Influence of rapeseed cake, linseed cake and hemp seed cake on laying performance of hens and fatty acid composition of egg yolk

Article *in* Journal für Verbraucherschutz und Lebensmittelsicherheit · September 2013

DOI: 10.1007/s00003-013-0822-3

Friedrich Schöne
Thuringian State Institute of Agriculture Investigation Unit - Laboratory

Influence of rapeseed cake, linseed cake and hemp seed cake on laying performance of hens and fatty acid composition of egg yolk

Ingrid Halle & Friedrich Schöne

Journal für Verbraucherschutz und Lebensmittelsicherheit
Journal of Consumer Protection and Food Safety

ISSN 1661-5751
Volume 8
Number 3

J. Verbr. Lebensm. (2013) 8:185-193
DOI 10.1007/s00003-013-0822-3

Springer

J. Verbr. Lebensm. (2013) 8:185–193
DOI 10.1007/s00003-013-0822-3

Journal für Verbraucherschutz und Lebensmittelsicherheit
Journal of Consumer Protection and Food Safety

RESEARCH ARTICLE

Influence of rapeseed cake, linseed cake and hemp seed cake on laying performance of hens and fatty acid composition of egg yolk

Ingrid Halle · Friedrich Schöne

Received: 21 November 2012 / Accepted: 30 April 2013 / Published online: 22 May 2013

Abstract In hens the effects of expeller/cakes from rapeseed, linseed, and hemp seed were investigated on feed intake, laying performance and fatty acid composition of egg yolk. A total of 216 individually caged laying hens were allocated to nine dietary treatments (5, 10, 15 % cake) and fed for six laying months. For feed intake, egg mass production and feed-to-egg mass ratio at 15 % dietary cake level a significant lower performance was shown compared with the 5 and 10 % cake level groups. Also interaction was significant for all these parameters, indicating that the cake level acted differently for the three cakes. The egg mass production was lower in the linseed cake than in the hempseed groups and the hens fed the linseed cake needed significantly more feed per kg egg mass compared with both the other cakes tested. Increasing dietary level of all oil seed cakes lowered the yolk percentage and increased the egg white percentage. Increased dietary levels of all three oil seed cakes lowered the percentages of most saturated fatty acids and also the percentages of the monounsaturated fatty acids. The linoleic acid and the linolenic acid as polyunsaturated fatty acids were heightened by increasing the cake levels from 5 to 10 and 15 % in the diet. The results allow the conclusion that compound feeds with up to 10 % of cakes does not negatively influence the laying performance of hens and provides the possibility of the enrichment of yolk fat with polyunsaturated fatty acids.

Keywords Rapeseed cake · Linseed cake · Hemp seed cake · Hen · Laying performance · Fatty acid composition

1 Introduction

Oil seed meals, solvent extracted, and oil seed expeller-cakes are important protein and energy sources in animal feeding. Plant oil supplements to laying hen diets are primarily used to satisfy the essential fatty acid requirement. The different shares of polyunsaturated fatty acids (PUFA) in the different lipid sources can be used to modify the fatty acid composition of egg yolk (Ohtake and Hoshino 1967; Noble et al. 1990; Hargis and Van Elswyk 1993; Halle 2000; Antruejo et al. 2011) which might have health benefits for the consumer (Simopoulos and Salem 1992; Jiang and Sim 1993; Blanch and Grashorn 1996; Lewis et al. 2000).

Rapeseed was grown on 1.46 million ha in Germany in 2010 contrasting with only 6.86 thousand ha linseed and 1.2 thousand ha hemp. Three million tons rape seed meal and 4.9 million tons other oil seed meals, mainly imported soya-bean meal, were used as protein feed (UFOP Bericht 2010/2011; EFSA 2011). Linseed and hemp seed feeds are of minor importance, however, they are listed in the "German Positive List of Feed Materials" (Normenkommission

I. Halle (✉)
Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, Braunschweig, Germany
e-mail: ingrid.halle@fli.bund.de

F. Schöne
Thüringer Landesanstalt für Landwirtschaft (TLL), Naumburger Str. 98, Jena, Germany

für Einzelfuttermittel 2011) in addition to the rapeseed feeds.

Besides the food and feed use the oil from rapeseed (*Brassica napus*) is also the basis for European biodiesel production. Therefore rapeseed meal and rapeseed cake are increasingly available as protein and energy feed components. Whereas the meal from "00-rapeseed" causes no problems in the feeding of ruminants, there is a maximum of 10 % rapeseed meal and 5 % rapeseed cake in the feed for hen strains laying white eggs (Spiekers et al. 2012; Jeroch and Dänicke 2012). For some brown-egg laying hen strains (Honkatukia et al. 2005), the inability to metabolize trimethylamin (TMA) originating from the bacterial degradation of rapeseed sinapine in the large intestine results in "fishy taint" eggs, and therefore rapeseed feeds are seen critically (Butler and Fenwick 1984). Meanwhile the Lohmann Tierzucht Company identified the gene which is responsible for the inability of some hens to metabolize TMA and these hens were eliminated from breeding. So, Lohmann Tierzucht announced that all commercial laying hens of Lohmann and H&N origin hatching from January 2007 onwards are free of the genetic defect and may receive diets containing rape seed products (Pottgüter 2006).

Linseed or flaxseed (*Linum usitatissimum*) and the respective meal and cake contain "mucilaginous gums" (Cheeke 1991) which prevent gastrointestinal disorders in piglets, calves and horses. Seeds and oil were both used in human nutrition. Due to the steam treatment, mainly in the meal, the content of cyanogenic glycosides is very low. Minor amounts of prussic acid, which is formed by plant own and/or microbial glucosidases in the intestine are effectively detoxified to thiocyanate by the enzyme sulfurtransferase (the former term is rhodanese). For this reason feeding linseed cake increased the concentration of thiocyanate in the blood serum of pigs (Schöne et al. 1997) or laying hens (Richter et al. 1998). In comparison with rapeseed feed, for linseed meal and cake, the uncertainty in feed value relevant constituents is high and therefore only a maximum of 3 % linseed meal or cake is recommended in the laying hen diets (Beynen 2004; Costa et al. 2008; Jeroch and Dänicke 2012).

In the EU, hemp seed (*Cannabis sativa*) production is only allowed for the varieties of the type list of regulation (EC) No. 2860/2000. In this regulation the maximum content of tetrahydrocannabinol (THC), which is the main psychoactive substance, is limited to 0.2 % (w/w). Although the total production of hemp in the EU in the last years increased (2006/06—13911 ha; 2009/10—14550 ha), this production represents a small part of the worldwide production of 360 thousand ha in 2005 (EFSA 2011). Hemp seed consists of >30 % oil and 25 % protein, with large amounts of fiber and minerals, and the seed oil contains more than 80 % polyunsaturated fatty acids (PUFA) with 56 % linoleic acid (C18:2, n-6), 22 % alpha-linolenic acid (C18:3, n-3) and 4 % gamma-linolenic acid (C18:3, n-6) (Callaway 2004). In hen experiments hemp seed or hemp seed oil significantly decreased expression of genes for hepatic fatty acid desaturase 1 and 2 which represents the desaturation of long-chain PUFA. The investigators conclude from this finding that the inclusion of hemp seed or hemp seed oil in hen feed should not exceed 20 and 12 %, respectively (Gakhar et al. 2012).

Among feeding stuffs from home-grown oilseeds, linseed and hemp cake are of very low importance, sharply contrasting with the rapeseed meal, solvent extracted, and rapeseed cake in connection with the "biodiesel boom" mentioned above. However, all three seeds are important as a side-product of oil extraction in smaller presses. In a given region, producing the seed, oil and cake, then feeding the cake and using the animal manure as fertilizer may "close the circle" of sustainability for the environment and climate.

Objectives of this study were to press the seeds from rape, linseed and hemp in the same screw press and to test the respective cakes in an experiment with laying hens. Besides the laying intensity and egg quality parameters, the fatty acid composition of egg yolk had to be investigated.

2 Material and methods

The seed cakes originated from the varieties "Lorenz" (rapeseed), "Lirina" (linseed) and "USO 31" (hemp). The seeds were processed in a screw press (IBG Oekotec Monforts GmbH&Co.KG) speed stage 2.5, 6 mm nozzle, pressure head temperature 32–35 °C.

A total of 216 laying hens (LB) were used in the feeding study. The hens were allocated to nine groups of 24 hens each. The hens were kept individually in a cage battery. The study commenced when the hens were 22 weeks old and continued until the sixth laying month (168 days). Each hen was offered the respective diet (Table 1) and water ad libitum. The number of eggs laid was recorded daily and the individual feed consumption was recorded monthly per hen. Each month the collected eggs were weighed four times within 2 weeks. All eggs laid over 3 consecutive days in the sixth laying month were collected per hen and the yolk weight, albumen weight and shell weight were measured. The color of the egg yolk was read with the Roche-Fan. Six yolk samples were drawn from a pooling the yolk of three eggs of 6 hens each for determination of fat and fatty acid methyl esters (FAME). In presscakes and compound feeds quality relevant parameters were determined according to VDLUFA-Methods (VDLUFA 1976–2009)—Dry matter (DM) according to method 3.1, ash and crude lipid according to methods 8.1 and 5.1.1 (using petrol ether), respectively. Neutral detergent fiber (NDF) was assayed after pretreatment with a heat stable amylase and expressed inclusive of residual ash (method 6.5.1). Contents of acid detergent fiber (ADF) were determined according to method 6.5.2 and expressed inclusive of residual ash. Determination of acid detergent Lignin (ADL), expressed as ADL (sa), was performed by solubilization of cellulose with sulphuric acid (method 6.5.3). Contents of Ca and P were determined by inductively coupled plasma optical emission spectrometry, ICP-OES (method 10.8.2), lysine, methionine and cystine using ion-exchange chromatography with postcolumn derivatization with ninhydrin (method 4.11.1). The glucosinolates of the rapeseed cake were determined with the official method of the European Commission (1990; Rothe et al. 2004) by HPLC (Dionex, Idstein, Germany). In hemp cake THC and cannabidiol were analyzed according to the official method of the European Commission (2000).

Yolk samples were homogenized and freeze dried and the fat was extracted. Analysis of FAME was made using a gas chromatograph equipped with flame ionization detectors (HP 6890 GC). The method included the separation and identification of FAME ranging from 4 to 20 carbon atoms and was conducted using a capillary column (Zebron 7HG-G009-11).

Data were analyzed with a two-way ANOVA: $yijk = \mu + SCi + Dj + SCDij + eijk$, with $yijk =$ observation, $\mu =$ general mean, $SCi =$ seed cake (rapeseed, linseed, hemp seed), $Dj =$ dose (50, 100 and 150 g/kg), interaction SCDij and $eijk =$ error term (random). Multiple comparisons of means were carried out using the Student-Newman-Keuls Test ($P \leq 0.05$). The statistical analyses were performed by the SAS software package (Version 9.2).

Table 1 Ingredient composition of the diets (g/kg)

Ingredients	Level of cake		
	50	100	150
Wheat	450	450	450
Corn	230	167	106
Soyabean meal, solvent extracted	151	146	139
Soyabean oil	12	20	28
Di-Calcium-phosphate	12	15	18.6
Calcium carbonate	80	88	95
Sodium chloride	3	3	3
DL-methionine	0.8	0.6	0.4
L-lysine-HCl	1.2	0.4	0
Premix[a]	10	10	10
Cake of rapeseed, linseed or hemp seed	50	100	150
Dry matter[b]	909	905	908
Crude protein[b]	159	165	169
ME, MJ/kg[c]	11.4	11.4	11.5
Lysine[b]	9.4	8.7	8.5
Methionine + Cystine[b]	7.2	6.8	7.0

[a] Vitamin-mineral premix provided per kg of diet: Fe, 40 mg; Cu, 10 mg; Zn, 80 mg; Mn, 100 mg; Se, 0.25 mg; I, 1.2 mg; Co, 0.21 mg; vitamin A, 10000 IE, vitamin D3, 2500 IE; vitamin E, 20 mg; vitamin K3, 4 mg; thiamine, 2.5 mg; riboflavin, 7 mg; pyridoxine, 4 mg; vitamin B12, 20 µg; nicotinic acid, 40 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 25 µg; choline chloride, 400 mg

[b] Analysed values

[c] Calculated values according to the equation of WPSA; 1985

3 Results

All three seed cakes are characterized by their high content of protein (28–32 %), fiber (NDF 15–45 %, ADF 17–33 %) and lipid (11–16 %; Table 2). The hemp seed cake had the highest content of NDF and ADF (45 and 32 %) und thus the content of metabolisable energy (ME) of hemp seed cake was only 9.21 MJ/kg compared to rapeseed cake with 12.08 MJ/kg and linseed cake with 10.78 MJ/kg. In relation to the total fatty acids the rapeseed oil contained the highest proportion of 60 % oleic acid (C18:1 n-9). The linolenic acid (C18:3 n-3) in linseed oil dominated with 51 % and the linoleic acid (C18:2 n-6) in hemp seed oil with 56 %. The glucosinolates of the rapeseed cake amounted to 21 mmol/kg. The THC and cannabidiol content of hemp was below the detection limit of 0.005 %.

There were significant effects of kind of cake and of the dietary cake level tested on the determined performance parameters (Table 3). A significant lower performance was shown for feed intake, egg mass production and feed-to-egg mass ratio at 15 % dietary cake level compared with the 5 and 10 % cake level groups. Also interaction was significant for all these parameters, indicating that the cake level acted differently for the three cakes: increasing the rapeseed cake level the feed intake decreased whereas in

Table 2 Analysed nutrients (g/kg) and fatty acid composition of oilseed cake (% of total FAME)

Nutrients (g/kg)	Cake of		
	Rapeseed	Linseed	Hemp seed
Dry matter	895	897	912
Crude protein	295	322	281
Crude ash	56	52	72
Crude lipid	158	130	110
NDF	209	194	447
ADF	163	130	304
Lignin	69	50	117
ME, MJ/kg[a]	12.08	10.78	9.21
Calcium	61	48	28
Phosphorus	97	87	148
Fatty acid (%)			
Lauric (12:0)	0.1	0.3	0.2
Palmitic (16:0)	4.4	5.9	6.0
Stearic (18:0)	0.6	2.9	1.6
Oleic (18:1 n-9)	59.6	22.1	11.4
Linoleic (18:2 n-6)	23.5	17.1	55.8
Linolenic (18:3 n-3)	10.6	51.5	24.7

[a] Calculated values according to the equation of WPSA; 1985

the linseed and hempseed groups no effect of dietary level was observed, and this at significantly higher intake of both these cakes compared with rapeseed cake. The egg mass production was lower in the linseed cake than in the hempseed cake groups and the hens fed the linseed cake needed significantly more feed per kg egg mass compared with both the other cakes tested.

Table 4 summarizes egg color, egg weight and the weight percentages of yolk, egg white and shell from total egg weight recorded during the sixth laying month. The egg weight showed the same differences between the cakes administered; however, it was higher due to collection only during the sixth month compared with total time of study. There was a tendency for the egg weight to decline at 10 and 15 % rapeseed cake in the hen feed and to increase at 10 and 15 % hemp seed cake. Increasing dietary level of all oil seed cakes lowered the yolk percentage and increased the egg white percentage. Higher egg shell content was obtained in the rapeseed cake compared with the linseed cake groups and the eggs from the groups with 15 % cake level tested showed higher shell percentage than these from the hens fed only 5 % cake in the diet. The differences in the yolk color were probably random.

Fatty acid compositions in yolk fat are shown in Table 5. Increased dietary levels of all three oil seed cakes lowered the percentages of most saturated fatty acids (SAFA): 12:0; 16:0; 18:0 ($P < 0.01$) and also the percentages of the monounsaturated fatty acids (MUFA) 16:1 and 18:1 n-9 decreased with higher cake levels in the diet. The highest proportion ($P < 0.001$) of oleic acid (C 18:1 n-9) was measured in eggs of the 5 % hemp seed cake group; of linoleic acid (C18:2 n-6) in the 15 % hemp seed cake group, and of linolenic acid (C18:3 n-3) in the 15 % linseed cake group. As opposite to the SAFA and MUFA the linoleic acid (18:2 n-6) and the linolenic acid (18:3 n-3) as main representatives of polyunsaturated fatty acids (PUFA) were heightened by increasing the cake levels from 5 to 10 and 15 % in the diet. The significant differences of almost all yolk fatty acids and the significance of interactions reflected the different fatty acid composition of cakes between the cakes fed (Table 2). Indeed, the hemp cake with "linoleic acid oil" created the highest yolk fat content of this n-6 PUFA, whereas the linseed cake with "linolenic acid oil" the highest yolk fat content of this n-3 PUFA.

4 Discussion

The fat and crude protein contents in the oilseed cakes tested (Table 3) were typical for the smaller oil presses. The ME results from fibre fractions, which are unacceptably high in hemp cake, were limited. However, also in rapeseed and linseed cake, the fibre with its high lignin portion causes the lower ME compared with soya-bean meal. The relatively high cake fat content can not compensate for this fibre-induced ME depression. Whereas the hemp feed was almost free of THC and cannabiol, significant amounts of glucosinolates as secondary plant compounds were found in rapeseed press cake. For 21 mmol GSL/kg and 158 g crude fat/kg the rapeseed cake contained on a fat free basis 25 mmol GSL/kg at 910 g DM/kg as the DM content standardized by rapeseed breeders. The value is below the 30 mmol glucosinolates/kg defatted matter (derived from 18 mmol/kg seed with 40 % oil) given as upper limit for rapeseed of "00" quality some time ago (Butler and Fenwick 1984) and it represents the upper third of the range in rapeseed cake monitoring: of 10–25 mmol GSL/kg DM, 11–29 mmol GSL/kg fat free DM (Schöne et al. 2012).

In the present hen trial, after cold pressing, a rapeseed cake resulted with 29.5 % crude protein, 15.8 % crude lipid and 12.08 MJ ME/kg. Studies of Roth-Maier and Kirchgessner (1995) and Jeroch et al. (1999) with laying hens and a diet with 15 % rapeseed

Table 3 Laying performance of hens (n = 24, mean, SD)

Treatments dose, % of diet	Feed intake (g/day)	Laying intensity (%)	Egg weight (g/egg)	Egg mass production (g/hen/day)	Feed conversion (kg/kg)
Rapeseed cake					
5	106.7 ± 7.7	96.4 ± 6.7	58.8 ± 5.0	56.8 ± 6.6	1.901 ± 0.19
10	105.5 ± 9.3	96.5 ± 6.5	57.8 ± 4.9	55.8 ± 5.9	1.903 ± 0.19
15	103.8 ± 10.4	95.7 ± 7.4	58.3 ± 4.7	55.8 ± 6.5	1.879 ± 0.26
Linseed cake					
5	110.1 ± 8.8	96.6 ± 5.6	58.8 ± 4.7	56.9 ± 5.4	1.954 ± 0.19
10	109.6 ± 10.7	94.8 ± 9.2	59.3 ± 4.8	56.4 ± 7.6	1.969 ± 0.26
15	105.9 ± 17.4	90.2 ± 13.4	58.3 ± 5.0	52.8 ± 9.8	2.043 ± 0.30
Hemp seed cake					
5	107.1 ± 8.4	95.7 ± 7.3	58.3 ± 4.8	55.9 ± 6.9	1.939 ± 0.24
10	110.9 ± 10.0	96.8 ± 5.2	59.8 ± 6.0	58.1 ± 6.8	1.927 ± 0.21
15	107.1 ± 9.0	95.4 ± 7.8	59.9 ± 4.6	57.2 ± 6.5	1.892 ± 0.22
Cake (ANOVA, P-value)	<0.001	<0.001	0.009	0.002	<0.0001
Rapeseed cake	105.3 b	96.2 a	58.3 b	56.1 ab	1.895 b
Linseed cake	108.4 a	93.8 b	58.9 ab	55.4 b	1.989 a
Hemp seed cake	108.4 a	96.0 a	59.4 a	57.0 a	1.919 b
Cake doses (ANOVA, P-value)	<0.001	<0.001	0.6	0.003	0.9
5	108.0 a	96.2 a	58.7	56.5 a	1.931
10	108.6 a	96.1 a	59.0	56.7 a	1.933
15	105.4 b	93.7 b	58.9	55.3 b	1.937
Cake × Cake doses (ANOVA, P-value)	<0.01	<0.001	0.007	<0.001	0.003

a, b Means with different letters differ significantly

showed no differences in the laying parameters compared with the control groups without rapeseed feeds. Richter et al. (1996) measured negative effects due to feeding of only 5 % dietary rapeseed. After a combined chemical and hydrothermical treatment of the rapeseed or rapeseed cake to reduce antinutritive compounds a higher percentage of rapeseed (22.5 %) or rapeseed cake (30 %) could be fed without negative impact on the laying performance (Jeroch et al. 1995, 1999, 2008).

After cold pressing, a linseed cake from linseed or flax resulted in the present trial with 32 % of crude protein, 13 % crude lipid and 10.78 MJ ME. In the hens, notably a dietary level of 15 % linseed cake reduced the daily feed intake and this affected the laying performance negatively (laying intensity, daily egg mass production, feed-to-egg mass ratio). Antruejo et al. (2011) also observed decreased ($P < 0.05$) feed intake, egg weight and increased feed-to-egg mass ratio after feeding 15 % flax seed per kg hen diet whereas 6 % flax seed oil did not depress feed intake and laying parameters. The laying parameters and feed efficiency were not significantly affected in two other trials testing 2 to 10 % linseed cake in hen diets from the 21st to the 44th weeks (Richter et al. 1998). Beynen (2004) noted that a linseed versus a soya-bean diet significantly reduced egg weight by 3.4 %. Eder et al. (1998) tested diets containing 0, 5, 10, 15 % whole and ground flaxseed with no effects up to the 10 % level on feed intake and laying performance. In contrast, Yannakopoulos et al. (1999) found a significantly higher feed intake and egg weight of hens fed a dietary level of 10 % ground linseed compared with this level of whole linseed as control. Levels of 15 % of flaxseed, however, increased the feed-to-egg mass ratio. Farrell and Gibson (1991) measured significantly increased laying intensity and egg weight in 30-week old hens after the feeding of 7 % linseed oil. In our previous two laying hen trials a supplementation of 0.7, 1.4 or 2.1 % linseed oil did not significantly influence feed intake and laying parameters (Halle 2000). Costa et al. (2008) did not find changed laying parameters after feeding diets with 0.5, 1.0, 1.5 and 2.0 % linseed oil either.

The hemp used for cake production was grown as fibre hemp as listed in Regulation (EC) 2860/2000 and the EU analysis method confirmed a non-occurrence of cannabioids. The hemp seed cake for

Table 4 Egg quality—sixth laying month (n = 62–69 eggs/group; mean, SD)

Treatments dose, % of diet	Egg weight (g/egg)	Colour of yolk Roche face	Yolk (%)	Egg white (%)	Egg shell (%)
Rapeseed cake					
5	62.1 ± 4.6	13.2 ± 0.6	27.9 ± 2.0	60.0 ± 2.1	12.1 ± 1.0
10	60.7 ± 3.9	13.0 ± 0.4	27.4 ± 2.0	60.4 ± 2.5	12.2 ± 1.2
15	60.8 ± 3.4	13.0 ± 0.5	26.1 ± 2.2	61.4 ± 2.4	12.4 ± 0.8
Linseed cake					
5	62.0 ± 3.6	13.4 ± 0.5	27.9 ± 2.2	60.1 ± 2.4	11.9 ± 1.0
10	62.4 ± 4.1	13.3 ± 0.4	26.7 ± 1.7	61.5 ± 2.0	11.7 ± 0.9
15	61.8 ± 4.2	13.4 ± 0.5	26.5 ± 1.5	61.4 ± 1.9	12.1 ± 1.0
Hemp seed cake					
5	61.2 ± 3.9	12.9 ± 0.5	28.6 ± 1.8	59.5 ± 2.3	11.8 ± 1.2
10	63.4 ± 5.9	13.2 ± 0.6	27.6 ± 2.2	60.1 ± 2.6	12.2 ± 0.9
15	62.7 ± 5.3	13.2 ± 0.5	27.1 ± 2.0	60.8 ± 2.2	12.1 ± 0.9
Cake (ANOVA, P-value)	0.02	<0.001	<0.001	0.001	0.01
Rapeseed cake	61.2 b	13.1 b	27.2 b	60.6 a	12.2 a
Linseed cake	62.0 ab	13.4 a	27.0 b	61.0 a	11.9 b
Hemp seed cake	62.4 a	13.1 b	27.8 a	60.1 b	12.0 ab
Cake doses (ANOVA, P-value)	0.5	0.2	<0.001	<0.001	0.01
5	61.7	13.2	28.2 a	59.9 c	11.9 b
10	62.2	13.1	27.2 b	60.7 b	12.0 ab
15	61.8	13.2	26.5 c	61.2 a	12.2 a
Cake × Cake doses (ANOVA, P-value)	0.01	<0.001	0.2	0.2	0.055

a, b, c Means with different letters differ significantly

the trial with laying hens contained 28 % crude protein, 11 % crude lipid and 9.21 MJ ME. Silversides and LefranCois (2005) measured no effect of 0, 5, 10 and 20 % hemp seed meal in the diet on feed intake, laying intensity, egg weight and feed conversion through a four-week hen trial. Gakhar et al. (2012) compared diets containing 10 or 20 % hemp seed with diets containing 4, 8 and 12 % hemp oil in a 12-week laying hen trial. Feed intake of the 4 % oil group was significantly lower compared to the other groups, but, laying intensity was not affected when the hens were fed either hemp seed or hemp seed oil. The egg weight of the 20 % hemp seed group was significantly higher than that of the control group.

The effect of increased supplementation of dietary PUFA on egg weight was significant in the hemp seed hen groups and also the percentage of yolk from eggs sampled in the sixth laying month was affected in all seed cake groups by increasing the dietary level of the oil and PUFA, respectively (Table 4). The reduction of the triacylglycerides in the blood as appears after the intake of n-3 PUFA (Phetteplace and Watkins 1989; Hargis et al. 1991; Van Elswyk et al. 1991; Fritsche et al. 1993) may significantly reduce egg yolk mass as was shown after feeding a hen diet with fish oil or linseed oil (Scheideler et al. 1998) or with fish oil plus linseed oil (Halle 2000).

As expected, increasing portions of all three cakes tested (5, 10, and 15 %) in the hen diet resulted in linear increases in the concentration of linoleic acid and linolenic acid and decreases in SAFA and MUFA in egg yolk Table 5. The ratio of n-6 fatty acid to n-3 fatty acid decreased from 17:1 to 11:1 with increased rape seed oil (8/16/24 g/kg) in the hen feed. Hämmal et al. (2000), Jeroch et al. (2001) and Bretschneider et al. (2006) also found a linearly decreased ratio of the n-6 to n-3 fatty acids in the yolk fat with increased rape seed oil in hen feed. In these three studies the ratio ranged from 4.1 to 5.4: 1 after supplementation of 4–8 % of rape seed oil.

The increased linseed cake level corresponded with rising dietary linseed oil levels (6.5/13/19.5 g/kg feed) and the highest proportion of linolenic acid (C18:3 n-3—2.36/4.04/5.14 %) in yolk fat compared with the rapeseed and hempseed cake feeding. In the yolk fat of the hens fed the linseed cake the lowest ratio of n-6 to n-3 fatty acids was found (8.5/5.6/4.4:1).

Table 5 Fatty acid composition in egg yolk (n = 6 samples of three eggs; mean, SD) (% of total FAME)

Treatments dose, % of diet	12:0	14:0	16:0	16:1	18:0	18:1 n-9	C 18:2 n-6	C 18:3 n-3
Rapeseed cake								
5	0.04 ± 0.03	0.34 ± 0.03	26.05 ± 1.00	3.15 ± 0.26	6.69 ± 0.28	44.18 ± 0.84	18.45 ± 0.51	1.10 ± 0.05
10	0.23 ± 0.11	0.65 ± 0.88	24.18 ± 0.68	2.21 ± 0.54	6.86 ± 0.23	43.22 ± 0.79	21.04 ± 0.70	1.61 ± 0.07
15	0.40 ± 0.04	0.25 ± 0.02	22.45 ± 0.46	1.90 ± 0.17	5.96 ± 0.24	44.50 ± 0.43	22.52 ± 0.65	2.03 ± 0.13
Linseed cake								
5	0.50 ± 0.02	0.31 ± 0.01	24.5 ± 0.61	2.53 ± 0.22	6.09 ± 0.32	43.60 ± 0.96	20.10 ± 0.50	2.36 ± 0.11
10	0.58 ± 0.06	0.26 ± 0.01	21.46 ± 0.62	2.22 ± 0.14	5.28 ± 0.27	43.67 ± 1.73	22.60 ± 2.11	4.04 ± 0.19
15	0.46 ± 0.02	0.40 ± 0.40	20.46 ± 0.62	2.14 ± 0.26	5.14 ± 0.28	43.80 ± 0.55	22.44 ± 0.90	5.14 ± 0.35
Hemp seed cake								
5	0.58 ± 0.02	0.33 ± 0.01	23.19 ± 0.38	2.71 ± 0.12	5.72 ± 0.39	46.74 ± 0.87	19.10 ± 0.83	1.62 ± 0.10
10	0.54 ± 0.09	0.29 ± 0.02	21.61 ± 0.64	2.19 ± 0.18	4.84 ± 0.15	45.08 ± 0.71	22.92 ± 0.62	2.55 ± 0.07
15	0.48 ± 0.04	0.27 ± 0.02	21.34 ± 0.73	1.85 ± 0.13	5.08 ± 0.25	42.50 ± 1.10	25.46 ± 0.83	3.02 ± 0.15
Cake (ANOVA, P-value)	<0.001	0.5	<0.001	0.12	<0.001	0.003	<0.001	<0.001
Rapeseed cake	0.23 b	0.41	24.2 a	2.4	6.5 a	43.9 b	20.7 c	1.58 c
Linseed cake	0.52 a	0.32	22.1 b	2.3	5.5 b	43.6 b	21.7 b	3.85 a
Hemp seed cake	0.54 a	0.30	22.0 b	2.2	5.2 c	44.8 a	22.5 a	2.40 b
Cake doses (ANOVA, P-value)	<0.001	0.6	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
5	0.38 b	0.32	24.6 a	2.8 a	6.2 a	44.8 a	19.2 c	1.69 c
10	0.45 a	0.40	22.4 b	2.2 b	5.7 b	44.0 b	22.2 b	2.73 b
15	0.45 a	0.31	21.4 c	1.9 c	5.4 c	43.6 b	23.5 a	3.40 a
Cake × Cake doses (ANOVA, P-value)	<0.001	0.3	<0.001	0.003	<0.001	<0.001	<0.001	<0.001

a, b, c Means with different letters differ significantly

Eder et al. (1998) measured linolenic acid levels in yolk lipids of 3.6/8.0/11.7 % caused by 5/10/15 % ground flaxseed and 2.8/6.3/10.4 % for whole flaxseed, respectively. In contrast, an identical proportion of 4.9 % linolenic acid in the yolk fat was found by Yannakopoulos et al. (1999) after feeding 10 % whole versus ground linseed. In another hen study, a diet with 4 % linseed oil resulted in 4.87 % of linolenic acid in yolk fat (Baucells et al. 2000). Antruejo et al. (2011) calculated the n-6 to n-3 PUFA ratio in yolk fat with 2.04 after feeding 15 % dietary flax seed and with 2.26 after feeding 6 % flax oil.

The 5, 10 and 15 % dietary level of hemp seed cake resulted in the highest egg yolk fat proportion of linoleic acid (C18:2 n-6—19.1/22.9/25.52 %) as compared with the other two oilseed cakes tested (Table 5). The yolk fat ratio of n-6 to n-3 fatty acids (11.8/9.0/8.4:1) was lower than in the previously mentioned rape seed cake groups. Silversides and Lefrancois (2005) tested hemp seed meal (0/5/10/20 %) in hen diets and found a linear increase of linoleic acid (15/17/19/22 %) and linolenic acid (2/5/7/12 %) in the yolk fat. Gakhar et al. (2012) also obtained a linearly increased egg yolk n-3 fatty acid content with diets based on hemp seed (10/20 %) or hemp seed oil (4/8/12 %).

The results allow the conclusion that compound feeds with up to 10 % of rapeseed cake, linseed cake or hemp seed cake do not negatively influence the laying performance of hens and also provide the possibility of the enrichment of yolk fat with n-3 type PUFA.

Acknowledgments The authors thank the members of the experimental research station Celle of the Institute of Animal Welfare and Animal Husbandry (FLI) for the execution of the hen trial as well as the laboratory of the Institute of Animal Nutrition (FLI) and of the Thüringer Landesanstalt für Landwirtschaft (TLL) for the different chemical feed analyses in the frame of the Project *Method Develoment and Quality Assessment* (No. 92.01) and Dr. agr. Frank Höppner, (Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für Pflanzenbau und Bodenkunde, Braunschweig) for analyses of the THC and cannabidiol content in hemp seed and Dr. U. Baulain (Institute of Farm Animal Genetics, FLI) for the support with the statistical evaluation of the results.

References

Antruejo A, Azcona JO, Garcia PT, Gallinger C, Rosmini M, Ayerza R, Coates W, Perez CD (2011) Omega-3 enriched egg production: the effect of α-linolenic ω-3 fatty acid sources on laying hen performance and yolk lipid content and fatty acid composition. Br Poult Sci 52: 750–760

Baucells MA, Crespo N, Barroeta AC, Lopez-Ferer S, Grashorn MA (2000) Incorporation of different polyunsaturated fatty acids into eggs. Poultry Sci 79:51–59

Beynen AC (2004) Fatty acid composition of eggs produced by hens fed diets containing groundnut, soya bean or linseed. J Life Sci 52:3–10

Blanch A, Grashorn MA (1996) Ernährungsphysiologische Bedeutung der Omega-3-Fettsäuren und Möglichkeiten der Anreicherung in Eiern. Arch Geflügelk 60:49–58

Bretschneider JG, Jankowski J, Kozlowski K, Jeroch H (2006) Influence of rape seeds in laying hen rations on the fatty acid profile of the egg yolk fat. Pol J Nat Sci Supplement 3:397–402

Butler EJ, Fenwick GR (1984) Trimethylamine and fishy taint in eggs. World's Poult Sci J 40:38–51

Callaway JC (2004) Hempseed as a nutritional resource: an overview. Euphytica 140:65–72

Cheeke PR (1991) Applied animal nutrition. Feeds and feeding. Macmillan Pub, New York

Costa FGP, de Souze JG, da Silva JHV, Rabello CBV, Goulart CD, Lima RD (2008) Influence of linseed oil on performance and egg quality of semi heavy laying hens. Brazilian J Animal Sci 37:861–868

Eder K, Roth-Maier D, Kirchgeßner M (1998) Laying performance and fatty acid composition of egg yolk lipids of hens fed diets with various amounts of ground or whole flaxseed. Arch Geflügelk 62:223–228

EFSA (2011) Scientific opinion on the safety of hemp (*Cannabis genus*) for use as animal feed. EFSA J 9(3):41

European Commission (1990) Oilseeds—determination of glucosinolates—HPLC. Off J Eur Comm L170:27–34

European Commission (2000) Commission Regulation (EC) No 2860/2000 of 27 December 2000 amending Regulation (EC) No 2316/1999 laying down detailed rules for the application of Council Regulation (EC) No 1251/1999 establishing a support system for producers of certain arable crops, to include flax and hemp grown for fibre, specifying the rules on set-aside areas and amending the base areas for Greece and Portugal O. J. Europ. Communities L332:43, 28 December 2000, 13 pp

Farrell DJ, Gibson RA (1991) The enrichment of eggs with omega-3 fatty acids and their effects in humans: recent advances. In: Farrell DJ (ed) Recent advances in animal nutrition in Australia 1991. University of New England, Armidale, pp 256–270

Fritsche KL, Huang SC, Cassity NA (1993) Enrichment of omega n-3 fatty acid suckling pigs by maternal dietary fish oil supplementation. J Animal Sci 73:1841–1847

Gakhar N, Goldberg E, Jing M, Gibson R, House JD (2012) Effect of feeding hemp seed and hemp seed oil on laying hen performance and egg yolk fatty acid content: Evidence of their safety and efficacy for laying hen diets. Poultry Sci 91:701–711

Halle I (2000) Effect of dietary fish oil and linseed oil on performance, egg components and fatty acid composition of egg yolk in laying hens. Arch Geflügelk 65: 13–21

Hämmal J, Tikk V, Tikk H, Kuusik S (2000) Suitability of the rapeseed oil for the production of the omega-eggs. Vet Zootech 10:47–49

Hargis PS, Van Elswyk ME (1993) Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. World's Poultry Sci 49:251–264

Hargis PS, Van Elswyk ME, Hargis BM (1991) Dietary modification of yolk lipid with menhaden oil. Poultry Sci 70:874–883

Honkatukia M, Reese K, Preisinger R, Tuiskula-Haavisto M, Weigend S, Roito A, Mäki-Tanilla A, Vilkki J (2005) Fishy taint in chicken eggs is associated within a conserved motif of the FMO3 gene. Genomics 86:225–232

Jeroch H, Dänicke S (2012) Faustzahlen zur Geflügelfütterung. Geflügeljahrbuch 2012, Ulmer Verlag, Stuttgart

Jeroch H, Dänicke S, Zachmann R (1995) Zum Futterwert und zur Eignung von Rapsexpellern in der Legehennenfütterung. Agribiol Res 48:248–256

Jeroch H, Dänicke S, Brettschneider JG, Schumann W (1999) Einsatz von behandelter Rapssaat bei braunen Legehennen. Die Bodenkultur 50:45–55

Jeroch H, Brettschneider W, Böttcher W, Peter W, Seskeviciene J, Prinz M (2001) Verbesserung des Nährwertes von Hühnereiern durch Verfütterung von Futtermischungen mit Rapsöl an Legehennen. Vet Zootech 16:122–124

Jeroch H, Jankowski J, Schöne F (2008) Rapsfuttermittel in der Broiler- und Legehennenfütterung. Arch Geflügelk 72: 49–55

Jiang Z, Sim JS (1993) Consumption of n-3 polyunsaturated fatty acid-enriched eggs and changes in plasma lipids of human subjects. Nutrition 9:513–518

Lewis NM, Seburg S, Flanagan NL (2000) Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans. Poultry Sci 79:971–974

Noble RC, Cocchi M, Turchetto E (1990) Egg fat—a case for concern? World's Poultry Sci J 46:112–118

Normenkommission für Einzelfuttermittel im Zentralausschuss der Deutschen Landwirtschaft, Positivliste für Einzelfuttermittel (2011) 9. Auflage

Ohtake Y, Hoshino Y (1967) Influences of dietary fat and oil on the fatty acid distribution in egg yolk lipids of laying hens. Jpn J Zootech Sci 47:431–440

Phetteplace HW, Watkins BA (1989) Effects of various n-3 lipid sources on fatty acid composition in chicken tissues. J Food Compos Anal 2:104–117

Pottgüter R (2006) New prospects for using rape seed (canola) in layer rations. Lohmann Information, Cuxhaven

Richter G, Lemser A, Bargholz J (1996) Rapssamen und Rapsextraktionsschrot als Komponenten in der Legehennenfütterung. Arch Anim Nutr 49:229–241

Richter G, Lüdke H, Ochrimenko W, Leiterer M, Huller M, Schenkel H (1998) Investigations about the use of linseed cake in laying hens. Arch Geflügelk 62:264–272

Rothe R, Hartung H, Marks G, Bergmann H, Götz R, Schöne F (2004) Glucosinolate content in vegetative tissues of winter rape cultivars. J Appl Bot 78:41–47

Roth-Maier DR, Kirchgessner M (1995) Untersuchungen zum Einsatz von 00-Rapssaat in der Geflügelfütterung. Arch Geflügelk 59:241–246

SAS Institute Inc (2002–2008) SAS 9.2. SAS Institute Inc, Cary

Scheideler SE, Jaroni D, Froning GW (1998) Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. Poultry Sci 77:192–196

Schöne F, Kirchheim U, Tischendorf F, Rudolp B (1997) Prüfung von Leinfuttermitteln (Saat, Extraktions-schrot)

an wachsenden Schweinen - Futterwert, Thiocyanat- und Schilddrüsenhormonstatus. Fett/Lipid 99:15–18
Schöne F, Weber M, Graf T (2012) Rapsmonitoring für eine bessere Positionierung von Rapsextraktionsschrot und Rapskuchen in der Tier-besonders Schweineproduktion. Proc 124th VDLUFA Congress (in press)
Silversides FG, LefranCois MR (2005) The effect of feeding hemp seed meal to laying hens. Br Poultry Sci 46:231–235
Simopoulos AP, Salem NJR (1992) Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. Am J Clin Nutrition 55:411–414
Spiekers H, Südekum K-H, Engelhardt T, Mahlkow-Nerge K, Pries M (2012) Einsatz von Rapsextraktionsschrot in der Milchkuhfütterung. UFOP-Praxisinformation, Union zur Förderung von Öl- und Proteinpflanzen e.V., Berlin
UFOP Bericht 2010/2011, UFOP-Praxisinformation, Union zur Förderung von Öl- und Proteinpflanzen e.V., August 2011, Berlin
Van Elswyk ME, Schake LE, Hargis BM, Hargis PS (1991) Effects of dietary menhaden oil on serum lipid parameters and hepatic lipidosis in laying hens. Poultry Sci 70(Suppl.):122
VDLUFA, Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (1976 bis 2009) Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln. (The chemical analysis of feeds) VDLUFA-Verlag, Darmstadt, Germany. 3. Auflage
Yannakopoulos AL, Tserveni-Gousi AS, Yannakakis S (1999) Effect of feeding flaxseed to laying hens on the performance and egg quality and fatty acid composition of egg yolk. Arch Geflügelk 63:260–263