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Effects of Long-Term Feeding Flaxseed on Growth and Carcass Parameters, Ovarian Morphology and Egg Production of Pullets

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Abstract: The growth and reproduction performance and egg production parameters in 128 pullet chicks were monitored during 42 days received 5, 7.5 and 10% Flaxseed (FS) in their diet as a treatment. One-day-old Hy-line W-36 pullet chicks were divided into four groups of Control (C) and treatments (T_1, T_2 and T_3) with 32 pieces in each one and 4 replicates. The chicks received *ad libitum* feed and water from the first day of age to week 22. Birds were individually penned from week 18 to determine time of oviposition and egg weight and quantity. The mean Body Weight (BW) and Feed Intake (FI) were recorded and calculated every 3 weeks. One pullet from each replicate on week 8 and day of oviposition was sacrified. Then, the weight of internal organs as percentage including: femur, FeW; breast, BrW; heart, HW; spleen, SW; liver, LW; gut, GW; fat cavity, FW; oviduct, ovary and Large Yellow Follicles (LYF) and number of LYF were measured. Our results indicated that as the level of FS increases from 5-7.5% and 10%, the BW and FI reduce, thus FCR increases in pullets. The evaluation of internal organs at 8^{th} week and 1^{th} oviposition, revealed no significant differences between treatments and control group. Although, in the 1^{st} time, FS treatments increased HW and LW, but decreased FW; whereas in the 2^{nd} time, FeW, BrW and GW induced and SW and LW reduced. Also, reproduction parameters showed reduction in weight of oviduct, ovary and LYF and number of LYF in treatment group. In addition, the weight of egg at oviposition, number of days to photo-stimulation and egg production on week 22 in T_2 increased in birds received FS. Overall, the results indicated that feeding 7.5% of FS to pullets may produce more eggs than other treatments.

Key words: Flaxseed, carcass growth, ovarian follicogenesis, egg production, pullet chicks

INTRODUCTION

Flaxseed is an excellent source of ALA, exploited by egg producers to achieve significant increase in n-3 fatty acid contents of eggs. Researchers suggested that most adults who follow a low-fat and low-cholesterol diet plan can eat between 4-12 of n-3-enriched eggs per week without an increase in blood total or LDL-cholesterol levels (Lewis *et al*., 2000). Similar reports determined that eating 3-14 regular n-3-enriched eggs per week has no effect on blood lipid levels, especially if the dietary intake of saturated fat is low (Ferrier *et al*., 1995; Oh *et al*., 1995). The n-3 fatty acids are Alpha-Linolenic Acid (ALA) which makes up about 58% of total FAs in flaxseed. It also contains linoleic acid, an n-6 FAs (14%) monounsaturated FAs (19%) and saturated FAs (9%) (Udo Erasmus, 1993). The essential fatty acid ALA is converted to eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6). The conversion is affected by many factors and differs among species. Chickens are good at converting ALA to EPA and DHA, whereas humans are not. The amount of conversion has been reported from 0.2-6% in humans (Pawlosky *et al*., 2001; Emken *et al*., 1994). The supplementation of diet with 4% maize oil providing 22.4 g linoleic acid (kg, diet) has increased egg weight in an experiment by Whitehead *et al*. (1993). The addition of 5% poultry-fat to broiler breeder diets has been reported to increase egg production and reduce feed intake (Brake, 1990). Other researchers reported that fat supplementation improved egg production, feed intake and feed efficiency in experimental groups compared to control group; whereas egg weight was not affected in fat supplementation (Saban and Necati, 2006).

There are a few studies that examine the effects of flaxseed on reproductive morphology in pullets. Some reports in broiler breeders showed that ovarian morphology is important, because an extra Large Yellow Follicle (LYF) at sexual maturity can be associated with a reduction in total egg production (Robinson *et al*., 1998a,b). Other investigators suggested the positive effect of dietary linoleic acid on plasma estradiol metabolism, which enhances the lipid and protein synthesis for egg formation (Whitehead *et al*., 1993). Our study is the first to evaluate the effects of flaxseed on reproduction and carcass morphology of pullets at sexual maturity and to indicate the follicogenesis response to n-3 and n-6 FA contents of flaxseed. The current study was designed to compare the effects of long-term feeding different levels of flaxseed on growth, carcass and ovarian parameters at sexual maturity and egg production up to week 22.

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MATERIALS AND METHODS

Birds and treatments: This study was conducted at experimental animal house and physiology laboratory of Ferdowsi University of Mashhad (FUM) Department of Animal Sciences in March, 2009. One-day-old Hy-line W-36 (Hy-Line Variety, 2007-2008) pullets were obtained from a commercial hatchery. One hundred twenty eight pullets (N = 128) from the 1st day randomly separated into 4 treatment groups (n = 32 chicks/trt.). Birds from the first day received *ad libitum* standard diet supplemented with 5, 7.5 and 10% Flaxseed (FS) as treatments (Table 1). The diets were formulated according to NRC (1994). The pullets were housed in standard cages up to week 18, then each bird was placed in laying cage separately to monitor the sexual maturity and individual egg laying records.

Carcass growth and reproductive performance: During the study, chicks in control and treatments were weighted on weeks 3, 6, 9, 12, 15, 18 and 21 of age for Body Weight (BW) (Table 2) and consequently Feed Intake (FI) (Table 3) according to standard feeding regime for pullets (starter: 0-6 weeks; grower: 6-9 weeks; developer: 9-16 weeks; pre-layer: 16-18 weeks; pre-peak 5-50% pro) (Table 1). The birds were photo-stimulated one hour (11L: 13D) at 18 weeks of age and then after, 30 min light added biweekly until the light was reached to 16 h a day (Robinson *et al*., 1998a,b). The date of oviposition and the weight of first egg for each hen in every treatment were recorded daily. The total number of eggs in each treatment was calculated on 21th and 22nd week of study. On the last day of week 8 and time of sexual maturity, one bird was randomly selected from each replicate of treatment and withdrawn from feed overnight to facilitate gut clearance, then sacrificed. First, BW of each bird was recorded to determine the percentage of carcass morphology by measuring the weights of Femur (FeW), Breast (BrW), Heart (HW), Spleen (SW), Liver (LW), Gut (GW), abdominal Fat (FW), (covering the gizzard) left oviduct, ovary, Large Yellow Follicles (LYF) and the number of LYF except the largest follicle (graafian follicle) (Table 4 and 5).

Statistical analysis: Data generated from experiment was carried out in a complete randomized design (Steel and Torrie, 1980). These data were subjected to ANOVA according to GLM procedure of SAS software (SAS, 1996). The significant differences among means were

Table 1: Formulation rations, feed ingredients (%) and schedule of feeding pullets during experiment

	Starter, 0-6 week				Pre-peak (5-50% production) week			
Ingredients	C[1]	T1[1]	T2[1]	T3[1]	C	T1	T2	T3
Corn	57.13	53.60	52.42	50.86	56.24	53.85	52.76	51.51
Soybean meal	33.16	30.89	29.84	29.16	28.57	26.53	25.5	24.44
Soybean oil	3.10	2.83	2.56	2.33	3.72	3.17	2.89	2.61
Wheat bran	2.15	3.20	3.20	3.20	0.0	0.0	0.0	0.0
Whole flaxseed	0.00	5.00	7.50	10.00	0.0	5	7.5	10.0
Limestone	1.22	1.21	1.20	1.20	8.18	8.17	8.17	8.16
DCP	1.93	1.92	1.92	1.91	2.02	2.01	2.01	2.01
Salt	0.42	0.42	0.42	0.40	0.42	0.41	0.41	0.4
DL-met	0.17	0.17	0.17	0.17	0.25	0.25	0.25	0.25
L-Lysine	0.12	0.16	0.17	0.18	0.0	0.0	0.0	0.02
Vitamin E[2]	0.10	0.10	0.10	0.10	0.1	0.1	0.1	0.1
Vitamin Premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated content								
ME (Kcal/kg)	3000	3000	3000	3000	2900	2900	2900	2900
Crude protein	20.00	20.00	20.00	20.12	17.50	17.50	17.50	17.50
Calcium	1.00	1.00	1.00	1.00	3.65	3.65	3.65	3.65
Av. Phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Ether extract	6.16	7.39	7.87	8.39	6.62	7.58	8.07	8.54
n-6	3.00	3.00	2.93	2.88	3.24	3.09	3.02	2.95
n-3	0.29	1.10	1.50	1.90	0.32	1.12	1.51	1.91
Lysine	1.15	1.15	1.15	1.15	0.91	0.89	0.88	0.88
Methionine	0.48	0.48	0.48	0.48	0.53	0.53	0.53	0.53
Met+Cys	0.81	0.81	0.81	0.81	0.82	0.82	0.82	0.82

[1]C = Control; T1 = 5% flaxseed; T2 = 7.5% flaxseed; T3 = 10% flaxseed

[2]per kilogram of diet: alpha-tocopherol acetate, 46 IU.

[3]per kilogram of diet: retinol acetate, 13,200 IU; cholecalciferol, 5,720 IU; alpha-tocopherol acetate, 17.6 mg; riboflavin, 13.2 mg; pantothenic acid, 18.7 mg; niacin, 55 mg; folic acid, 0.44 mg; vitamin B12, 0.022 mg.

[4]per kilogram of diet: Mn, 140 mg; Cu, 12 mg; Fe, 79 mg; Zn, 140 mg; Se, 0.88 ppm; I, 6,600 ppm; Ca, 133 mg

Table 2: Mean body weight (g) and overall BWG of pullets fed different levels of flaxseed during 21 weeks

	Weeks							
Treatment[1]	3	6	9	12	15	18	21	BWG (g)
C	152[a]	409[a]	669	886	1124[a]	1249[a]	1442[a]	1195[a]
T1	143[a]	379[b]	653	874	1029[b]	1134[b]	1386[ab]	1096[ab]
T2	143[a]	369[b]	629	885	1034[b]	1174[b]	1376[ab]	1134[ab]
T3	131[b]	377[b]	613	843	985[b]	1039[b]	1325[b]	1075[b]
±SEM	3.28	8.9	21.65	19.87	25.36	29.21	23.32	27.01
p-value	0.01	0.04	0.31	0.5	0.01	0.02	0.03	0.04

[a,b]Means within a column and within a source with no common superscript differ significantly. [1]C = Control; T1 = 5% flaxseed; T2 = 7.5% flaxseed; T3 = 10% flaxseed, [2]BWG = overall body weight gain from 0-18 week

Table 3: Mean feed intake (g) and FCR of pullets fed different levels of flaxseed during 18 weeks

	Weeks						
Treatment[1]	3	6	9	12	15	18	FCR[2] (g)
C	15	24[a]	44	52	56	61	4.47[b]
T1	14	22[b]	45	52	58	58	4.85[a]
T2	14	22[b]	43	54	55	58	4.6[ab]
T3	14	21[b]	42	53	55	58	4.8[ab]
±SEM	0.36	0.46	1.02	0.47	1.95	1.98	0.08
p-value	0.42	0.01	0.22	0.90	0.68	0.76	0.02

[a,b]Means within a column and within a source with no common superscript differ significantly

[s1]C = Control; T1 = 5% flaxseed; T2 = 7.5% flaxseed; T3 = 10% flaxseed, [2]FCR = overall feed conversion ratio from 1-18 week

determined by using Duncan's multiple range tests. Differences among treatment means were compared at p<0.05.

RESULTS

Body weight and weight gain: Table 2 shows means BW of pullets fed different levels of flaxseed and control group on weeks 3, 6, 9, 12, 15, 18 and 21. The overall trend of BW illustrates that control group stands at the highest point with significant level on weeks 3, 6, 15, 18 and 21 (p<0.04). The 10% FS treatment shows lowest level of BW between all treatments except week 6 throughout the study. The BW was similar in T1 and T2 on week 3 and did not differ significantly with control group ($C>T_1 = T_2>T_3$). Similar pattern occurred on week 6 for control with exception of T_2 which was the lowest ($C>T_1>T_3>T_2$) but not significantly. The treatment comparisons on week 9 showed regressive trend of BW ($C>T_1>T_2>T_3$) but on week 12, this effect was reversed for T_2 and T_1 ($C>T_2>T_1>T_3$). On weeks 15 and 18, the BW between treatments did not show any significant differences, but their trends decreased in this manner ($C>T_2>T_1>T_3$). On week 21, the mean BW differed significantly (p<0.03) between T1 and T2 VS T3 ($C>T_1>T_2>T_3$). Overall, BWG from 1-8 weeks showed significant increase in control group in comparison with treatments ($C>T_1>T_2>T_3$). Our results indicated that as the level of FS increases from 5-7.5% and 10%, the BW reduces in pullets.

Feed intake: Table 3 shows mean FI of different levels of treatments and control group on weeks 3, 6, 9, 12, 15 and 18. The overall result indicated higher amount of FI for control birds on week 6 when compared to treatment group (p<0.01). However, control chicks ate more feed than treatment group with exception of weeks 9 and 15. The comprehensive result also showed higher FCR for treatment group ($T_1>T_3>T_2>C$) compared to control group (p<0.02). Within treatment comparison, FCR shows significant increase in T_1 with lowest for T2 ($T_1>T_3>T_2$) in pullets.

Carcass morphology at week 8 and sexual maturity: Table 4 shows growth performance and weight of internal organs at 8[th] week and sexual maturity as a percentage of body weight on corresponding days. During the first 8 weeks of study, the mean BW of control animals has increased significantly in comparison with treatment group (p<0.02). The overall mean weight of internal organs (%) did not differ significantly between treatment and control groups. The percentage of FeW in T_2 was highest between all groups ($T_2>C>T_1>T_3$). The % BrW was higher in control group compared to treatment group ($C>T_3>T_2>T_1$) and inversely, the % HW in treatment group was higher than control group ($T_3>T_1>T_2>C$). The % SW and % LW did not differ between all groups, but the % GW was highest for T_1 and T_3 ($T_1>T_3>C>T_2$). The % FW increased in control group when compared to treatment group.

During the second phase of study, organ collection on oviposition on week 18, showed no significant differences between control and treatment groups. The mean BW in T_1 was highest between all treatments ($T_1>T_3>C>T_2$). The % FeW in a treatment group increased compared to control group ($T_2>T_3>T_1>C$). The % BrW, % HW, % LW, % GW and % FW did not differ

Table 4: Mean body and organ weight (g) of pullets fed different levels of flaxseed at 8th week and 1th oviposition

		% of BW[2]						
Treatment[1]	BW (g)	Femur	Breast	Heart	Spleen	Liver	Gut	Fat
Week 8								
C	670[a]	17.6	14.99	0.39	0.21	2.23	9.99	1.89
T1	605[b]	17.15	13.85	0.43	0.22	2.36	10.71	1.51
T2	603[b]	17.97	14.45	0.42	0.20	2.31	9.85	1.41
T3	601[b]	17.08	14.82	0.47	0.23	2.27	10.62	1.45
± SEM	15.77	0.22	0.44	0.43	0.09	0.13	0.33	0.26
P Value	0.02	0.27	0.5	0.4	0.25	0.91	0.21	0.57
1th oviposition								
C	1263	15.87	14.68	0.33	0.20	1.95	4.34	4.14
T1	1337	17.48	15.28	0.30	0.12	1.93	4.67	3.5
T2	1220	18.09	14.81	0.37	0.13	1.87	4.76	4.48
T3	1287	17.99	15.24	0.32	0.12	1.76	4.67	3.94
± SEM	78.59	0.93	0.72	0.01	0.02	0.08	0.23	0.51
P Value	0.76	0.36	0.91	0.10	0.21	0.46	0.61	0.61

[a,b]Means within a column and within a source with no common superscript differ significantly

[1]C = Control; T1 = 5% flaxseed; T2 = 7.5% flaxseed; T3 = 10% flaxseed, [2]% of BW = organ weight/BW*100

Table 5: Mean egg weight (g) production and reproduction characteristics of pullets fed flaxseed

Parameters	Egg weight at SM	Days from PS to SM[2]	% Egg Pro 21 week[3]	% Egg Pro 22 week[3]	Oviduct (g)	Ovary (g)	LYF (n)[4]	LYF (g)[5]
Treatment[1]								
C	43.67	12.2	50.95	77.12	55.07	37.07	7.66	26.35
T1	45.02	16.7	37.00	61.5	45.52	26.16	4.00	15.31
T2	42.98	13.2	50.12	80.05	47.24	27.97	4.66	15.14
T3	43.82	15.2	40.05	69.4	43.23	21.51	3.66	12.13
±SEM	1.36	1.92	6.06	6.88	3.41	8.74	1.08	6.26
P Value	0.77	0.38	0.30	0.27	0.16	0.66	0.10	0.44

[a,b]Means within a column and within a source with no common superscript differ significantly

[1]C = Control; T1 = 5% flaxseed; T2 = 7.5% flaxseed; T3 = 10% flaxseed

[2]Days from photo-stimulation to sexual maturity, [3]Percentage of egg production at 21 and 22 week of experiment

[4]LYF (n) = large yellow follicles (>10 mm diameter) [5]LYF (g) = LYF weight without F1 follicles (graaffian follicle)

significantly between treatment and control groups, but the trend of % BrW was $T_1 > T_3 > T_2 > C$.

Ovarian morphology at sexual maturity: Table 5 shows, weights of reproductive organs, Large Yellow Follicles (LYF) and 1st egg at oviposition, number of LYF and egg production of hens on weeks 21 and 22. No significant differences between treatment and control groups for reproduction and production parameters were observed. The mean egg weight at Sexual Maturity (SM) was highest for T_1 compared to other groups ($T_1 > T_3 > C > T_2$). The days of Photo Stimulation (PS) to SM was lowest in control group when compared to treatment group ($T_1 > T_3 > T_2 > C$). The % egg production on week 21 was highest for control group in comparison with treatment group ($C > T_2 > T_3 > T_1$) but on week 22, the T_2 gained highest % of egg production between treatment and control groups ($T_2 > C > T_3 > T_1$). The weights of oviduct, ovary and LYF were highest at oviposition in control group when compared to treatment group respectively ($C > T_2 > T_1 > T_3$) ($C > T_2 > T_1 > T_3$) and ($C > T_1 > T_2 > T_3$). The number of LYF was highest in control in comparison with treatments ($C > T_2 > T_1 > T_3$).

DISCUSSION

Several studies have shown the effects of FS on hen's production. In our trail, the effects of FS on pullet's performance and reproduction parameters from day one to week 22 were evaluated. In fact, the effects of different levels of FS on BW, BWG, FI, FCR, organ weights on weeks 8 and 18, time of oviposition, days from photo-stimulation to oviposition, % egg production on 21st and 22nd weeks and weight and number of LYF were examined.

Body weight and weight gain were significantly reduced ($p<0.05$) in flaxseed-fed pullets compared to control in this trail, which may have been the result of anti-nutritional factors, such as linatine (trypsin inhibitor). Klosterman *et al*. (1967) reported that linatine in mucilage can decreases the productivity of animal by decreasing the amount of endogenous enzymes released from the pancreas, resulting in reduced digestion of feed particles. Also, the non-starch polysaccharide in mucilage of flaxseed increases intestinal viscosity in monogasteric animals and decreases nutrient availability. Flaxseed mucilage (composes 8% of the seed) contains polysaccharides such as rhamanose, fucose, arabinozylans, zylose, galacturonic acid and glucose (Erskine and Jones, 1957). Classen and Bedford (1991) also reported that arabinozylans increases intestinal viscosity, which in turn decreases nutrient availability by increasing passage rate, reducing rates of diffusion of endogenous enzymes and nutritional substrates.

Feed consumption was reduced in treatment group when compared to control pullets, which may be due to underestimating the energy supplied by FS or the fiber content of the seed. Therefore, the FCR significantly was highest for flaxseed-fed pullets in comparison with control group ($p<0.05$). Caston *et al*. (1994) reported opposite results with a significantly lower metabolizable energy value for 10% and 20% FS diets than expected. Similar results were also reported by Barbour and Sim (1991) who investigated the true metabolizable energy in canola and FS diets fed to poultry. A decrease in metabolizable energy in the rations containing FS perhaps contributed to a reduction in weight gain for flaxseed-fed pullets in our study. We found in this study lower BW ($p<0.05$) and some of the internal organs in treatment groups on week 8 compared to control animals which are agreed to latest study. Following collection of the internal organs on the first oviposition, the results have changed for some of the organs inversely.

Recently, a few trials showed the effects of FS on egg parameters including egg weight. In our study, we evaluated the effects of different levels of FS on number of days responded to photo stimulation, days of oviposition, egg weight at oviposition, weight and number of follicles at oviposition, weight of ovary and oviduct and number of eggs on 21st and 22nd weeks of feeding pullets. Several researchers reported that feeding FS to 16 mo-old hens for 4 weeks had no detrimental effects on egg production, egg weight and egg specific gravity (Jiang *et al*., 1991). Scheideler and Froning (1996) found a positive effect of feeding FS (10-20%) on egg production at 43-weeks-old hens for 6 weeks, but a decrease in feed consumption that resulted in a decrease of hen weights. Aymond and Van Elswyk (1995) using 22-weeks-old hens, reported a decrease in egg production within 2 weeks when hens fed 15% FS diet for 5 weeks. Caston *et al*. (1994) reported no change in egg production with feeding 10% or 20% FS diet to 19-weeks-old Leghorns for 48 weeks. Egg production was not affected by different levels of FS supplementation in our study. Also, the pullets fed 7.5% FS reached to 80% egg production by week 22 which was higher than control group. However, the number and weight of LYF in control group were higher in comparison with treatment group.

The 5% and 10% FS diets showed higher egg weight at oviposition and higher number of days to oviposition, but other parameters were not affected by flaxseed. Also, the first egg weight in pullets fed 7.5% FS was lowest. Decreased egg size may result from lower body weights due to reduced feed intake. These results are in contrast with those reported by Scheideler and Froning (1996). They found a decrease in egg weights with FS supplementation at 5% and 15% ground or whole flaxseed. Caston *et al*. (1994) reported that during the last period (44-48 weeks) of their trail, flaxseed-fed hens had significantly bigger eggs compared to controls. The FS we used in this study might have been supplying more or less protein (AAs) or energy (not analyzed) which provided needed nutrients for a balanced ration (met or expected the requirements for production and egg protein synthesis). Since we only collected the eggs for a short period of time, the trend of egg production may increase to higher level at older age.

Whitehead *et al*. (1993) reported decreased egg weight in response to dietary fish oil. They hypothesized that synthesis of yolk and an albumin precursor takes place at different sites and apparently shows different responses to long-chain fatty acids and consequently, a decrease in esteradiol synthesis. Flaxseed contains phytoesterogen components that may elicit the same response of lowering serum estrogen levels. Our results showed lower number and weight of LYF in flaxseed-fed birds when compared to control group. While, Scheideler and Froning (1996) reported significant increase in egg yolk n-6 FA when feeding a 10% FS diet compared to a corn/soybean meal control diet. Supplementation of FS to hens' diet is very important factor in producing nutritious eggs with high level of n-3 FA for consumers.

Conclusion: In conclusion, the long-term effects of feeding diet with 7.5% FS may improve egg production and quality with less feed consumption. The Sarabi variety of FS used in this study was mainly based on quality and availability.

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