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Effect of linseed oil supplementation on growth performance, digestibility coefficient, carcass characteristics and some blood parameters in growing rabbits.

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A total of 60 male White New-Zealand rabbits (WNZ) after weaning were allotted among 4 groups with 3 replications, 5 rabbits each, using a completely randomized design, for 56 d. The 1st group received the basal diet. The other three groups (2nd – 4th) received the basal diet with linseed oil (LO) as feed additive at 1, 2 and 3% level respectively, till age 8 wks. Supplementing diets with LO at 1, 2 and 3% level significantly decreased the feed intake by 11.7, 12.0 and 16.5%, respectively, compared to the control group. Feeding rabbits LO at the 1% level significantly improved the digestibility of DM by 7.1%, CF by 18.5% and NFE by 3.5%, respectively, compared to the control group. Rabbits received LO at the 3% level significantly decreased the digestibility of DM by 11.5, OM by 14.7%, CP by 9.5%, CF by 19%, NFE by 10.4 and TDN by 7.8%, respectively, compared to the control group. Feeding rabbits LO at the levels of 2 and 3% significantly increased the back fat weight by 17 and 31%, the liver weight by 11 and 19.4% and the fur weight by 7.7, respectively, compared to the control group. Feeding rabbits LO at the level of 3% significantly increased the hind part weight by 7.4% and the heart weight by 32% compared to the control group, while showed the lowest significant values of all digestibility coefficient parameters compared to the control group. Feeding rabbits LO% at the 1, 2 and 3% level showed non-significant reductions in total lipids, TC, LDL, HDL, TG, AST and ALT, respectively, compared to the control group. Feeding rabbits LO % at 1,2 and 3% levels significantly increased the unsaturated fatty acid at the form of the gondoic acid by 16, 25 and 31%, the linoleic acid by 16, 25 and 31% and the α -linolenic acid by 5, 11 and 14% as well as significantly decreased the saturated fatty acid at the form of the myristic acid by 25, 25 and 38 %, the stearic acid by 2, 4 and 6 % and the arachidic acid by 12, 17 and 33%, respectively, compared to the control group. In conclusion, dietary LO as feed additives did not affect growth performance and most blood parameters, while significantly improved the hind weight of the carcass and blood fatty acid profiles.

Keywords: White New-Zealand rabbits, linseed oil, growth performance, digestibility, carcass characteristics and blood parameters.

INTRODUCTION

Linseed oil used is cold-pressed extracted from reddish brown seeds of *Linum usitatissimum* L., with nutty flavor which considered a key part of

the improvement of meat quality and composition is regulation of the animal's diet (Rodriguez et al. 2013). Linseed oil feeding has been proposed to have beneficial effects on the outcome of the

metabolic syndrome due to the high n-3 fatty acid content of linseed oil (Chechi et al. 2010). Alpha-linolenic acid (ALA; 18:3 n-3) comprises about 55% of the total fatty acid content of linseed fatty acids, Flax Council (2015). Linseed oil, is a strategy to improve the fatty acid composition of healthiest broiler carcass (Lopes et al. 2013) and hen eggs (Elkin et al. 2015) in particular to increase the n-3 and improve the n-6: n-3 ratio (Oliveira et al. 2012) with a desirable tissues fatty acid composition (Fernandes et al. 2009). The partial substitution of the fatty acid component of linseed oil (rich in C18:3) corrects whole body adiposity (Cintra et al. 2012) and act as antioxidants that more efficient than a single type of antioxidant (Darvin et al. 2006).

Our theoretical hypothesis, how to get benefit from dietary linseeds oil particularly ω -6/ ω -3 as important factor in determining the capacity of bone for synthesis prostaglandin, thereby reducing bone resorption and improving bone mass during growth (Doha et al. 2014), as well as polyunsaturated fatty acids (PUFAs) are necessary for the body's metabolism, growth and development (Feng et al. 2015).

This study aimed to evaluate the effects of diets with partial substitution of linseed oil as feed additives on White New-Zealand rabbits performance, carcass traits and some blood parameters.

MATERIALS AND METHODS

This experiment was carried in Nubaria research and production station, National Research Centre and was conducted to study the substitute replacement effect of three different levels of LO in growing rabbit diets. Sixty male WNZ rabbits after weaning with an average body weight of 672 ± 82 g. Rabbit housed in individual wire cages and divided into four equal treatment groups of fifteen rabbits each (three replicates of five each). The basal experimental diet was formulated and pelleted to cover the nutrient requirements of rabbits as a basal diet according to NRC, 1977 as shown in Table (1).

The feeding period was extended for 56 days, and the experimental groups were classified as follow: Group 1: basal diet without additive (G1) control, group 2: basal diet with 1 % LO additive (G2), group 3: basal diet with 2 % LO additive (G3) and group 4: basal diet with 3 % LO additive (G4).

The cold-pressed linseed oil is characterized by high PUFA content, especially n – 3 fatty acids, which represent about 45 to 55% of total fatty acids, a moderate monounsaturated fatty acids

MUFA content and consists chiefly of three glycerides, called, respectively, linolein, linolenin, and olein.

Table (1): Composition of the basal diets* without linseed oil.**

Item	%
Clover hay	18
Yellow corn	32.9
Barley grain	20
Wheat bran	10
Soybean meal (44% CP)	16
Lime stone	1.7
Di-Ca-Phosphate	0.7
DL-methionine	0.1
Vit. &min. mixture*	0.2
Sodium chloride	0.3
Anti fungal agent	0.1
Total	100
Chemical composition of the experimental diets %	
DM	91.88
OM	91.36
CP	16.53
CF	13.45
EE	4.00
NFE	57.38
Ash	8.64
Calculated analysis DE** (Kcal/Kg DM)	2889

*** Linseed oil were added to basal diet as 1%, 2% and 3% level for each treatment

* Vit. & Min. mixture: Each kilogram of Vit. & Min. mixture contains: 2000.000 IU Vit. A, 150.000 IU Vit.D, 8.33 g Vit.E, 0.33 g Vit.K, 0.33 g Vit.B1, 1.0 g Vit.B2, 0.33g Vit.B6, 8.33 g Vit.B5, 1.7 mg Vit. B12, 3.33 g Pantothenic acid, 33 mg Biotin, 0.83g Follic acid, 200 g Choline chloride, 11.7 g Zn, 12.5 g Fe, 16.6 mg Se, 16.6 mg Co, 66.7 g Mg and 5 g Mn. **DE (Kcal/Kg DM)= 4253-32.6 (CF%) – 144.4 (ash%), according to Fekete and Gippert (1986).

Rabbits were individually housed in galvanized wire cages (30 x 35 x 40 cm). Stainless steel nipples for drinking and feeders allowing recording of individual feed intake for each rabbit were supplied for each cage. Feed and water were offered *ad libitum*. Rabbits of all groups were kept under the same administrative conditions and were individually weighed. Feed consumption was individually recorded weekly during the experimental period.

All rabbits were used in digestibility trials over period of 7 days to determine the nutrient digestibility coefficients and nutritive values of the tested diets. Feed intake of experimental rations and weight of feces were recorded daily. Representative samples of feces were dried at 60°C for 48 hrs, grinded and stored for chemical analysis later.

Table (2): Effect of different levels of LO on (WZR) rabbit's growth performance.

Item	Control	LO level		
		1%	2%	3%
Initial weight (kg)	0.734± 0.19	0.701±0.19	0.74±0.21	0.70±0.31
Final weight (kg)	2.40 ±0.61 ^a	2.04± 1.14 ^b	2.29 ±0.30 ^a	2.25 ±0.52 ^{ab}
Feed intake (kg)	6.29± 2.52 ^a	4.03± 0.64 ^b	4.07± 0.66 ^b	5.91± 0.65 ^a
Daily feed intake (g)	112.3±9.2 ^a	72.0±11.4 ^b	72.6±11.7 ^b	105.6±11.8 ^a
Total weight gain (kg)	1.67 ±0.55 ^a	1.34 ±0.97 ^b	1.54 ± 0.45 ^{ab}	1.55 ± 0.32 ^{ab}
Daily weight gain (g)	29.8 ± 9.89 ^a	23.9 ± 7.35 ^b	27.6 ±7.97 ^{ab}	27.6 ±5.63 ^{ab}
Feed conversion (ratio)	3.89 ±2.21 ^a	3.53 ± 3.56 ^{ab}	2.68 ± 0.86 ^b	3.85 ± 0.87 ^a

A & b: In each column means having different superscripts are significantly different ($p < 0.05$).

Table (3): Effect of different levels of LO on (WZR) rabbit's digestibility.

Digestibility coefficient	Control	LO level		
		1%	2%	3%
Dry matter (DM)	73.2±2.3 ^b	78.4±1.85 ^a	74.9± 4.4 ^{ab}	64.8± 4.6 ^c
Organic matter (OM)	74.8±2.60 ^a	80.3±3.84 ^a	77.2± 2.8 ^a	63.8±2.9 ^b
Crude protein (CP)	78.9±5.82 ^a	81.1± 3.55 ^a	77.7 ± 2.90 ^a	71.4±7.71 ^b
Crude fiber (CF)	41.0 ±5.39 ^b	48.6± 2.6 ^a	48.1 ±3.53 ^a	33.2± 2.03 ^c
Ether extract (EE)	82.5±3.6 ^{ab}	86.0±5.03 ^a	75.0±7.83 ^c	78.4± 3.2 ^{bc}
Nitrogen free extract (NFE)	81.1±1.9 ^b	83.9 ± 0.81 ^a	76.6 ± 4.01 ^c	72.7±1.6 ^d
Total digestible nutrients (TDN)	72.7±2.55 ^a	74.4± 1.4 ^a	64.6 ±0.65 ^b	67.0±1.2 ^c
Digestible crude protein (DCP)	12.9±0.96	12.2 ±2.98	11.7 ±2.98	11.1±2.46

a,b,c & d: In each column means having different superscripts are significantly different ($p < 0.05$).

Table (4): Effect of different levels of LO on (WZR) rabbit's carcass characteristics.

Item	LO level			
	Control	1%	2%	3%
Live body weight	2125±50 ^a	2112±21 ^a	2125±50 ^a	2100±30 ^a
Slaughter wt	1565±86	1544±57	1526±40	1538±28
Digestive tract wt / live wt %	228±43 ^a	229±43 ^a	219±14 ^a	229±36 ^a
Front part wt %	751±52 ^a	720±34 ^a	682±41 ^a	659±30 ^a
Hind part wt %	803±99 ^b	815±70 ^{ab}	833±21 ^{ab}	867±67 ^a
Fur wt %	297±21 ^a	285±70 ^{ab}	274±20 ^b	248±5.3 ^c
Back fat wt	70±2.0 ^c	77±2.0 ^{bc}	82±4.0 ^{ab}	92±3.1 ^a
Head %	128±22 ^a	141±35 ^a	132±22 ^a	137±18 ^a
Heart	9.11±2.0 ^b	9.50±0.9 ^b	10.3±1.5 ^b	12.0±2.0 ^a
Liver	79.0±2.0 ^c	81.3±3.1 ^c	87.7±7.6 ^b	94.3±5.0 ^a
Giblets	216±24 ^a	230±34 ^a	232±13 ^a	243±23 ^a

a, b & c: In each column means having different superscripts are significantly different (p<0.05)

Table (5): Effect of different levels of LO on (WZR) rabbit's blood parameters.

Item	Total lipids	TC	LDL	HDL	TG	AST	ALT
Control	906±4.1	205±8.7	316±8.1	83±117	208±15	31±2.0	126±11
1% LO level	905±5.3	206±9.5	315±5.0	84±2.0	205±10	30±2.0	121.3±4.6
2% LO level	904±5.3	206±3.1	314±4.2	82±1.2	204±7.2	29.7±64	118.7±3.0
3% LO level	902±5.7	208±1.4	312±5.0	81±1.2	204±7.2	29.5±14	119.5±4.2

Table 6: Effect of different levels of LO on (WZR) rabbit's blood fatty acids profile.

Item	Saturated fatty acid			Unsaturated fatty acid		
	Myristic acid	Stearic acid	Arachidic acid	Gondoic acid	Linoleic acid	α -Linolenic acid
Lipid Numbers	C14:0	C18:0	C20:0	C20:1	C18:2	C18:3
Control	0.08 \pm 0.01 ^a	38.6 \pm 0.53 ^a	0.69 \pm 0.04 ^a	23.5 \pm 1.7 ^c	0.22 \pm 0.01 ^c	9.7 \pm 0.65 ^d
1% LO level	0.06 \pm 0.01 ^b	37.8 \pm 0.61 ^b	0.61 \pm 0.04 ^b	24.6 \pm 1.0 ^b	0.24 \pm 0.01 ^c	10.1 \pm 0.09 ^c
2% LO level	0.06 \pm 0.01 ^c	36.9 \pm 0.31 ^c	0.57 \pm 0.04 ^c	25.8 \pm 0.31 ^a	0.27 \pm 0.03 ^b	10.7 \pm 0.5 ^b
3% LO level	0.05 \pm 0.01 ^d	36.6 \pm 1.02 ^c	0.46 \pm 0.1 ^d	26.4 \pm 0.8 ^a	0.32 \pm 0.02 ^a	11.3 \pm 0.5 ^a

a,b,c & d: In each column means having different superscripts are significantly different ($p < 0.05$).

Chemical analysis of experimental rations and feces were analyzed according to AOAC (2000) methods. Gross energy (mega calories per kilogram DM) was calculated according to Blaxter (1968), where, each g of crude protein (CP) = 5.65 kcal, each g of ether extract (EE) = 9.40 kcal, and each g crude fiber (CF) and nitrogen-free extract (NFE) = 4.15 kcal. Digestible energy (DE) was calculated according to Fekete and Gippert (1986) using the following equation: DE (kcal/ kg DM) = 4253 – 32.6 (CF %) – 144.4 (total ash). Diets were offered pelleted and the diameter of the pellets was 4 mm.

Three representative rabbits from each treatment were randomly chosen and fasted for 12 hours before slaughtering according to Blasco et al. (1993) to determine the carcass measurements. These were removed and individually weighed. Full and empty weights of digestive tract were recorded and digestive tract contents were calculated by differences between full and empty digestive tract. Weights of edible and external offal's were calculated as percentages of slaughter weight (SW). Hot carcass was weighed and divided into front, middle and hind parts.

Blood samples were collected in tubes from the brachial vein (3 rabbit /group), and centrifuged at 3000 rpm for 15 minutes to separate clear serum which stored at 20°C for determination of some blood constituents as total lipids (T.L), total

cholesterol (T.C), high density lipoprotein (HDL), low density lipoprotein (LOW), triglyceride (TG), aspartate transaminases (AST) and alanine transaminase (ALT) as well as the blood some fatty acids profile by spectrophotometer using available commercial kits. Collected data were subjected to statistical analysis as one way classification analysis of variance using the general linear model procedure of SPSS (1998). Duncan's Multiple Range Test (1955) was used to separate means when the dietary treatment effect was significant.

RESULTS AND DISCUSSION

Growth Characteristics:

Effect of LO on rabbit's growth performance

Feeding rabbits at 1, 2 and 3% LO levels significantly decreased the feed intake by 11.7, 12.0 and 16.5%, respectively, compared to the control group (Table 2). These results may be attributed to the increases in the monounsaturated fatty acids (MUFAs) in LO (Bertola et al. 2013) which activate receptors in the satiety center of the hypothalamus (Wanapat et al. 2011) and confirms the fact that the rabbit growth performance can be attributed to the feed intake (Mertens, 1994).

Supplementation LO at the three levels used did not change the final live body weight, the

body gain and the feed conversion ratio. These non-significant results may be attributed to the adhering effect of LO fatty acids to food particles and create a physical barrier which prevents the caecum microorganisms and microbial enzymes action, which consequently caused malfunction growth rabbit performance as noticed in lactating dairy cows (Sullivan et al. 2004). Or may be due to the effect of LO that correct the body insulin resistance and its adiposity (Cintra et al. 2012). Similar result showed no significant difference in broiler growth rate feeding on the diet containing LO (Bond et al. 1996) or as in lamb performance that could possibly attributed to the low level of lipid inclusion below 6.5% DM (Meale et al. 2014).

Effect of LO on rabbit's digestibility

Rabbits received LO at 1% level significantly improved the digestibility DM by 7.1%, CF by 18.5%, NFE by 3.5%, respectively, compared to the control group (Table 3). Feeding rabbit LO at the 3% level significantly decreased the digestibility of DM by 11.5, OM by 14.7%, CP by 9.5%, CF by 19%, NFE by 10.4 and TDN by 7.8%, respectively, compared to the control group (Table 3). In other word, feeding rabbits LO at the level of 1% significantly improved all digestibility coefficient parameters followed by the control group compared to the other two levels used, in contrast, rabbits received LO at the level of 3% showed the lowest significant values of all digestibility coefficient parameters (Table 3). These results may be attributed to the decrease of appetite induced by the gradually increase the concentrations of unsaturated fatty acids in diet, which activate receptors in the satiety center of the hypothalamus (Wanapat et al. 2011).

Effect of LO on rabbit's carcass characteristics

Although dietary rabbits LO at 1, 2 and 3% level showed no significant differences on slaughter weight, digestive tract weight, front part weight, head weight and giblets weight, while rabbits received LO at the 3% level showed the only one significant value in increasing the hind part weight by 7.4% compared to the control group (Table 4). This result may be attributed to the increase of rabbit bone weight as a reflect of the long-chain PUFAs in LO as a potent in reduction of the concentration of prostaglandin which may result in reduction of bone resorption (Raisz et al. 1989) and enhancement of bone formation (Watkins et al. 2000). Similar result showed that dietary PUFAs, particularly ω -6/ ω -3

ratios are important factors in improving bone mass during growth in growing rabbits (Doha et al. 2014).

Feeding rabbits LO at the level of 2 and 3% significantly increased the back fat weight by 17 and 31%, respectively, compared to the control group (Table 4). This result may be attributed to the deposition of some ingested fatty acids directly into the tissues resulting in changes in the omega-6/omega-3 ratio and/or in the melting point of the fats (Juárez et al. 2010). In other words it may be due to the catalyze rate limiting step induced by delta-9 desaturase enzymes in the conversion of saturated to MUFAs; mainly stearic acid to oleic acid and palmitic acid to palmitoleic acid (Alarcón et al. 2016). Also, it has been known that fat deposition in the carcass may be influenced by the degree of fat saturation, genotype and gender (Olivares et al. 2009). Similar results in calves cleared that the total n-3 polyunsaturated fatty acids increased in longissimusdorsi and subcutaneous fat when feeding linseed diet (González et al. 2014).

Feeding rabbits LO at the levels of 3% significantly increased the heart weight by 32% compared to the control group. This result may be due to the LO protective effect via an improvement of lipid profile and reduction in inflammatory markers in rabbits (Pan et al. 2009). Feeding rabbits LO at the levels of 2 and 3% significantly increased the liver weight by 11 and 19.4% and the fur weight by 7.7 and 16.5%, respectively, compared to the control group (Table 4). These results may be due to that liver mainly reflected feed fatty acid profile, except stearic acid, which increased as feeds contained higher doses of vegetable fat, which could be related to an inhibition of the activity of the stearoyl-CoA-desaturase (Tres et al. 2009).

Effect of LO on rabbit's blood parameters.

Feeding rabbits LO% at the 1,2 and 3% level showed non-significant reductions in total lipids, TC, LDL, HDL, TG, AST and ALT, respectively, compared to the control group (Table 5). This lack effect of these diets on blood rabbit parameters may possibly be attributed to the effect of some MUFA- or n-6 PUFA enriched LO that has been masked its effects. Similar results are shown in bovines by Fernandez and West (2005), or may be due to the low levels of OL inclusion at below 6.5% DM as noticed of some vegetable oils in lambs used by Meale et al. (2014). However, feeding LO revealed a significant reduction in hepatic triglyceride and cholesterol concentrations

in obese rats compared with the low fat rats (Chechi et al. 2010).

Effect of LO on rabbit's blood fatty acids profile.

Feeding rabbits LO % at 1,2 and 3% levels significantly increased the unsaturated fatty acid at the form of the gondoic acid by 16, 25 and 31%, the linoleic acid by 15.6, 25 and 31% and the α -linolenic acid by 5.3, 10.6 and 14.2%, respectively, compared to the control group (Table 6). Feeding rabbits LO % at 1,2 and 3% levels significantly decreased the saturated fatty acid at the form of the myristic acid by 25, 25 and 37.5% the stearic acid by 2.1, 4.4 and 5.5% and the arachidic acid by 11.6, 17.4 and 33.3%, respectively, compared to the control group (Table 6).

These results may be reflect the ability of linseed oil to induce a significant increase in the proportion of n-3 fatty acids (Kaul et al. 2008) and a decrease in the ratio of n-6 to n-3 (Simopoulos et al. 2008) which may be confirm the fact that as LO increased in feeds, the n-6/n-3 FA ratio was decreased in serum as a result of the incorporation of fatty acids from diets, and also may be due to the different selectivity of desaturase enzymes (Tres et al. 2009) which catalyze the rate limiting step in the conversion of saturated fatty acids mainly myristic acid, stearic acid and arachidic acid to monounsaturated fatty acids (Alarcón et al. 2016). In other words, the metabolism of some individual fatty acids may convert to other fatty acids differs; depending on many factors as sex, the use of some fatty acids for the synthesis of hormones and prostaglandins (Kloareg et al. 2007).

CONCLUSION

Supplementation linseed oil in the rabbit diets tends to increase the unsaturated fatty acids versus the saturated fatty acids as well as receive more emphasis than other nutrients, such as protein, fiber, minerals and vitamins and therefore, it can be recommended that there is no single universal optimum amount of added dietary linseed oil would be suitable across all feed sources because the additives is variable and depends on the type of source used, the rabbit breed, the adding methods and other characteristics.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example WEP designed and performed the experiments and also wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

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