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Effect of Dietary Supplementation of Safflower (*Carthamus tinctorius* L.) Seed on the Growth Performance, Blood Lipid and Meat Quality of Broiler Chickens

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ABSTRACT

Background: Broiler diets frequently include fats and oils to improve energy density, feed palatability and functionality associated with PUFAs and MUFAs. The purpose of this research was to see how dietary supplementation of safflower seed affected broiler growth, lipid profile and meat color.

Methods: In this study total two hundred male broiler chicks of one-day-old were fed a basal diet over one week. After one week, randomly chicks were distributed into five treatment groups, *i.e*. one which were fed with basal diet (control) and other fed with different ratios (2.5%, 5%, 7.5% and 10%) of safflower seed, respectively. Each treatment included five pens, each with eight chicks. The diet was formulated into two phases: starter phase (7-21 d) and the finisher phase (22-42 d).

Result: The birds fed rations supplemented with different levels of safflower seed resulted in increased (P<0.05) BWG and FCR during the last 21 days of the trial. In this experiment, the highest and lowest BWG were observed in 5% safflower seed and 0% safflower seed, respectively. No differences were observed in feed intake during the starter phase and finisher phase among the groups. In blood lipid profile, HDL-C was not different in all treatment, while CHO, LDL-C and VLD-C concentrate levels were decreased (P<0.05) when fed with different levels of safflower seed. Dietary supplement of safflower seed showed no significant (P>0.05) effect on meat color, pH and WHC. Hence, safflower seed could be used as a natural energy source to boost growth development, decrease cholesterol levels and improve meat color.

Key words: Growth performance, Functional foods, Lipid profile, Meat color, Safflower seed.

INTRODUCTION

Chicken meat is preferred over other meats because it contains more protein and less cholesterol (Kralik *et al.* 2018). Poultry meat, particularly broiler meat, is widely accepted in almost all the countries despite cultural taboos about meat consumption (Sudharsan *et al.* 2021). The poultry and related industries are facing problems because of high prices of feed ingredients such as maize, soybean, chickpea and other cereals, especially in developing countries. Presently, people are more health-conscious and want functional food to improve their long-term health goals (Bharath *et al.* 2017). Therefore, there is a need to look for alternative cheaper, easily available, nontoxic oilseed with some functional properties. In this context, safflower seed might be an important alternative source of fats and oils enriched with functional PUFA and MUFA.

Oil of Safflower is rich in polyunsaturated fatty acid (PUFA) linoleic acid (77%) (Singh and Nimbkar 2016). The number of studies demonstrating the positive effects of PUFA, including fats of animal origin, on human health has recently increased (Djuricic and Calder 2021). PUFA decreases blood cholesterol which is one of the contributory factor for coronary heart disease. Specifically, conjugated linoleic acid has inhibitory effects on cardiovascular diseases, diabetes, cancer, obesity and many more (Karabacak *et al.* 2015). However, nutritional importance of safflower seeds has not been extensively tested in poultry. Therefore, the current investigation was performed to study the effect of safflower seeds supplementation on lipid profile, growth performance and meat color of broiler chicken.

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MATERIALS AND METHODS

The feeding trial was conducted in the poultry farm of the Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi,

India. Two hundred one day old broiler male chicks (cobb-400) were procured from a commercial company. The chicks were kept in deep litter system and fed with basal diet for one week just before starting the trial. After one week, weight of the chicks were taken and they were divided into five separate groups. Each group comprised of 40 broilers each, with five replicates of eight broilers per replicate. Required amount of feed with drinkable water was provided *ad libitum.* The brooder's temperature was maintained at 33±1°C from day one of the trial to 14th day and then gradually decreased to 26±1°C by 28 days old, after that the chicks were kept at room temperature. Through out the 35-days of the experiment, all the birds were kept under the same management settings until they reached the age of 42 days. The chicks were vaccinated in accordance to the vaccination protocol.

Table 1 shows five iso-caloric and iso-nitrogenous diets that were formulated to meet the broilers' nutritional needs as per NRC (1994). Besides the basal control diet, four experimental diets were formulated to contain safflower seed (2.5%, 5%, 7.5% and 10%). The diets were formulated for two-phases, first starter phase (7-21 d) and finisher phase (22-42 d). The experimental diets fed to the birds were evaluated for proximate principles as per AOAC (2000).

The gain in body weight of birds and weight of feed provided to them were recorded each week up to 42 days. Under each dietary regimen, the cumulative weekly and overall body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined. Furthermore, during the trial, growth efficiency parameters such as energy efficiency ratio (EER) and protein efficiency ratio (PER) were determined as per Rokade *et al.* (2017).

PER = Weight gain ÷ Protein intake

EER = ME intake (kcal) ÷ Weight gain (g)

At the end of the experiment, two birds were chosen at random from each replication and 5 mL of blood sample was drawn out from the wing veins and placed in non-heparinized tubes without anticoagulants. The serum from the blood was separated by centrifugation process at 2000 rpm for 10 minutes and the samples were kept at -20°C for biochemical analysis. Commercial diagnostic kits (Qualigens India Pvt. Ltd.) were used to measure triglyceride (TRI) levels, high-density lipoprotein-cholesterol (HDL-C) and total cholesterol

Table 1: Ingredient composition and nutritional content of starter and finisher experimental diets.

Ingredients (%)	Level of safflower seed (%)									
	Starter diets					Finisher diets				
	0	2.5	5	7.5	10	0	2.5	5	7.5	10
Maize	50.7	49.76	48.24	47.28	45.61	59.44	58.06	57.4	55.22	53.39
Soybean meal	38.75	37.1	36.12	35.1	34.24	25.14	23.98	22.14	21.87	21.16
Wheat bran	5	5	5	5	5	5	5	5	5	5
Vegetable oil	2	2	2	2	2	7	7	7	7	7
Dicalcium phosphate	1.25	1.25	1.25	1.25	1.25	1	1	1	1	1
Shell grit	1	1	1	0.5	0.5	1.25	1.25	1.25	1.25	1.25
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Minerals premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamins premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.1	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0	0
DL-Methionine	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
Vitamin E	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Safflower seed	0	2.5	5	7.5	10	0	2.5	5	7.5	10
Chemical composition										
Dry matter (%)	88.93	89.42	89.88	89.94	90.14	89.96	90.01	90.05	90.08	90.16
Crude protein (%)	21.76	22.1	22.68	22.32	22.11	19.33	19.36	19.35	19.38	19.24
Crude fibre (%)	4	4.37	5.13	5.47	5.7	4.26	4.55	4.92	5.17	5.44
Lysine (%)	1.29	1.33	1.43	1.36	1.36	1.06	1.09	1.03	1.11	1.11
Methionine (%)	0.5	0.51	0.55	0.56	0.661	0.4	0.45	0.45	0.47	0.41
Calcium (%)	1.13	1.11	1.16	1.19	1.177	1.1	1.14	1.33	1.33	1.09
Phosphorus (%)	0.63	0.64	0.68	0.68	0.7	0.64	0.68	0.7	0.58	0.57
ME (kcal/kg)	3063.21	3016.82	3032.62	3012.69	3024.66	3200.23	3226.65	3218.58	3225.17	3224.7

*Data reflecting per KG: Niacin - 45 mg, Panthotenic acid - 20 mg, Riboflavin - 8 mg Thiamin - 5 mg, Vitamin B_{12}- 4.5 mg, Pyrodoxin - 4 mg, Biotin - 0.2 mg, Vitamin D_3- 8560 IU, Vitamin A - 8000 IU, Vitamin E - 95 IU.˙Supplied per kilogram: Manganese as Manganese sulfate monohydrate 100 mg, Zinc as Zinc sulphate monohydrate 70 mg, Iron as Ferrous sulphate monohydrate 55 mg, Copper (Cupric sulfate pentahy-drate) 8 mg, Iodine (Calcium iodate) 2 mg, Selenium as Sodium selenite 0.15 mg.

(CHO) according to the manufacturer's recommendations. Friedewald's formula was used to analyze both low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) (Aba *et al.* 2019).

$$LDL\text{-}C = CHO - (HDL\text{-}C + TRI \div 5)$$

$$VLDL\text{-}C = 1 \div 5 \text{ of } TRI$$

The color of broiler meat (L*, a*, b*) were measured by Color Flex, Hunter Lab. Raw meat samples were taken out from the left thigh and breast and were kept in Petri plate and then placed to the sensors of the colorimeter to analyze their color. Three color coordinate values were expressed as the mean values of lightness (L*), redness (a*) and yellowness (b*). After 24 hours of slaughter, meat sample (10 g) from the left side of thigh and breast was taken and homogenized with 100 ml of distilled water to measure the pH value. Buffer solution of pH 4 and pH 7 was prepared for calibration. The pH values were recorded by dipping the glass electrode of digital pH meter (Systrong μ pH system) into the homogenized solution. The water-holding capacity (WHC) was also measured as per the procedure described by Wilhelm *et al.* (2010).

The study involved a completely randomized design (CRD) with five treatments and five replications in each treatment. Data of this study were examined using one way ANOVA and Duncan's multiple post-hoc range tests, which is a common statistical approach Snedecor and Cochran (1989). Statistical treatments were performed by using SPSS version 25. Significance levels were declared at ($P<0.05$).

RESULTS AND DISCUSSION

Growth performance

Weekly body weights (BW) of broiler chickens are shown in Fig 1. The results indicate that there was no significant difference in body weight between the trial groups during first two to three weeks. While, a progressive increase in BW was observed with an increased level of safflower seed from the fourth to sixth week of the trial. At the end of the feeding trial *i.e.* during sixth week, the highest BW was recorded in 5% safflower seed group and the lowest was observed in the control group with 0% safflower seed. The body weight with 5% safflower seed was found significantly higheras compared to all other tested levels. Compared to 2.5% and 5% safflower seed groups, the BW gain in the last three weeks was modest to low in the 7.5% and 10% safflower seed groups. The reason behind this may be due to the higher fiber content in the 7.5% and 10% safflower seed groups, which lead to decrease in the diet intake.A similar pattern was observed in the BW of the birds up to five weeks of age which were fed with 5 g and 10 g of safflower oil (Amer *et al.* 2021). Malakian and Hassanabadi (2010) reported higher BW of broiler chicken fed with 5% safflower seed during 21-42 days. In another study, the chickens body weight did not get significantly affected when they were fed with 0%, 10% and 20% safflower but numerically the BW were higher in the group that were fed with 10% safflower (Oguz and Oguz 2007).

The performance data for the starter and finisher phases (Table 2) revealed that there was no ($P>0.05$) difference in FI and FCR between all groups during the trial period of 7-21 days of birds age. However, there was a significant difference in FCR when correlated between the treated groups and the control group during the finisher phase (22-42 days of age). FCR of birds which were fed with supplemented diet of 5% safflower seed was found better as compared to other groups. EER was significantly higher in 0% safflower seed group and lowest in 5% safflower seed group during starter and finisher phase. PER was significantly higher in 5% safflower seed group, while the lowest PER was observed in 0% safflower seed group. Moreover, there was no ($P>0.05$) difference between groups of 7.5% and 10% safflower seed. This could be because the 5% safflower seed group has more lysine than the other groups, while the 0% safflower seed group had the least. Lysine is an essential amino acid that improves the conversion of protein into metabolic activities, energy and tissue production. This is possible because higher amino acid present in the diet or body synthesize the muscle in much better way (Nasr *et al.* 2011). Increased level of safflower seed in the diet gave poor results of PER, EER and FCR indicating poor efficiency of feed with respect to protein and also poor energy utilization by the birds.

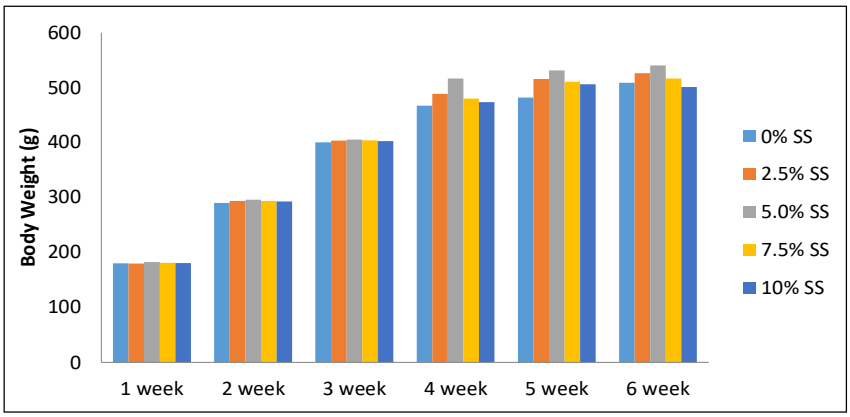

Fig 1: Effect of supplementation of safflower seed (SS) on weekly body weight gain of broiler chickens.

Table 2: Effect ofsupplementation of safflower seed on growth and production performanceof broiler chickens.

Attributes	Level of safflower seed (%)					SEM	P-value
	0	2.5	5	7.5	10		
Body weight (g)							
Initial (7 d)	180.35	179.67	182.55	181.08	180.61	2.05	0.890
21 d	871.04	877.07	883.60	878.59	875.47	2.65	0.065
42 d	2328.67^{c}	2407.47^{b}	2471.52^{a}	2385.35^{bc}	2355.35^{c}	13.25	0.010
Live weight gain (g)							
7-21 d Phase-I	690.69^{b}	697.40^{a}	701.04^{a}	697.51^{a}	694.58^{ab}	1.95	0.018
22-42 d Phase-II	1457.63^{d}	1530.39^{b}	1587.92^{a}	1506.75^{bc}	1480.39^{cd}	12.64	0.010
7-42 d overall	2148.32^{d}	2227.80^{b}	2288.96^{a}	2204.27^{bc}	2175.25^{cd}	13.18	0.010
Feed intake (g)							
7-21 d Phase-I	991.22	987.21	985.73	978.92	981.85	3.06	0.082
22-42 d Phase-II	2504.80	2503.16	2506.91	2503.09	2496.03	2.60	0.079
7-42 d overall	3496.02^{a}	3490.37^{ab}	3492.64^{ab}	3482.02^{bc}	3477.89^{c}	4.15	0.031
Feed conversion ratio (FCR)							
7-21 d Phase-I	1.43	1.41	1.40	1.40	1.41	1.79	0.64
22-42 d Phase-II	1.71^{a}	1.63^{c}	1.57^{d}	1.66^{bc}	1.68^{ab}	0.01	0.01
7-42 d overall	1.62^{a}	1.56^{c}	1.52^{d}	1.58^{bc}	1.59^{b}	0.00	0.00
Energy efficiency ratio (EER)							
7-21 d Phase-I	4.39^{a}	4.27^{b}	4.17^{c}	4.17^{c}	4.23^{b}	0.01	0.00
22-42 d Phase-II	5.50^{a}	5.23^{c}	5.06^{d}	5.36^{bc}	5.43^{ab}	0.14	0.10
7-42 d overall	5.14^{a}	4.92^{c}	4.79^{d}	4.98^{bc}	5.05^{b}	0.02	0.06
Protein efficiency ratio (PER)							
7-21 d Phase-I	3.20^{a}	3.19^{a}	3.13^{bc}	3.11^{c}	3.15^{b}	0.01	0.00
22-42 d Phase-II	3.01^{b}	3.15^{a}	3.14^{a}	3.10^{a}	3.08^{ab}	0.02	0.07
7-42 d overall	3.06^{c}	3.14^{ab}	3.16^{a}	3.10^{bc}	3.10^{bc}	0.02	0.01

$^{a,b,c,d.}$Means bearing different superscripts within a row differ significantly ($p<0.05$).

These results agree with Jalali *et al.* (2015) who reported that supplementation of soybean oil containing high PUFA to the broilers diet significantly increased (P<0.05) the FCR and increase in body weight was also observedin broilers during entire rearing phase and growing period. Manohar and Edwin (2015) also found that omega-3 PUFA supplementation to the quail diet significantly affects weight gain without significant difference in feed efficiency and intake. Abdulla *et al.* (2017) compared soybean oil and linseed oil and found that soybean oil supplementation increased (P<0.05) BW at sixth weeks while no such significant increase was found in linseed oil supplemented diet. However these findings are in contrast with Ebeid *et al.* (2011), who had reported that growth performance like BW, FI, or FCR is not adversely affected by diet supplemented with omega-3 PUFAs in Japanese quail. PUFA in the diets enhances growth performance of broiler birds. Oils like rapeseed oil, sunflower oil and canola oil are rich in unsaturated fatty acids which have better intestinal absorption due to hydrolysis of monoglyceride and micelle formation compared to saturated fatty acids Sudharsan *et al.* (2021).

Serum lipid profile

Increasing levels of safflower seed supplementation in the diet (P<0.05) affected serum lipid profile except for HDL (Table 3). The level of total cholesterol (CHO) concentration tended to be lower (P<0.05) when fed with higher levels of safflower seeds. Saleh and Alzawqari (2021) observed that the serum concentration of CHO level decreased in birds fed with different levels of olive cake meal. A similar type of result was found in the study of Saleh *et al.* (2020) and Cayan and Erener (2015) in which the level of cholesterol decreased significantly in birds fed with different levels of olive cake meal and olive leaf powder. It was also observed by Rama Rao *et al.* (2004) that serum concentration of CHO levels decreased in birds fed with different levels of sunflower meal. Safaa *et al.* (2014) reported that on addition of oat hulls in broiler diets caused a linear reduction lipid profile, like total cholesterol and triglycerides in 3 weeks of broilers. This might be due to high fiber contained in the diet. The decrease in the levels of serum lipid metabolite which are found to be by the dietary supplements of insoluble fiber is because of bile lipid binding ability of fibers in the animal gut Sarikhan *et al.* (2009). These findings are in contrast with Oguz and Oguz (2007) and Selvaraj *et al.* (2004) whose observation showed that there was no significant difference in CHO levels when birds fed different levels of full-fat safflower and full-fat sunflower seeds. During the present study, there were no significant differences between 7.5% and 10% safflower seed groups in TRI, LDL-C and VLDL-C. Malakian and Hassanabadi

Table 3: Effect of supplementation of safflower seed on lipid profile of broiler chickensat 42-day.

Level of safflower seed (%)	Attributes				
	CHO (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TRI (mg/dl)	VLDL-C (mg/dl)
0	139.91[a]	36.94	79.09[a]	119.34[a]	23.86[a]
2.5	127.36[b]	37.22	75.65[b]	119.12[a]	22.44[b]
5	116.37[c]	37.58	74.20[b]	116.72[b]	21.76[b]
7.5	113.53[cd]	36.44	67.89[c]	110.74[c]	18.90[c]
10	109.04[d]	38.41	68.21[c]	110.32[c]	17.69[c]
SEM	0.82	0.30	0.88	0.56	0.45
P-value	<0.01	0.630	0.001	<0.01	0.001

[a,b,c,d]Means bearing different superscripts within a column differ significantly (p<0.05).

*CHO- Total cholesterol, HDL-C- High density lipoprotein-cholesterol, LDL-C- Low density lipoprotein-cholesterol, TRI- Triglyceride, VLDL-C- Very low density lipoprotein-cholesterol, SEM-Standard error mean.

Table 4: Effect of supplementation of safflower seed on meat quality parameters of broiler chickens.

Attributes	Level of safflower seed (%)					SEM	P-value
	0	2.5	5	7.5	10		
Breast meat							
L*	52.28	52.29	52.31	52.30	52.28	0.01	0.05
a*	5.06	5.12	5.17	5.11	5.10	0.04	0.52
b*	5.64	5.63	5.62	5.63	5.64	0.01	0.24
pH	5.61	5.62	5.63	5.62	5.61	0.01	0.93
WHC (%)	63.79	63.82	63.84	63.81	63.80	0.01	0.99
Thigh meat							
L*	53.19	53.22	53.23	53.21	53.20	0.68	0.09
a*	8.38	8.40	8.43	8.41	8.39	0.62	0.14
b*	7.01	6.98	6.95	6.96	6.97	0.60	0.15
pH	5.74	5.73	5.68	5.67	5.69	0.02	0.35
WHC (%)	62.52	62.55	62.54	62.50	62.48	0.01	0.98

L*- Lightness; a*- Redness; b*-Yellowness; WHC- Water holding capacity.

(2010) also observed that the concentration of plasma TRI was lower in the birds which were fed with higher amount of full-fat safflower seed during 21-42 days and this effect was found not significantly different (P>0.05). The lower TRI values found in chickens fed with the highest levels of safflower seeds may be due to the specific fatty acids that stimulate oxidative beta pathway enzymes. Furthermore, in birds, using an oil seed crop increases the activities of S-3-hydroxy acyl-CoA dehydrogenase and carnitine palmitoyltransferase-1. As a result, enhanced carnitine palmitoyltransferase-1 activity would increase the availability of fatty acids for oxidation (Sanz *et al.* 2000). By feeding various levels of safflower seed, HDL-C did not significantly differ between control and treatment groups, but LDL-C and VLDL-C were significantly decreased. The result are suggertive of the fact that diets containing high linoleia cid poditively effect on plasma CHO TRI, LDL-C and VLDL-C cholesterol in broiler birds but has a negative effect on HDL-C in birds.

Meat quality

The different level of safflower seed supplemented diet showed significant effect on meat quality in broiler chickens (Table 4). This study elucidated that increasing levels of safflower seed in the supplemented diet does not pose significant effect (P>0.05) on pH, meat color and water holding capacity during the entire experimental period. The color of meat is an important parameter deciding the quality of meat and is one of the most influential parameter determining customer acceptability of meat and meat products (Adeyemi *et al.* 2014). The intense dark or light colors have been reported to be indicators of the poor meat quality (Benli *et al.* 2016). Myoglobin and pH affect meat color and meat pH is favorably connected to redness, but negatively to yellowness and lightness (Han *et al.* 2012). Decrease in water-holding capacity lead to a drop in surface light reflectance also. The WHC is the most important parameter of meat which decide its quality particularly its texture, palatability and cooking characteristics.

CONCLUSION

The supplementation of the diets with different levels of safflower seed (2.5, 5.0, 7.5 and 10.0%) resulted into increased growth performance of the broiler birds. The best

result in terms of enhanced growth performance, improved serum lipid profile and meat quality was obtained with supplementation of 5% safflower seed. The PUFAs and MUFAs of safflower add to the functional health benefits from the meat obtained from the broiler birds fed on diets supplemented with this oilseed.

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Conflict of interest: None.

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