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RIGHT CHOICE AND PROPORTION CAN MAKE BLEND OF EDIBLE OILS A GOOD GROWTH PROMOTER AND A POTENTIAL SOURCE FOR DESIGNER MEAT PRODUCTION IN CHICKEN

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In this study we investigated the comparative efficacy of blend of Olive oil, Black seed oil and Flax seed oil versus solitary use of these oils on growth performance and meat quality in broiler chicken. For this purpose chicks (n=210; strain-Ross) were offered following treatments in antibiotic free diet: A; Olive oil at rate of 1.0%, B; Black seed oil at rate of 0.5%, C; Flax seed oil at rate of 0.5%, and treatments D, E and F were prepared adding blend of oils (i.e. 50% Olive oil+25% Black seed oil+25% Flax seed oil) at rate of 0.5, 1.0 and 1.5%, respectively. The treatment G (control) was comprised of broiler feed with added antibiotic growth promoter. The treatments were offered from 1 to 35th day of broiler age. The poorest ($P<0.05$) body weight, feed intake and FCR were recorded in treatments A and B compared with all other treatments, while, rest of the treatments did not differ significantly for these parameters. No detectable differences for hematological parameters were observed except for WBCs and RBCs between the treatments. Serum cholesterol, triglycerides and low-density lipoproteins (LDL) were significantly higher, conversely high density lipoproteins (HDL) were the lowest ($P < 0.05$) in treatment G compared with all to other treatments. Treatments A and G had the lowest ($P<0.05$) intestinal (whole) weights compared with all other treatments. The concentration of oleic acid was highest in treatment F, linoleic acid in B and linolenic acid in C, however from the blend of oils F had reasonably good concentrations of these fatty acids. The profit was highest in treatment D (i.e. 0.5% of blend of oils) and lowest in treatment A. We concluded that addition of blend of 50% Olive oil+25% Black seed oil+25% Flax seed oil to broiler diet can give comparable growth to that of the diet supplemented with antibiotic growth promoters and it enriches the meat with reasonable amount of desirable fatty acids.
K**eywords:** Gut health, growth promoters, PUFA, meat quality, designer meat.

INTRODUCTION

The use of antibiotics in poultry and other livestock is a matter of growing concern around the globe. Whereas, the ever-rising demand for organic meat products increasingly stressing for antibiotic free meat production. So not far from now, this will eventually lead to ban on use of antibiotic growth promoters even in developing countries as observed in Europe, a decade ago. This is due to the fact that antibiotics make certain bacteria insensitive to some drugs while those which are less sensitive get eliminated, hence, make room for insensitive one to grow even better. So, every long or short application of low-dosage of antibiotics may give better adoption chance to insensitive bacteria that lead to increase resistance (Levy, 1998).

In developed world and even in some developing countries the meat supplied in the markets is generally free from antibiotic residues because a specific withdrawal period is followed after the administration of antibiotics. However, in most developing countries this practice is not common and antibiotic withdrawal period is often compromised in an effort to avoid anticipated risk of disease out breaks. So, the antibiotic use is continuing in poultry production and honestly speaking this is not without need, but the demand for elimination of antibiotic use will continue as developed world is now moving towards a relatively new concept of "No Antibiotic Ever (NAE)", that means no use of antibiotics during the whole production cycle. The NAE production, however, is largely affected by pathogenic load in certain areas where high infestation challenge

accomplishment of this goal. Nevertheless in future with the increase in intensive production in developing countries, the demand for compounds/products having antimicrobial properties will increase because the resistance bacteria due to mutation will grow, new bugs emerge, and overall pathogenic load will increase which require continuous use of sub-therapeutic level of antimicrobial agents. Thus, the need to explore alternatives i.e. safe options, as has been observed in the recent past, will grow. However, the focus will be on the solutions that are natural, commonly available, and do not add any extra cost to the feed.

Fats and oils are common feed constituents/ingredients that are essentially required for balance feed formulation in poultry since they function as a source of energy, help in decreasing dust of diet, and increase in digestion and absorption of fat-soluble vitamins and lipoproteins (Leeson and Atteh, 1995). Previously, the supplementation of oils in feed has shown contradictory results where some studies reported better growth with the addition of oils (Moura, 2003; Baiao and Lara, 2005; Ashraf *et al*., 2017), while other showed poor performance in terms of body weight gain, however, the studies with less encouraging growth results are predominant (Olomu and Aracos 1991; Zollitsch *et al*., 1997; Bou *et al*., 2005; Febel *et al*., 2008; Gonzalez-Esquerra and Leeson 2000; Ebeid *et al*., 2011). So the contradiction exists whether the oils should be used or not. Meanwhile a recent study in rats has shown an improvement in population of desirable gut microbiota by Olive oil supplementation (Isabel *et al*., 2018), hence there exists possibility that certain oils may be effective to enhance growth in chicken.

The aromatic plants are abundantly available in certain regions and historically being used as anthelminthic, nerve tonic, antispasmodic, antiseptic, appetizer, antiarthritic, and anti-parasitic purposes. There is wide range of candidate plants and amongst them *Nigella sativa* (Black seed), Olive and Flax seed are the best examples. *Nigella sativa* or Black seed is found in Mediterranean region (Rouhou *et al.,* 2007). It contains alkaloids, saponins, volatile oil, quinolone and sterols (Al-Homidan *et al.,* 2002; Ramadan, 2007). Whereas Olive contains monounsaturated fatty acid that inhibits lipid peroxidation and provides protection against the development of atherosclerosis (Gokce *et al.,* 2000). The Flax seed oil has been reported to improve meat quality by increasing level of omega-3 fatty acids and maintaining concentration of serum triglycerides and total cholesterol (Zelenka *et al.,* 2008; Ahmed *et al.,* 2013). Previously significant higher body weight gain was observed in chicken broilers which were fed on diets supplemented with Olive oil, Black seed oil, or Flax seed oil on solitary basis (Ashraf *et al*., 2017). From this we hypothesized that if these oils are mixed in certain ratios and added in the feed, even better growth promoting activity may be observed and better quality meat may be obtained. Thus, this study was planned to investigate comparative efficacy of blend of Olive oil, Black seed oil and Flax seed oil versus solitary use of these oils on growth performance, gut function and meat quality in chicken broilers.

MATERIALS AND METHODS

Day-old chicken Broiler chicks (n=210, strain; Ross) were housed in an open sided house containing floor pens of 3.5 x 3.5 sq. ft each. Rice husk was used as litter material and natural environmental conditions were provided. Daily light was provided with 23 hours light/01hour dark cycle. Feed and water were provided at *ad-libitum*. Is-ocaloric and iso-nitrogenous starter (ME=2800 kcal/kg & CP=19.2%) and finisher (ME=2925 kcal/kg & CP=18%) diets were offered to all treatments (Table 1). The experiment lasted from 1 to 35th day of broiler age.

Table 1. Chemical composition of broiler starter and finisher rations fed to the broilers as a basal feed and to prepare different diets.

Nutrient composition	Starter ration	Finisher ration
CP (%)	19.25	18.00
ME kcal/kg	2800	2925
CF (%)	5.50	5.50
Ash (%)	6.90	6.25
Phosphorus (%)	0.44	0.42
Ca (%)	1.05	1.00
Lysine (%)	1.15	1.05
Methionine (%)	0.52	0.50

Seven treatments were applied and each treatment was administered in three replicates of 10 chicks each as followed: treatment A; basal diet plus added Olive oil at rate of 1.0%, B; basal diet plus added Black seed oil at rate of 0.5%, C; basal diet plus added Flax seed oil at rate of 0.5%, and treatments D, E and F were offered blend of "50 % Olive oil+25% Black seed oil+25% Flax seed oil" at rate of 0.5 %, 1.0%, and 1.5% respectively in basal diet. Treatment G served as control comprising of commercial broiler feed with added antibiotic growth promoter. Data on weekly body weight gain and feed consumption were recorded to calculate feed conversion ratio. For collection of slaughter data two broilers from each replicate were randomly weighed and killed by decapitating. For hematological analysis, collected blood samples were immediately poured in vacutainers containing anticoagulant and analyzed using hematology analyzer (Elite-3, Erba Mennheim, Germany). Following hematology parameters were recorded; total white blood cells (WBCs), total red blood cells (RBCs), granulocytes count (GRA), lymphocytes count (LYM), hemoglobin (HGB), monocytes count (MID), platelets distribution width (PDW), red cells distribution width (RDW), mean corpuscular hemoglobin concentration

(MCHC), mean platelet volume (MPV), hematocrit (HCT), lymphocytes (LYS), platelet large cell ratio (P-LCR), total platelet count (PLT), platelets larger than 12 fl and smaller than 30fl (P-LCC), mean corpuscular volume (MCV), granulocytes percentage (GRA%) and monocytes percentage (MID%). Serum triglycerides, cholesterol, HDL and LDL were determined by enzymatic calorimetric method using automated random access clinical chemistry analyzers Erba XL-180 (Erba Mannheim, Germany). The regents used for enzymatic reactions i.e. triglycerides-LS, cholesterol-LS, HDL-cholesterol and LDL- cholesterol were from BioMed Diagnostics, Germany. Weight of whole intestine and different sections of intestine were recorded and the pH of duodenum, jejunum and ileum were determined by the method described by Al-Natour and Alshawabkeh (2005). To measure the fatty acid concentration, the fats were isolated using standard method (i.e. AOAC, 1990). The fatty acids were then converted into fatty methyl esters using method described by Chin *et al.* (1992) and Gas Chromatography (GC) procedure was used to estimate the concentration of oleic acid, linoleic acid and linolenic acid as per method described by Robert and Barry (2004).

***Statistical analysis*:** The data were analyzed using General Linear Model (GLM) procedure of SPSS 18.0. The treatment means were compared by Least Significant Difference (LSD) test (Montgomery and Runger, 2010). All significant values are presented at ($P<0.05$), unless specified otherwise.

RESULTS

Body weight, feed intake and feed conversion ratio were significantly poor in treatments A and B compared with all other treatments, while non-significant differences were observed between remaining treatments (Table 2). Highest body weight (1966±17.2) and best FCR (1.62±0.01) were observed in treatment D, conversely the lowest values were observed in treatment A.

The intestinal weight (whole) was significantly lower in A (164±6) and G (166±6) compared with treatments B, C, D, E and F. The intestinal weight did not differ significantly between B, C, D, E and F treatments. Moreover weight of different intestinal sections i.e. duodenum, jejunum, ilium and large intestine as well as the pH of intestinal parts did not differ significantly between the treatments (Table 3).

Oleic Acid concentration was significantly higher in treatments A (0.16±0.03) and F (0.22±0.1) compared with all other treatments while the concentration did not differ

Table 2. Body weight, feed intake and feed conversion ratio of broilers fed on different diets from 1 to 35 days.

Treatments	Weight gain (g)	Feed intake (g)	FCR
A	1750±6.4^{b}	3163±9.6^{b}	1.81±0.01^{b}
B	1791±8.5^{b}	3157±1.9^{b}	1.76±0.02^{b}
C	1893±8.2^{a}	3193±7.7^{a}	1.69±0.06^{a}
D	1966±7.2^{a}	3193±6.5^{a}	1.62±0.01^{a}
E	1902±4.2^{a}	3192±3.5^{a}	1.68±0.04^{a}
F	1953±4.4^{a}	3198±1.3^{a}	1.64±0.01^{a}
G	1910±8.8^{a}	3198±5.9^{a}	1.67±0.01^{a}

$^{a-b}$Means without common superscripts in a column varysignificantly ($P<0.05$). *Blend composition = 50% Olive oil + 25% Black seed oil + 25% Flax seed oil. A; 1.0% Olive oil, B; 0.5% Black seed oil, C; 0.5% Flaxseed oil, D; 0.5% blend*, E; 1.0% blend*, F; 1.5% blend*, and G (control).

Table 4. Concentration of polyunsaturated fatty acid (i.e. Oleic acid, Linoleic acid, Linolenic acid) in meat of broilers fed on different diets from 1 to 35 days.

Treatment	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
A	0.16±0.03^{a}	0.09±0.01^{b}	0.00±0.00^{c}
B	0.07±0.02^{b}	0.22±0.06^{a}	0.07±0.00^{bc}
C	0.04±0.01^{b}	0.16±0.02^{a}	0.15±0.03^{a}
D	0.06±0.01^{b}	0.05±0.01^{bc}	0.07±0.01^{b}
E	0.08±0.01^{b}	0.13±0.04^{a}	0.08±0.01^{b}
F	0.22±0.10^{a}	0.16±0.03^{a}	0.09±0.01^{b}
G	0.01±0.00^{b}	0.01±0.01^{c}	0.003±0.0^{c}

$^{a-c}$Means without common superscripts in a column vary significantly ($P<0.05$). *Blend composition = 50% Olive oil + 25%

Table 3. Weights of whole intestine and its parts i.e. Duodenum, Jejunum, Ilium & large intestine, and pH of intestine of broilers fed on different diets from 1 to 35 days.

Treatment	Duodenum	Jejunum	Ilium	Large Intestine	Whole intestine Weight	pH of Intestine
A	25±1.4^{a}	69±0.8^{a}	58±2.1^{a}	12±0.6^{a}	164±6^{a}	6.10±0.03^{a}
B	29±0.7^{a}	74±0.9^{a}	67±4.6^{a}	11±0.6^{a}	181±10^{b}	6.05±0.03^{a}
C	27±1.0^{a}	71±1.0^{a}	66±1.0^{a}	10±0.8^{a}	174±6^{b}	6.08±0.03^{a}
D	28±1.3^{a}	73±4.6^{a}	71±10^{a}	10±0.6^{a}	182±6^{b}	6.05±0.03^{a}
E	27±0.5^{a}	71±4.2^{a}	70±3.2^{a}	11±0.5^{a}	179±5^{b}	6.05±0.03^{a}
F	26±1.0^{a}	75±5.1^{a}	67±3.7^{a}	10±0.1^{a}	178±8^{b}	6.05±0.03^{a}
G	26±0.7^{a}	69±4.3^{a}	61±3.9^{a}	10±0.9^{a}	166±6^{ac}	6.03±0.03^{a}

$^{a-c}$Means without common superscripts in a column vary significantly ($P<0.05$). *Blend composition = 50% Olive oil + 25% Black seed oil + 25% Flax seed oil. A; 1.0% Olive oil, B; 0.5% Black seed oil, C; 0.5% Flaxseed oil, D; 0.5% blend*, E; 1.0% blend*, F; 1.5% blend*, and G (control)

Black seed oil + 25% Flax seed oil. A; 1.0% Olive oil, B; 0.5% Black seed oil, C; 0.5% Flaxseed oil, D; 0.5% blend*, E; 1.0% blend*, F; 1.5% blend*, and G (control).

significantly between treatments B, C, D, E and G. The treatment G had the lowest (0.01±0.0) value for Oleic Acid concentration. The concentration of linoleic Acid was significantly lower in treatments A, D and G compared with all other treatments. The linoleic Acid concentration was highest in treatment B (0.22±0.06) and the lowest in treatment G (0.01±0.01).

The concentration of linolenic Acid was significantly higher in treatment C (0.15±0.03) compared with all the treatments while lowest values were observed in treatments A (0.003±0.00) and G (0.003±0.00) (Table 4). The treatments D, E and F also had significantly higher linolenic acid concentration compared with treatments A and G.

The WBCs were significantly higher in treatments D (63.3) compared with A, C, E, F and G. In addition to that treatments C and F had significantly higher WBCs compared with treatments A, E and G. The highest RBCs were observed in treatment E (2.70) while the lowest in G (2.20). Except for treatments B and D, all other treatments differ significantly for RBC count. Nevertheless for all other hematology parameters non-significant differences were

Table 5. Complete blood count of broilers fed on different diets from 1 to 35 days.

Parameters	Diets							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**SEM**
WBC	50.0^{a}	61.1^{cd}	57.3^{bc}	63.3^{d}	52.0^{a}	56.4^{ab}	49.1^{a}	1.09
RBC	2.30^{b}	2.40^{c}	2.50^{d}	2.40^{c}	2.70^{f}	2.60^{e}	2.20^{a}	0.06
GRA	7.90^{a}	7.80^{a}	6.50^{a}	8.60^{a}	8.10^{a}	7.10^{a}	7.60^{a}	0.09
LYM	38.9^{a}	40.0^{b}	38.1^{a}	38.2^{b}	38.2^{a}	37.1^{a}	39.1^{a}	1.04
HGB	11.9^{a}	13.8^{a}	12.5^{a}	12.7^{a}	11.5^{a}	12.2^{a}	13.2^{a}	0.08
PDW	7.90^{a}	7.80^{a}	6.80^{a}	7.20^{a}	6.90^{a}	8.10^{a}	6.70^{a}	0.07
RDW	84.2^{a}	86.1^{a}	82.1^{a}	83.9^{a}	80.2^{a}	83.0^{a}	84.3^{a}	1.17
MID	17.2^{a}	18.4^{a}	15.8^{a}	15.6^{a}	16.0^{a}	17.0^{a}	19.1^{a}	0.09
MCHC	45.1^{a}	47.7^{a}	45.9^{a}	47.2^{a}	45.2^{a}	46.1^{a}	47.5^{a}	1.09
MPV	8.70^{a}	9.80^{a}	7.50^{a}	8.50^{a}	9.70^{a}	8.80^{a}	8.01^{a}	0.07
HCT	33.5^{a}	36.0^{a}	34.0^{a}	31.9^{a}	32.1^{a}	32.3^{a}	35.2^{a}	0.08
LYS	0.93^{a}	0.94^{a}	0.93^{a}	0.95^{a}	0.97^{a}	0.97^{a}	0.96^{a}	0.01
P-LCR	16.2^{a}	15.0^{a}	17.9^{a}	17.5^{a}	15.2^{a}	18.1^{a}	15.9^{a}	0.07
PLT	18.3^{a}	23.5^{a}	19.1^{a}	19.5^{a}	22.0^{a}	23.0^{a}	20.1^{a}	0.09
P-LCC	3.50^{a}	4.10^{a}	3.50^{a}	3.50^{a}	4.08^{a}	3.60^{a}	3.90^{a}	0.05
MCV	169.5^{a}	164.0^{a}	166.0^{a}	159.0^{a}	161.5^{a}	164.2^{a}	160.1^{a}	1.19
GRA%	17.4^{a}	16.7^{a}	21.7^{a}	21.9^{a}	19.2^{a}	18.4^{a}	20.1^{a}	0.09
MID%	33.5^{a}	33.9^{a}	32.8^{a}	31.05^{a}	34.1^{a}	31.3^{a}	32.2^{a}	0.09

$^{a-f}$Means without common superscripts in a row vary significantly (P<0.05). *Blend composition = 50% Olive oil + 25% Black seed oil + 25% Flax seed oil. A; 1.0% Olive oil, B; 0.5% Black seed oil, C; 0.5% Flaxseed oil, D; 0.5% blend*, E; 1.0% blend*, F; 1.5% blend*, and G (control). WBC=total white blood cells, RBC=Total red blood cells, GRA=Granulocytes count, LYM=Lymphocytes count, HGB=Hemoglobin, MID=Monocytes Count, PDW=Platelets distribution width, RDW=Red cells distribution width, MCHC=Mean corpuscular hemoglobin concentration, MPV=Mean platelet volume, HCT=Hematocrit, LYS=Lymphocytes, P-LCR=Platelet large cell ratio, PLT=Total Platelet Count, P-LCC= Platelets larger than 12 fl and smaller than 30fl, MCV=Mean Corpuscular Volume, GRA%=Granulocytes Percentage, MID%=Monocytes Percentage.

Table 6. Concentration of serum cholesterol, triglycerides, high density lipoprotein and low-density lipoprotein of broilers fed on different diets from 1 to 35 days.

	Diets							
Parameter (mg/dl)	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**SEM**
Cholesterol	130.9^{a}	132.5^{a}	138.7^{a}	134.0^{a}	138.8^{a}	140.9^{a}	168.1^{b}	1.14
Triglycerides	45.6^{a}	55.1^{a}	52.0^{a}	41.9^{a}	47.2^{a}	39.8^{a}	78.3^{b}	1.09
High Density lipoprotein	43.3^{a}	49.0^{a}	49.7^{a}	52.8^{a}	56.7^{a}	52.3^{a}	32.1^{b}	1.01
Low density lipoprotein	89.8^{a}	93.1^{a}	102.2^{a}	95.6^{a}	105.5^{a}	88.2^{a}	141.3^{b}	1.12

$^{a-b}$Means without common superscripts in a row vary significantly (P<0.05). A; 1.0% Olive oil, B; 0.5% Black seed oil, C; 0.5% Flaxseed oil, D; 0.5% blend*, E; 1.0% blend*, F; 1.5% blend*, and G (control). *Blend composition = 50% Olive oil + 25% Black seed oil + 25% Flax seed oil.

observed between the treatments (Table 5).

The values for serum cholesterol, triglycerides, and LDL were significantly higher for treatment G compared with all other treatments and vice versa for high density lipoproteins (i.e. HDL) (Table 6). The C (0.17$/kg meat), D (0.22$/kg meat) and G (0.22$/kg meat) treatments fetch significant higher profit compared with all other treatments while treatment A (-0.07$/kg meat) had the poorest economic returns (Fig. 1).

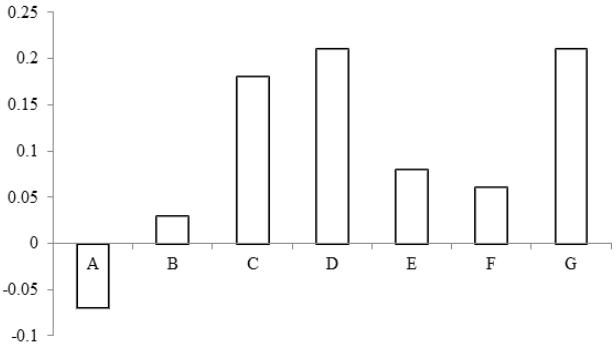

Figure 1. Profit margins (US $) against different diets from 1 to 35 days. *Blend composition = 50% Olive oil + 25% Black seed oil + 25% Flax seed oil. A; 1.0% Olive oil, B; 0.5% Black seed oil, C; 0.5% Flax seed oil, D; 0.5% blend* of oil, E; 1.0% blend* of oil, F; 1.5% blend* of oil, G; control

DISCUSSION

Supplementation of blend of oils improved body weight gain, feed intake and FCR whereas when the oils were used on solitary basis, the growth rate was poorer compared with the control. Solitary use of oils increased cost of production hence the profit margins got reduced. This suggests that with the current cost/per unit price of each of these oils, they should not be supplemented on solitary basis as energy source/growth promoter in broiler diet. Nevertheless, the blend of these oils may be used as good source of energy and as well as a good replacement of antibiotic growth promoters. The increase in concentration of poly unsaturated fatty acids (PUFA) in meat; however, indicated that meat enrichment with desirable PUFA is possible with supplementation of these oils and in a situation where customers are willing to pay more for extra benefits i.e. meat enriched with PUFA, the blend of oils is a good option to accomplish that.

The broilers supplemented with Olive oil and Black seed oil showed the lowest body weight. In addition to that concentration of oleic acid and linoleic acid was highest in Olive oil and Black seed oil supplemented groups respectively. The lower body weight gain in Olive oil and Black seed oil supplemented groups might be due to the higher concentration of unsaturated fatty acids. This is evident from previous studies that diets enriched with high percentage;.

e of unsaturated fatty acids (i.e. Black seed & Olive oil) are associated with a lower stimulatory effect on weight gain and hepatic lipid accumulation compared the diets having higher percentage of saturated fatty acids. It is now well known that the intestinal health largely depends on the diet, where certain type of favorable and non-favorable gut microbiota influences the growth. The fats influence the pattern of gut microflora which control body weight gain by affecting the body energy balance (Shen *et al*., 2013; Fava *et al*., 2013; Le Roy *et al*., 2013). A recent study has demonstrated that the supplementation of virgin Olive oil was linked to colonization of specific microbiota that triggered some unknown physiological parameters and reduced obesity in rats (Isabel *et al*., 2018). So, the lower body weight gain in Olive or Black seed oil supplemented group in this study is not that surprising. However, previous studies have shown better growth results by addition of oils to the feed. The possible explanation for contradictory results might be the presence of different microbiota and their inability to trigger the specific "unknown physiological parameters" that were linked to reduce obesity. Nevertheless, further studies are required to quantify the microflora that is associated with production of good quality meat in chicken and to investigate the physiological parameters that are triggered with supplementation of diets enriched in PUFA's such as Olive and Black seed oil.

The Flax seed oil produced comparatively better results in terms of body weight gain than that of the Olive and Black seed oil, however, compared with the control the outcome was not encouraging. Contradictory results on body weight gain have previously been reported with the supplementation of Flax seed oil in chicken (Lopez-Ferrer *et al*., 1999; El-Sayed *et al*., 2013; Al-Anbari *et al*., 2013; Starcevic *et al*., 2014; Goudarzi *et al*., 2015). However, the studies with non-significant gain in body weight are more prevalent. The possible reason for poor body weight gain could be the processing damage or the rancidity issue that are associated with the Flax seed oil. During processing, most Flax seeds are damage by heat or friction or cold-press. While, within few minutes after processing the oil begins to go rancid and during feed mixing/processing/conditioning more damage and rancidity is caused that effects digestion negatively. The non-significant difference in body weight gain, feed intake and FCR between control and Flax seed oil supplemented group reflects that the Flax seed oil we used in this study was of good quality as it did not affect feed consumption and digestion. In a recent study Flax seed oil was found to cause no impact on growth of broilers; however, its effect on fatty acid deposition in breast and thigh meat was observed with the increase expression of genes i.e. $FADS_2$. However, the expression of $FADS_2$ was correlated with the duration of Flax seed oil administration and it was recommended that if

Flax seed oil is use to improve fatty acid profile of the meat it should be administered for short period of around two weeks before slaughtering (Mirshekar *et al*., 2015). So, for weight gain, if Flax seed oil need to be supplemented in broiler diet it may be started early, while if the aim is to improve meat quality it may be supplemented two weeks prior to marketing age of broilers.

When the blends of oils were used the results for body weight gain, feed intake and FCR were comparable to the control diet. The control diet was supplemented with commercially available antibiotic growth promoter. The combination or blend of oils did similar action in terms of growth as that provided by antibiotic growth promoters in the control diet and in addition to that they increase the concentration of polyunsaturated fatty acids in the meat. This dictates that instead of using oil from single source a blend of oil from different sources may be beneficial. However, there is need to explore other combinations of oils that can give even better growth and may be more cost effective. In addition to that it is also required to investigate the population of gut microflora that may be associated with good health and growth in broilers and get benefitted by the supplementation of blend of different oils.

***Conclusion*:** Supplementation of Blend of oils in broiler feed has more beneficial effect on growth and meat quality compared with oil from single source. The 0.5% blend of 50% Olive oil+25% Black seed oil+25% Flax seed oil gives comparable results to antibiotic growth promoter supplemented feed. it also enriches the meat with reasonable amount of Oleic acid, linoleic acid and linolenic acid, and also increases HDL contents of meat.

REFERENCES

Al-Anbari, A.A. Abbas, F.R. Al-Samarai, J.S. Al- Shamire and F.H. Al-Zaidi. 2013. Effect of using cumin oil (*Cuminum syminum*) as feed additives on profile analysis and growth curves of broiler. G.J.B.B. 2:326-330.

Ahmed, A.S., S.M. El-Bahr and A.A. Al-Azraqi. 2013. Effect of canola and olive oils on productive, immunological and some biochemical parameters of broiler chickens fed iso-caloric and high caloric diets. Int. J. Poult. Sci. 12:726-734.

Al-Homidan, A., A.A. Al-Qurawi, S.A. Al-Waily and S.E.I. Adam. 2002. Response of broiler chicks to dietary *Rhazya satricata* and *Nigella sativa*. Br. Poult. Sci. 43:291-296.

Al-Natour, M.Q. and K.M. Alshawabkeh. 2005. Using varying levels of formic acid to limit growth of *Salmonella gallinarum* in contaminated broiler feed. Asian-Aust. J. Anim. Sci. 18:390-395.

AOAC. 1990. Official Method of Analysis, 16th Ed. Association of Official Analytical Chemists, Washington, DC.

Ashraf, M., A. Haq, F. Ahmad and Z. Sindhu. 2017. Effect of supplementing Olive, Black seed and Flaxseed Oils on growth performance and serum biochemistry of broiler and economics of its production. Int. J. Agric. Biol. 19:951-956.

Baiao, N.C. and L.J.C. Lara. 2005. Oil and fat in broiler nutrition. Rev. Bras. Cienc. Avic. 7:129-141.

Bou, R., F. Guardiola, A.C. Barroeta and R. Codony. 2005. Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. Poult. Sci. 84:1129-1140.

Chin, S.F., W. Liu, J.M. Storkson, Y.L. Ha and M.W. Pariza. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Compos. Anal. 5:185-197.

Ebeid, T., A. Fayoud, S. Abou-el-Soud, Y. Eid and M. Al-habbak. 2011. The effect of omega-3 enriched meat production on lipid peroxidation, antioxidative status, immune response and tibia bone characteristics in Japanese quail. Czech. J. Anim. Sci. 56:314-324.

El-Sayed, M.R., I. Doaa and B. M. El-Sayed. 2013. Effect of supplementation of broiler diets with Guava leaves and/or olive oil on growth, meat composition, blood metabolites and immune response. B.V.M.J. 25:23-32.

Fava, F., R. Gitau, B.A. Griffin, G.R. Gibson, K.M. Tuohy and J.A. Lovegrove. 2013. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. Int. J. Obes. 37:216-23.

Febel, H., M. Mezes, T. Palfy, A. Herman, J. Gundel, A. Lugasi, K. Balogh, I. Kocsis and A. Blazovics. 2008. Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. J. Anim. Nutr. 92:369-376.

Gokce, R., I. Akhus, M. Yontem, A.Y.A. Gurel, O. Caglayan, S. Bodur and S. Ergun. 2000. Effects of dietary oils on lipoproteisn, lipid peroxidation and thromboxame A_2 production in chicks. Turk. J. Vet. Anim. Sci. 24:473-478.

Gonzalez-Esquerra, R. and S. Leeson. 2000. Effects of menhaden oil and flaxseed in broiler diets on sensory quality and lipid composition of poultry meat. Br. Poult. Sci. 41:481-488.

Goudarzi, P., A. Nikkah, P. Shawarang and M. Chamani. 2015. Effect of different sources of lipids on performance and immune response in broiler chicken. Int. J. Biocsi. 6:179-189.

Isable, P., M. Hidalgo, A.B. Segarra, A.M. Martinez-Rodriguez, A. Cobo, M. Ramirez, H. Abriouel, A. Galvez and M. Martinez-Canamero. 2018. Influence of a diet enriched with virgin olive oil or butter on mouse

gut microbiota and its correlation to physiological and biochemical parameters related to metabolic syndrome. PLoS ONE 13:e0190368.

Le Roy, T., M. Llopis, P. Lepage, A. Bruneau, S. Rabot and C. Bevilacqua. 2013. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut. 62:1787-94.

Leeson, S. and J.O. Atteh. 1995. Utilization of fats and fatty acids by turkey poults. Poult. Sci. 74:2003-10.

Levy, S.B. 1998. The challenges of antibiotic resistance. Sci. Am. 278:46-53.

Lopez-Ferrer, S., M.D. Baucells, A.C. Barroeta and M.A. Grashorn. 1999. n-3 enrichment of chicken meat using oil: Alternative substitution with rapeseed and linseed oils. Poult. Sci. 78:356-365.

Mirshekar, R., F. Boldaji, B. Dastar, A. Yamchi and S. Pashaei. 2015. Longer consumption of flaxseed oil enhances n-3 fatty acid content of chicken meat and expression of $FADS_2$ gene. Eur. J. Lipid. Sci. Technol. 117:810-819.

Montgomery, D.C. and G.C. Runger. 2010. Applied Statistics and Probability for Engineers, 5th Ed. Jhon Wiley and Sons. Inc; USA.

Moura, B.H.S. 2003. Performance and carcass composition of broiler cutting pad with different energy levels with and without oil [dissertation]. Belo Horizonate: Vet. Sch., Fed. Uni. Min. Gera., Brazil.

Olomu, J.M. and V.E.B. Aracos. 1991. Influence of dietary flaxseed oil on the performance, muscle protein deposition, and fatty acid composition of broiler chicks. Poult. Sci.70:1403-1411.

Ramadan, M.F. 2007. Nutritional value, functional properties and nutracetuical application of black cumin (*Nigella sativa* L.). Int. J. Food. Sci. Tech. 42:1208-1218.

Robert, G.L. and E.F. Barry. 2004. Modern Practice of Gas Chromatography, 4th Ed. John Wiley & Sons, USA.

Rouhou, S.C., S. Besbes, B. Hentati, C. Blecker, C. Deroanne and H. Attia. 2007. *Nigella sativa* L. chemical composition and physicochemical characteristics of lipid fraction. Food Chem. 101:673-681.

Shen, J., M.S. Obin and L. Zhao. 2013. The gut microbiota, obesity and insulin resistance. Mol Aspects Med. 34:39-58.

Starcevic, K., T. Masek, D. Brozic, N. Filipovic and Z. Stojevic. 2014. Growth performance, serum lipids and fatty acid profile of different tissues in chicken broiler fed a diet supplemented with linseed oil during a prolonged fattening period. Vet. Arhin. 84:75-84.

Zelenka, J., D. Schneiderova, E. Mrkvicoa and P. Dolezal. 2008. The effect of dietary linseed oil with different fatty acids pattern on the content of fatty acids in chicken meat. Vet. Med. 53:77-85.

Zollistch, W., W. Knaus, F. Aichnger and F. Lettner. 1997. Effects of different dietary fat sources on performance and carcass characteristics of broilers. Anim. Feed. Sci. Technol. 66:63-73.