quantitatively eight major fatty acids of egg yolks (g FA/100g FA) from control or reference dietary treatments or commercial diets of different studies as indicated

Fat source of diet		3% animal fat (Hammershoj, 1995)	2.1% lard (Lopez-Bote, et al., 1998)	Conventional eggs (Samman, et al., 2009)	0.6% animal and vegetable fat (Karsten, et al., 2010)	Conventional eggs (Woods & Fearon, 2009)
Palmitic acid	C16:0	25.8	24.0	25.1	24.3	24.0
Palmitoleic acid	C16:1	2.6	2.3	3.2	3.1	-
Stearic acid	C18:0	9.5	13.1	8.4	8.0	8.4
Oleic acid	C18:1	44.4	36.0	46.7	42.0	42.8
Linoleic acid	C18:2 n-6	12.0	18.7	13.1	14.2	17.2
Linolenic acid	C18:3 n-3	0.7	0.4	0.5	0.3	0.9
Arachidonic acid	C20:4 n-6	1.8	2.1	1.8	2.1	-
Docosahexaenoic acid (DHA)	C22:6 n-3	0.3	0.6	0.9	0.8	-
	Sum n-3	1.0	1.2	1.4	1.3	0.9
	Sum n-6	12.2	21.6	15.0	16.3	17.2
	n-6/n-3	12.2	18.7	11.0	12.1	19.1

From the data in Table 1, it is clear that the major fatty acids in egg yolk composition are oleic acid > palmitic acid > linoleic acid > stearic acid > palmitoleic acid > arachidonic acid > linolenic acid ≈ DHA.

2.2.1 Effect of grass and herbs on fatty acid composition of eggs

Mugnai et al. (2013) found that egg laying hens with access to 10 m^2 per hen grass pasture in the spring season had the highest effect on the yolk fatty acid content compared both with other seasons (winter, summer, and autumn) and with hens without access to pasture. The eggs produced on the spring grass pasture had in comparison with eggs from hens without access to pasture a reduced palmitic acid content, an increased palmitoleic acid content, a reduced stearic acid content, an increased oleic acid content, decreased linoleic acid content, increase linolenic acid content, decreased arachidonic content, and increased DHA content. Especially, the changes in long polyunsaturated fatty acids (>C20) were significant by 3-5 fold changes (Mugnai, et al., 2013). The changes in saturated fatty acids were minor and insignificant, whereas changes in monounsaturated fatty acids were significant for oleic acid.

Other studies have found that grass pastured hens lay eggs with somewhat other alterations of the fatty acid composition as illustrated in Table 2. Here the oleic acid content is decreased for grass pastured hens compared with the values in Table 1 of eggs from hens without access to pasture, while the linolenic acid and the DHA increases around 2-3 fold. The other fatty acids remain more or less at the same levels. The sum of n-3 fatty acids is higher, when hens have access to grass, which results in a decrease in the ratio of n-6/n-3. The ratio of n-6/n-3 has been paid much attention by dietary scientist the recent 20-30 years, as the modern lifestyle increases the n-6 intake for humans on account of the n-3, where an optimal ratio of n-6/n-3 is ~3-5 (Gerzilov, Nikolov, Petrov, Bozakova, Penchev, & Bochukov, 2015), which is achievable for eggs from grass pastured hens (Table 2) compared with eggs from hens without grass pasture (Table 1).

Feeding broiler chickens with the herb plantain resulted in a reduction of the meat fatty acid ration of n-6/n-3 together with lower peroxide values of the meat (Tamura, et al., 2010).

Mixing powders of garlic, yarrow, oregano, thyme, basil, rosemary and cinnamon to a total of 0.23% in the diet of egg laying hens resulted in unchanged egg yolk fatty acid composition except an increased arachidonic acid content and a decreased eicosapentaenoic acid (C20:5) content in triglycerides, and increased linolenic acid and DHA content in phospholipids (Gerzilov, et al., 2015).

Table 2. Fatty acid composition of the quantitatively eight major fatty acids of egg yolks (g FA/100g FA) from grass fed or grass pastured egg-laying hens as indicated

Fat source of diet		Grass pasture (own data unpublished, 2012)	Grass pasture (Karsten, et al., 2010)	Grass free range (Lopez-Bote, et al., 1998)	Trend in relation to eggs from hens without grass pasture (Table 1)
Palmitic acid	C16:0	23.2	24.6	27.0	-
Palmitoleic acid	C16:1	1.9	3.4	2.6	-
Stearic acid	C18:0	8.7	7.7	14.1	-
Oleic acid	C18:1	37.4	38.9	36.9	↓
Linoleic acid	C18:2 n-6	19.3	14.8	12.0	-
Linolenic acid	C18:3 n-3	1.6	1.2	1.0	↑
Arachidonic acid	C20:4 n-6	2.5	2.1	2.0	-
Docosahexaenoic acid (DHA)	C22:6 n-3	3.6	1.5	1.6	↑
	Sum n-3	5.3	3.0	3.0	↑
	Sum n-6	21.7	16.9	14.7	-
	n-6/n-3	4.1	5.7	5.2	↓

Hemp seed meal included in laying hens diet altered the fatty acid composition of the egg yolks with significantly higher linoleic and linolenic acid contents (Silversides, et al., 2005). The increase in egg yolk linolenic acid was confirmed by feeding whole hemp plants as silage to egg laying hens (own data, unpublished), in comparison to hens on diet without forage material. The linoleic acid content did not differ. On the other hand, feeding with dried and grinded marigold flowers in the diet at levels of 10-20 g/kg feed to laying hens increased the total content of saturated fatty acids and decreased the monounsaturated fatty acids of the egg yolks (Altuntas & Aydin, 2014). Hence, this indicates that the flowers have a less favorable effect on egg yolk fatty acid composition compared to many of the other green leave herbs. In this area, the nettle leaves appear to have very high content of linolenic acid, which ranges from 30-40 g/100 g sample (Guil-Guerrero, et al., 2003), but the effect hereof on egg yolk fatty acid composition has not been reported.

Hens on a pasture with mix of red clover (variety Cinnamon) and white clover (variety California Ladino) making out 70% of the pastures botanical composition produced egg yolks compared with caged hens, with significantly lower percentage of oleic acid (38.9% vs. 42.0%), higher linoleic acid (15.0% vs. 14.2%), higher linolenic acid (1.6% vs. 0.3%), higher EPA (0.04% vs. 0.02%), and higher DHA (1.6% vs. 0.8%), respectively (Karsten, et al., 2010). The n-6/n-3 ratio of clover pasture produced eggs was 4.8, comparable to alfalfa pasture produced eggs of 4.4, but lower than grass pasture eggs of 5.7 (Table 2) and significantly lower than eggs from caged hens of 12.1 (Table 1) (Karsten, et al., 2010). Another study, where broilers were raised with free access to pasture of white clover resulted in the breast meat fatty acids linoleic acid and linolenic acid to decrease, and at the same time the EPA increased significantly, suggesting a higher conversion of linolenic acid into EPA in these broilers compared to meat from broiler without access to pasture (Ponte, et al., 2008).

The fatty acid content of salad burnet is relatively high, and linolenic acid accounts for the majority (~47%) of the composition followed by linoleic acid (~22%) (Elgersma, et al., 2013). Furthermore, the quantitative content of fatty acid is in the high end regarding herbs as 22.2 g/kg DM is fatty acid. Although, no experimental studies on pastures with salad burnet for egg laying hens are reported, this fact could make it worthwhile to include salad burnet in a grass-herb pasture for organic egg production.

Likewise for birdsfoot trefoil, there are no reports on its use in pastures for egg laying hens, although it has been reported to efficient against parasite infections in lamb, deer, pigs and cattle (Marley, Cook, Barrett, Lampkin, & Keatinge, 2004). However, due to its high content of fatty acids, and especially linolenic acid, together with at high content of lutein 206 mg/kg DM compared with other herbs and legumes of 129-174 mg lutein/kg DM (Elgersma, et al., 2013), it appears promising for including in a grass-herb pasture for organic laying hens and the effect on egg quality.

Even though, chicory has been studied as feed ingredient and as pasture crop for different farm animals including laying hens, the effect hereof on egg yolk fatty acid composition has not been analyzed. While the fatty acid composition of the leaves resembles something in-between the salad burnet and the alfalfa leaves (Elgersma, et al., 2013), chicory is expected to influence the egg yolk fatty acid composition likewise.

2.3 Egg yolk color of eggs as affected by grass and herbs

It is well documented that grass and other plant materials containing carotenoids as xanthophylls affect the egg yolk color, when fed to egg laying hens (Hammershoj, 2014). There are studies on the fatty acids and carotenoids in nettle and its use for egg laying hens as pigmenting source for egg yolk (Guil-Guerrero, et al., 2003; Loetscher, et al., 2013).

Comparison of eggs from hens receiving the same standard feed, but differed in with or without access to outdoor pasture with grass, showed significant differences in the yolk color parameters L* (lightness), a* (redness) and b* (yellowness), where hens with outdoor access laid eggs with significantly darker, more reddish/yellowish egg yolks (Dvorak, Suchy, Strakova, & Kopriva, 2012). Likewise, for hens with outdoor access to either grass pasture or bare ground showed a 2-fold increase in content of carotenoids in the egg yolk for both lutein, zeaxanthin and beta-carotene, for hens with access to grass pasture (Skrivan, et al., 2014). Here the yolk color was measured by the RYC-scores, resulting in 8.6 for yolks from hens with access to bare ground and 10.3 for those with access to grass pasture, and the egg yolk darkness L* and redness a* increased correspondingly, however not the yellowness b*.

Besides the EU regulation for organic egg production requiring 4 m^2 outdoor area per hen (The Council of the European Union, 2007), a further increase of the area available with attractive grass-herb cover seems to have an effect on the egg yolk color as well. By increasing the area from 4 to 10 m^2 per hen the egg yolk color increased by RYC-scores from 9.7 to 11.2, respectively (Mugnai, Dal Bosco, & Castellini, 2009), and the content of lutein in the egg yolks increased in parallel from 0.55 to 1.39 mg/100g (Mugnai, et al., 2013). However, it should be noted that in flock sizes of 3000 hens, in practice it is a challenge for the hen flock to spread evenly on a correspondingly larger outdoor area.

Alfalfa has a good pigmenting effect of the egg yolk, which is comparable to corn gluten meal (Fletcher, Papa, Halloran, & Burdick, 1985; Leeson & Caston, 2004). Feeding quails with concentrate of alfalfa resulted in a carotenoid content in the egg yolk, which was at a level comparable to the result of feeding with marigold, i.e. 0.22-0.39 mg/100 g (Karadas, et al., 2006).

Feeding with alfalfa silage is very common to dairy cows, and can be used for organic egg laying hens also. Alfalfa silage has a lutein content comparable to grass and 2-15 fold higher than maize silage dependent on the quality and season of the alfalfa (Hammershoj, et al., 2015). Feeding with alfalfa silage resulted in egg yolks with higher redness a* and yellowness b*.

Dried and milled whole nettle plants were included in the feed at levels of 6.25-25 g/kg for egg layers, and the egg yolk color parameter yellowness increased, while darkness and redness was not significantly affected (Loetscher, et al., 2013). This resembled a RYC-score increase from ~2 when no nettles were included in the diet to ~6 at 25 g nettles/kg feed.

Chicory leaves contain lutein at an intermediate level of ~150 mg/kg DM, which is a bit higher than alfalfa and salad burnet (~130 mg/kg DM) but lower than birdsfoot trefoil of ~200 mg /kg DM (Elgersma, et al., 2013). One study with organic laying hens on a pasture with chicory, showed that the hens do consume a high quantity of the chicory leaves, and that the egg yolk color tended to be darker, more reddish and less yellow compared with an egg yolks from hens on a grass-clover pasture (Horsted, et al., 2006).

A study by (Narahari, Manohar, Suba, & Thiruvengadam, 2009) showed that two types of herbs belonging to the basil family (*Ocimum sanctum* and *Ocimum album)* Holy basil and White Basil, respectively had a significant (P<0.05) effect on egg yolk color when fed to egg laying hens. The hens did not have access to outdoor pasture and the basil was included in the feed at 3 g/kg.

2.4 Sensory properties of eggs – effect of grass and herbs

Only very few studies have focused on the effect of grass and herbs in the hen yard of egg laying hens for the sensory properties regarding flavor of the eggs. Most literature deal with inclusion of dried herbs or flavor components in the diet of the hen or given as silages.

As dried meal or dried herb, the inclusion of thyme and basil to egg layers diets may have an impact on the sensory properties of the eggs produced (Hammershoj, et al., 2012; Tserveni-Gousi, 2001). However, no reports of feeding these aromatic herbs as fresh for egg layers are found.

Feeding alfalfa silage in comparison with maize silage plus carrots as forage material for egg layers, resulted in sensory evaluation of hard boiled eggs mainly differing in yolk color, with only minor differences in egg albumen taste being more positively perceived when hens were given alfalfa silage (Hammershoj, et al., 2015).

A study with broilers given dried herbs and aroma compounds in the diet including garlic, rosemary, thyme and yarrow (røllike), resulted in only very small effects on the flavor intensity of the chicken meat compared with a control without any herbal addition (Cross, McDevitt, & Acamovic, 2011). However, the garlic and thyme essential oil included diets were characterized to result in high sensory scores on 'abnormal' flavor of cooked meat, and garlic included diet gave resulted in a high score of 'garlic' flavor (Cross, et al., 2011).

Eggs from hens on a grass-clover pasture were evaluated for sensory properties in comparison with eggs from hens without access to outdoor range fed on the same layer diet (Horsted, et al., 2010). Here, both the taste and aroma of eggs were found to be more watery, less fresh, more sulfurous, more animal and more cardboard-like from hens on pasture, however without a direct linkage to the plant material fed. The study illustrated the complexity in combining feed with high plant material intake for egg laying hens.

2.5 Intake of grass and herbs by egg laying hens

The amount of forage material eaten by the hen is directly linked to its possible impact on the egg quality parameters described above. A large variety in the intake of grass and herbs from a pasture and from forage material offered as cut, fresh or silages are reported.

In Table 3, reported intakes of a range of different forage materials are shown. It is underlined, that the intakes from pastures are estimated by different methods, which may have some uncertainty (Lorenz, et al., 2013). Furthermore, different conditions may affect the intake of forage material such as hen age and adaptation to forage material, season of the pasture (highest intakes in the spring) (Mugnai, et al., 2013), content of dietary fibres (Steenfeldt, et al., 2015), dry matter and nutrient concentration of forage material versus basal feed (Horsted, et al., 2006). There are however, no rule of thumb regarding the intake amount of forage material, which may range from 6-126 g/hen/day (Table 3), and the corresponding basal feed intake ranges from of 102-155 g/hen/day. Hence, a high forage material intake does not necessarily account for a low intake of basal feed and vice versa. However, in most cases does the forage material intake make out <50% of the total intake by the hen, and if calculating on a dry matter basis it is evenly less, as the forage material or pasture contains less dry matter than the basal layer feed.

Table 3. Intake of basal feed and different forage materials (g/hen/day) by egg laying hens

Pasture or forage material type	Intake of forage material	Intake of basal layer feed	Reference
Grass-clover-herb pasture	73	95	(Skrivan, et al., 2014)
Maize silage	112	139	(Hammershoj, et al., 2012)
Kale, fresh	95	163	(Hammershoj, et al., 2012)
Grass pasture (4 m^2/hen)	6-18	102-115	(Mugnai, et al., 2013)
Grass pasture (10 m^2/hen)	14-59	96-116	(Mugnai, et al., 2013)
Maize silage, carrots	104	113	(Steenfeldt, et al., 2015)
Alfalfa silage	57	124	(Steenfeldt, et al., 2015)
Barley-pea silage	58	107	(Steenfeldt, Kjaer, & Engberg, 2007)
Grass-clover pasture	111	129	(Horsted, et al., 2006)
Chicory pasture	126	155	(Horsted, et al., 2006)

3. Conclusion and recommendations for grass and herbs in organic egg production

Based on the literature it is concluded that a range of herbs and grasses can affect the quality of the animal products i.e. meat and egg of the animals foraging on the plants either as pasture or as forage material.

Especially, the fatty acid composition, egg yolk color, and sensory properties may be influenced by grass and herb composition. For favorable fatty acid composition of the egg yolk, a grass pasture or feeding with grasses as forage material is proven a potential source of n-3 fatty acid. The herb stinging nettle is interesting in several aspects; it grows easily in temperate climate, has a high content of linolenic acid in the leaves, and can increase the yolk color yellowness. However, the level of intake as fresh from a pasture by poultry is uncertain. In addition, alfalfa is a good source of both n-3 fatty acids, especially linolenic acid, EPA and DHA, and of pigmenting carotenoids for the egg yolk. The conclusion on sensory quality is scarce as only few studies have analyzed the effect of fresh grass and herbs intake of laying hens for the egg flavor properties, however, there are indications that pasture may affect the sensory properties, but not in a predictive and straight forward way.

In consideration of the Danish climate and cultivating conditions, and to achieving a combined optimal effect on the above described egg quality parameters, it is recommended to cultivate a grass-herb pasture with single herbs or combinations of stinging nettle, alfalfa, salad burnet and birdsfoot trefoil for organic egg laying hens.

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