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Effects of addition of flaxseed to diets of laying hens on some production characteristics, levels of yolk and serum cholesterol, and fatty acid composition of yolk

Einfluss des Zusatzes von Leinsaat zu Legehennenrationen auf die Leistung, die Cholesterolgehalte in Serum und Dotter sowie auf das Fettsäuremuster des Dotters

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Introduction

Foods of animal origin such as meat, milk, and egg take a very important place in human diets due to their nutritional qualities. However, these types of foods containing different amounts of unsaturated/saturated fatty acids and cholesterol are sometimes associated with health problems. The past suggestion that egg cholesterol caused atherosclerosis which in turn results in health complications such as heart attacks and paralyses is arguable at the present (HANSEN and THORLING, 1994; MARSHALL et al., 1994; CHERIAN and SIM, 1996; FARELL, 1997). Cholesterol and fatty acids contents of yolks vary depending especially on dietary manipulations, the genetics of chickens, age, and egg production rate (BEYER and JENSEN, 1989; HARGIS and VAN ELSWYK, 1993; AHN et al., 1995; MELUZZI et al., 1995; VAN ELSWYK et al., 1997; SCHARF and ELMADFA, 1998).

In the past, studies have been conducted to reduce the cholesterol level of eggs. One idea to reduce egg cholesterol is to change the fatty acid (FA) composition of the diet and by this of the egg yolk reducing the cholesterolaemic effect. The hypocholesterolaemic effects of mono unsaturated fatty acids, e.g. oleic acid (OA), and poly unsaturated fatty acids, e.g. linoleic acid (LA), have been discussed for a long time (GRUNDY, 1986). In recent years researches have been focused on Ω-3 polyunsaturated fatty acids (PUFA) such as α-linolenic acid (LnA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (WATKINS, 1992; FARRELL, 1993, 1997; HANSEN and THORLING, 1994; BLANCH and GRASHORN, 1996; CHERIAN and SIM, 1996).

In literature it was shown that diets rich in Ω-3 PUFA reduced blood cholesterol levels in rats (CASTON and LEESON, 1990; JIANG *et al.*, 1991; WATKINS, 1992; FERRIER *et al.*, 1995; SCHEIDELER and FRONING, 1996). Comparable data on layers are lacking.

Therefore, this experiment was carried out to determine the effects of addition of flaxseed (FS) to laying hens' diets on some performance characteristics, yolk and blood cholesterol levels, and fatty acid compositions of egg yolks.

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Material and Methods

In the experiment 256 commercial Isa Brown laying hens, 26 wk of age, were used. The hens were randomly placed in groups of 4 into 64 cages. The four treatments comprised supplementation levels of flaxseed of 0, 5, 10, and 15% (C, FS 5, FS 10, FS 15). Four cages were asigned as one replicate. Therefore, the design of the experiment was 4 treatments × 4 replicates × 4 cages × 4 hens = 256 layers. The experimental diets were calculated on an isonitrogenic and isoenergetic level. The composition of the diets is given in Table 1. Diets were fed for 8 weeks. Feed

Table 1. Composition of experimental diets
Zusammensetzung der Versuchsrationen

Ingredient, %	C	FS 5	FS 10	FS 15
Flaxseed	0.00	5.00	10.00	15.00
Corn	60.10	54.00	46.60	42.10
Soybean meal	26.40	23.10	20.30	17.50
Sunflower meal	2.20	6.60	12.00	13.70
Vegetable oil	1.50	1.50	1.50	1.50
Dicalcium phosphate	1.50	1.50	1.50	1.50
Limestone	7.60	7.60	7.50	8.00
Salt	0.25	0.25	0.25	0.25
Vitamin/mineral premix*	0.35	0.35	0.35	0.35
Methionin	0.10	0.10	0.10	0.10
Chemical analyses, %				
Dry matter	90.63	91.10	91.67	91.50
Crude protein	17.19	16.91	17.01	17.04
Crude fat	3.68	5.00	6.86	8.41
Crude fiber	5.08	5.04	5.15	6.26
Calcium	4.02	4.05	4.36	4.00
Total phosphorous	0.75	0.73	0.76	0.78
Metabolisable energy, kcal/kg**	2827	2842	2834	2844

* For each kg of the diet; Vitamin A 12000 IU; Vitamin D_3 2000 IU; Vitamin E 35.0 mg; Vitamin K_3 5.0 mg; Vitamin B_1 3.0 mg; Vitamin B_2 7.0 mg; Calcium D-Pentotenat 10.0 mg; Vitamin B_6 5.0 mg; Vitamin B_{12} 0.015 mg; Folic acid 1.0 mg; D-Biotin 0.045 mg; Choline chloride 125.0 mg; Vitamin C 50.0 mg; Mn 80 mg; Fe 60.0 mg; Zn 60.0 mg; Cu 5 mg; Co 0.2 mg; Se 0.15 mg.
** The metabolisable energy content was calculated (Anonymous, 1991).

consumption (g/hen/day) and egg mass (g/hen/day) were calculated by dividing total feed consumed and total egg mass in each week by days of week, respectively. Feed conversion and egg production were calculated as kg feed consumped/kg egg mass in each week and hen-day in each week, respectively. All measurements were calculated for each replicates in groups. Mortality and rate of damaged egg were determined for treatments. At the end of the experiment, the total of 48 and 24 eggs (12 and 6 samples from each group) were randomly selected for yolk cholesterol and yolk fatty acids analyses, respectively (POYRAZ, 1987). The cholesterol levels and the ratio of fatty acids were determined by gas chromatography[5] (A.O.A.C., 1990). In addition, in order to determine the blood cholesterol levels, a total of 40 blood samples (10 samples from each group) were taken by puncturing the wing vein of hens, after feed withdrawal for 12 h. After coagulation of blood, blood samples were centrifuged at 2,000 rpm, and serum of samples were collected. The serum cholesterol levels were determined spectrophotometrically[6] by using commercial kits[7].

During the experimental period, the temperature and the relative humidity of the poultry house were determined by a thermohygrograph. The mean temperature and relative humidity were 16.7 °C and 67.1%, respectively.

Data were statistically analysed by one-way analysis of variance. The differences between groups were determined by Duncan's test. All statistical analyses were performed using SAS statistical programme (SAS, 1985).

Results

The calculated nutrient compositions of isonitrogenous and isocaloric diets given to laying hens are presented in table 1. The effects of diets on feed consumption, feed conversion ratio, egg mass, egg production, weight of egg yolk, cholesterol levels in egg yolk and blood are given in table 2. The addition of FS significantly ($P < 0.01$) affected the parameters investigated. Daily feed consumption was significantly reduced ($P < 0.01$) with increasing levels of FS in diets. This reduction was not significant between groups C and FS 5, but was significant in groups FS 10 and FS 15. The feed conversion ratios in groups C, FS 5, FS 10, and FS 15 were found to be 2.13, 2.04, 1.99, and 1.96, respectively, and FS improved feed conversion ratio significantly. Apart from group FS 15, there was no significant difference between groups for egg mass. The highest egg mass was found to be 63.2 g in group FS 5, whereas, the lightest one was 60.0 in group FS 15. The egg production usually differed between groups and the highest production was found in the group FS 10 (91.0%), whereas, the lowest production was found in group C (84.1%).

The addition of FS to diets resulted in significant differences in egg yolk cholesterol levels ($P < 0.05$), weights of egg yolks, and cholesterol levels in blood ($P < 0.01$) between treatments. The weight of egg yolk was reduced in groups FS 5, FS 10, and FS 15 in comparison to C and this reduction was far more noticeable in group FS 15 ($P < 0.01$). The level of cholesterol in blood and egg yolk was reduced with increasing supplementation level of FS. The difference between group FS 15 and group FS 10 was more pronounced than for the control group. The lowest cholesterol values of yolk and blood were detected in group FS 15: 12.6 mg/g and 104.8 mg/100 ml, respectively, whereas the highest values were detected in group C: 13.6 mg/g and 150.7 mg/100 ml, respectively.

The effects of diets on yolk FA are given in table 3. The addition of FS to the basal diet reduced the level of saturated fatty acids and MUFA, whereas, increased the PUFA level ($P < 0.01$) in yolk. The level of PUFA increased linearly with increasing FS levels. The level of LA (Ω-6 fatty acid) was not affected by addition of FS, the level of arachidonic acid (AA) was reduced, and the level of Ω-3 FAs increased progressively and noticeably. The ratios of Σ PUFA : Σ SAT in yolks of groups C, FS 5, FS 10, FS 15 were 0.77, 0.97, 1.08, and 1.17, respectively.

During the experimental period, no mortality and no damaged eggs were observed.

Discussion

In the experiment, the effects of addition of various levels of FS (0, 5, 10 and 15%) to diets on various production aspects, blood and egg yolk cholesterol levels, and egg yolk fatty acid composition were investigated (tables 2 and 3).

Feed consumption values did not change in groups C and FS 5, whereas, this value decreased progressively and noticeably in groups FS 10 and FS 15 ($P < 0.01$). This may be caused by the presence of some toxic substances in raw FS (AJUYAH et al., 1991). The feed conversion ratio

[5] Carlo Erba Fraktovap Series 2350 Model.
[6] Ilab TM[900] Clinical Chemistry Instruments.
[7] IL Test TM Cholesterol Kits.

Table 2. The effect of addition of flaxseed to layers' diets on feed consumption, feed conversion, egg mass, egg production, blood and egg yolk cholesterol level
Einfluss des Zusatzes von Leinsaat zum Futter auf Futterverbrauch, Futterverwertung, Eimasse, Legeleistung sowie Blut- und Dottercholesteringehalte

Diets	Feed consumption, g/hen/d	Feed conversion, kg feed/kg egg	Egg mass, g/hen/d	Egg production, % (Hen-day)	Weight of egg yolk, g	Egg yolk cholesterol, mg/g	Blood cholesterol, mg/100 ml
C	110.5^{a}	2.13^{a}	62.2^{a}	84.1^{c}	14.2^{a}	13.6^{a}	150.7^{a}
FS 5	111.3^{a}	2.04^{b}	63.2^{a}	87.1^{bc}	14.0^{a}	13.5^{a}	106.3^{b}
FS 10	108.3^{b}	1.99^{cb}	62.2^{a}	91.0^{a}	13.8^{a}	13.0^{ba}	124.9^{ab}
FS 15	104.8^{c}	1.96^{c}	60.0^{b}	87.9^{ba}	12.8^{b}	12.6^{b}	104.8^{b}
SEM	0.77	0.02	0.73	1.11	0.33	0.52	10.25
N	128	128	128	128	48	48	40
Significance	**	**	**	**	**	*	**

$^{a-c}$ Means within a row with no common superscripts differ significantly different. SEM: Poolled Standard Error Mean.
$^{*}P < 0.05$; $^{**}P < 0.01$

Table 3. The effect of flaxseed on fatty acids levels of the yolks (% of total fatty acids)
Einfluss des Zusatzes von Leinsaat zum Legehennenfutter auf das Fettsäuremuster des Dotters (in % der Gesamtfettsäuren)

Fatty Acids	C	FS 5	FS 10	FS 15	SEM	N obs.	Significance
C 14 : 0 Miristic	0.30^{a}	0.24^{b}	0.19^{c}	0.20^{c}	0.01	24	**
C 16 : 0 Palmitic	23.45^{a}	21.47^{b}	20.38^{c}	19.28^{d}	0.30	24	**
C 16 : 1 Palmitoleic	2.83^{a}	2.51^{ab}	2.32^{b}	1.61^{c}	0.13	24	**
C 18 : 0 Stearic	8.67^{b}	8.74^{b}	8.99^{b}	10.58^{a}	0.17	24	**
C 18 : 1 Oleic	38.66^{a}	37.12^{b}	36.05^{b}	33.11^{c}	0.47	24	**
C 18 : 2 Linoleic	20.94^{a}	20.80^{a}	22.01^{a}	21.56^{a}	0.51	24	N.S.
C 18 : 3 Linolenic	0.70^{d}	4.29^{c}	5.37^{b}	9.05^{a}	0.18	24	**
C 18 : 4 Stearidonic	0.19^{a}	0.17^{b}	0.20^{a}	0.21^{a}	0.01	24	**
C 20 : 4 Arachidonic	2.38^{a}	1.65^{b}	1.57^{b}	1.32^{c}	0.04	24	**
C 20 : 5 Eicosapentaenoic	0^{c}	0.08^{b}	0.08^{b}	0.14^{a}	0.01	24	**
C 22 : 5 Docosapentaenoic	0.12^{b}	0.33^{a}	0.33^{a}	0.38^{a}	0.04	24	**
C 22 : 6 Docosahexaenoic	0.79^{b}	2.21^{a}	2.36^{a}	2.42^{a}	0.09	24	**
Σ Ω 3	1.80^{d}	7.07^{c}	8.35^{b}	12.20^{a}	0.15	24	**
Σ Ω 6	23.32^{a}	22.48^{a}	23.51^{a}	22.88^{a}	0.54	24	N.S.
Σ Ω 6/Σ Ω 3	13.12^{a}	3.19^{b}	2.76^{c}	1.88^{a}	0.14	24	**
Σ Saturated (S)	32.68^{a}	30.45^{b}	29.59^{b}	30.04^{b}	0.33	24	**
Σ MUFA	41.51^{a}	39.79^{b}	38.37^{b}	34.91^{c}	0.49	24	**
Σ PUFA (P)	25.12^{d}	29.48^{c}	31.91^{b}	35.08^{a}	0.62	24	**
Σ PUFA/Σ S	0.77^{d}	0.97^{c}	1.08^{b}	1.17^{d}	0.03	24	**

Σ: Total, MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; SEM: Polled Standard Error Mean; N.S.: Not Significant; ** ($P < 0.01$)
$^{a-d}$ Means within a row with no common superscripts are significantly different ($P < 0.05$).

values of treated groups were found to be better than in the control. The crude fat amount of diets increased by addition of FS (0, 5, 10 and 15%). It might be expected that a low 'specific dynamic effect' of fats leads to an improvement in feed conversion in groups fed with diets including FS. The results observed from this study were similar to those reported by Scheideler and Froning (1996).

The addition of 15% FS reduced significantly egg mass in comparison to the other experimental groups ($P < 0.01$). Scheideler and Froning (1996) reported noticeably lower egg weight values in comparison to the control when 15% FS was added to laying hens' diets for 8 weeks. Egg production differed significantly ($P < 0.01$) between group C and the other experimental groups (FS 10, FS 15). This may be caused by the reduced feed intake, or by the presence of some toxic substances in raw FS (Ajuyah et al., 1991), or by the lower true amino acid availability of glutamate, serine, valine, phenylalanine, isoleucine, leucine, and methionine of flax products in respect to soybean meal (Barbour and Sim, 1991). Some data from literature support these results (Jiang et al., 1991; Scheideler and Froning, 1996; Farrell, 1997), whereas some others do not (Caston et al., 1994; Aymond and Van Elswyk, 1995; Balevi and Coskun, 1999). Consequently, it was concluded that there was no negative effect of additional FS on egg production and egg weight of laying hens (Caston and Leeson, 1990; Jiang et al., 1991).

In the experiment, the weight of egg yolk was reduced with increasing supplements of FS in diets. But, this reduction was not significant between groups C, FS 5 and FS 10, whereas, it was significant ($P < 0.01$) in group FS 15. In another word, the difference was more pronounced in the diet with the highest FS addition. These results agree with the results of Scheideler and Froning (1996) and do not agree with those of Caston and Leeson (1990).

It was shown that egg yolk cholesterol level decreased by increasing FS addition and this reduction was significant in group FS 15 ($P < 0.05$). This result agrees with the results of Jiang et al (1991) conducted with a similar approach on rats. But, experiments of Caston and Leeson (1990), Scheideler and Froning (1996) showed that cholesterol level was not affected noticeably by FS level in the diet.

The blood cholesterol level usually decreased progressively and regularly in FS added diets ($P < 0.01$). This result was in agreement with the results of experiments conducted on rats with diets including 10% additional FS (Jiang and Sim, 1992).

The effects of FS added diets on egg FA profiles were given in table 3. These data agree with classical expectations (Jiang et al., 1991) and the statement regarding the effectiveness of the diet composition on the fatty acid composition of egg (Hargis, 1988; Farrell, 1997). The level of saturated fatty acids and MUFA (palmitoleic and oleic) generally decreased progressively when FS increased ($P < 0.01$). This observation was supported by several similar studies (Caston and Leeson, 1990; Jiang et al. 1991).

Considering PUFAs the addition of FS did not alter the level of Ω-6 FAs ($P > 0.05$), the level of Aa decreased progressively and the level of Ω-3 FA (LNA, EPA, DPA and DHA) increased linearly and significantly with increasing FS levels in diets ($P < 0.01$). As a result of this, the level of Lna in group FS 15 was found to be thirteen times higher than in group C. All these results (table 3) corresponded with data from literature (Caston and Leeson, 1990; Jiang et al., 1991; Ferrier et al., 1995; Aymond and Van Elswyk, 1995; Scheideler and Froning, 1996). However, Caston and Leeson (1990) and Aymond and Van Elswyk (1995) showed that EPA and DPA levels did not change significantly with addition of FS to the diets.

The level of total Ω-3 fatty acids increased significantly ($P < 0.01$), the level of Ω-6 fatty acids did not change, and the level of saturated and Mufa decreased significantly depending on the level of FS in diet ($P < 0.01$). Experiments on saturated fatty acids and MUFA (Caston and Leeson, 1990; Jiang et al., 1991; Aymond and Van Elswyk, 1995) showed similar results. The ratio of total Ω-6 to Ω-3 FA (Ω 6/Ω 3) decreased with increasing amounts of FS in the diets. Works related to this subject showed similar results (Caston and Leeson, 1990; Jiang et al., 1991; Ferrier et al., 1995; Scheideler and Froning, 1996).

The ratio Σ PUFA : Σ SAT in yolks of groups C, FS 5, FS 10, and FS 15 was 0.77, 0.97, 1.08 and 1.17, respectively. It was reported that blood cholesterol level decreased when this ratio was about 1.00 (CHERIAN and SIM, 1994). Experiments with FS showed that this ratio was around 1.00 and that the treated groups had 0.20–0.37 units lower values than the control group (CASTON and LEESON, 1990; AYMOND and VAN ELSWYK, 1995). The present results agree with those results. Therefore, it can be concluded that yolks can be enriched in Ω-3 fatty acids by dietary actions and the level of cholesterol in the egg can be manipulated in the same way.

Summary

This trial was conducted to investigate the effects of the addition of ground flaxseed to the diets of laying hens on performance, on cholesterol levels in blood and yolks, and on fatty acid composition of yolk. In total, 256 laying hens were fed diets containing ground flaxseed at the levels of 0, 5, 10 and 15%. Feed consumption, feed conversion, egg production of hens, egg mass, egg yolk weight, egg and blood cholesterol levels and fatty acid composition of yolk were investigated. The duration of the experiment was 8 weeks.

Compared to the control diet (0% flaxseed), diets including flaxseed decreased feed consumption, egg mass and yolk weights, levels of serum and yolk cholesterol. On the other hand, flaxseed improved feed conversion and egg production (87.1%, 91.0% and 87.9%) for 5, 10 and 15% flaxseed diets, respectively, and egg production increased relatively compared to control diet (84.1%). Levels of total Ω-3 fatty acids linearly increased in yolks as the levels of dietary flaxseed increased (1.80%, 7.07%, 8.35% and 12.20% of egg yolk fatty acids for 0, 5, 10 and 15% flaxseed diet, respectively). Producing eggs with a high level of Ω-3 FA and with a lower Ω-6/Ω-3 ratio meets the needs of individuals concerned with health benefits.

Key words

Nutrition, layers, flaxseed, performance, yolk, cholesterol, fatty acids

Zusammenfassung

Einfluss des Zusatzes von Leinsaat zu Legehennenrationen auf die Leistung, die Cholesterolgehalte in Serum und Dotter sowie auf das Fettsäuremuster des Dotters

Das Ziel dieses Versuches war es, den Einfluss des Zusatzes von Leinsaat zum Legehennenfutter auf die Leistung, die Gehalte an Cholesterol in Serum und Dotter sowie auf das Fettsäuremuster des Dotters zu bestimmen. Insgesamt 256 Legehennen wurden mit verschiedenen Futterrationen gefüttert, die 0, 5, 10 bzw. 15% Leinsaat enthielten. Als Leistungsparameter wurden Futteraufnahme, Futterverwertung, Legeleistung, Eimasse und Dottergewicht erfaßt. Ferner wurden die Cholesteringehalte in Ei und Blut sowie das Fettsäuremuster im Dotter bestimmt. Die Versuchsdauer betrug 8 Wochen.

Bei den Leinsaatgruppen wurde im Vergleich zur Kontrolldiät (0% Leinsaat) geringere Futteraufnahmen, Eimassen, Dottergewichte sowie Serum- und Dotter-Cholesteringehalte registriert. Andererseits waren Futterverwertung und Legeleistung bei der Fütterung mit Leinsamen besser. Bei den Behandlungen mit einem Zusatz von 5, 10 und 15% Leinsaat betrug die Legeleistung 87,1%, 91,0% und 87,9% gegenüber 84,1% in der Kontrollgruppe. Der Zusatz von Leinsaat zu den Futterrationen erhöhte linear den Gehalt an Ω-3 Fettsäuren im Dotter. Der Gesamtgehalt an Ω-3 Fettsäuren betrug 1,80, 7,07, 8,35% und 12,20% bei einem Zusatz von 0, 5, 10 oder 15% Leinsaat. Zwischen den Gehalten an Linolsäure und Eicosapentaensäure bestanden positive Korrelationen. Ähnliche Beziehungen wurden zwischen der Docosapentaensäure und der Docosahexaensäure beobachtet. Die Versuchsergebnisse belegen, dass durch den Einsatz von Leinsaat in den Legehennenrationen die Gehalte an Ω-3 Fettsäuren bzw. das Verhältnis zwischen Ω-3 und Ω-6 Fettsäuren in den Eiern erhöht werden kann.

Stichworte

Fütterung, Legehenne, Leinsaat, Leistung, Dotter, Cholesterin, Fettsäuren

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