Indian Journal of Comparative Animal Physiology
Vol.25(2). (2007) p.12-18

EFFECT OF DIETARY OIL ON THE HEALTH OF THE GROWING BIRD, *GALLUS DOMESTICUS*

PUJARI S and R ROY *

Department of Zoology, Govt. College Sanquelim, Goa* Department of Zoology, Goa University, Goa

ABSTRACT

The seven days old chicks *Gallus domesticus* (Vencobb broilers) were fed with a diet supplemented with 5% dose of coconut oil, sunflower oil and fish oil for period of 30 days. The coconut oil contains around 26% of monoenoic acid and 61% saturated fatty acid. Sunflower oil contains 58% of linoleic acid (omega 6 fatty acid), while the fish oil contains about 20% monoenoic fatty acids, 10% linoleic acid and 35% of long chain polyunsaturated fatty (omega 3 fatty acids). Dietary supplementation of these oils greatly influenced the activities of liver function and cardiac function enzymes as well as tissue fatty acid profiles of the chicks. Supplementation of sunflower oil and fish oil enhanced the level of omega 6 (linoleic, arachidonic acids) fatty acids respectively in liver and serum. A reduction in the serum LDL cholesterol and increase in HDL cholesterol was noticed in the chicks fed with a diet supplemented with fish oil and sunflower oil, best results were obtained with the supplementation of fish oil. The cardiac and liver function test confirmed the well being of the chick supplemented with fish oil.

Key words: chicks, health, linolenic acid, dietary oils, PUFA

INTRODUCTION

Both linoleic (9,12, Octadecaenoic acid) and linolenic (9,12,15 Octadecatrienoic acid) acids cannot be synthesized de novo by animals[1] but are very essential for animals to be in physiologically well being state. These two fatty acids undergo further elongation and desaturation to produce various PUFAs of both ω3 and ω6 series. PUFA further metabolize to produce large amounts of prostaglandins and thromboxanes of diene and triene series, which are the key regulatory factors to maintain the animals in well being state[2].

Klinger[3] suggested that dietary lipid effect several hematological factors of culture channel cat fish. Fish fed with fish oil diet had significantly lower hematocrits, higher thrombocyte count and higher serum iron concentration. Dietary lipid affects the fatty acid composition of blood leucocytes and plasma eicosanoid concentration in European Sea Bass[4].

1% conjugated linoleic acid supplemented diet significantly increase the body mass gain along with the increased ratio of HDL – Cholesterol and total cholesterol ratio in rat[5]. Lopez[6] reported that high fish oil concentration decreases the saturated and monoenoic fatty acid content in the thymus sample. Production of platelet thromboxane A2 and aortic prostacyclin decreased in rat with higher intake of n3 fatty acid[7]. Castillo[8] showed that fish oil produced a significant reversion of the hyper cholesterolemia previously induced by coconut oil feeding. Fish oil also produces a clear decrease in plasma triacylglycerine level. PUFA reduces the incident of NEC (nectorzing enterocolitis) by modulating PAF (platelet activating factor) metabolism and endotoxin trans location. Dietary administration of γ- linolenic acid increased in vitro production of prostaglandin E1 derived from dehomo γ- linolenic acid but did not significantly influenced the production of prostaglandin – E2 derived from Arachidonic acid in rat[9].

There is obsolute dearth of knowledge about what is the exact quantity of the linoleic and linolenic acid should be there in the diet in order to maintin animal in a well being condition. Although the poultry science in India and other countries is well established with regard to improvement of the meat and production of eggs through dietary manipulation, feed formulation of the poultry has not been aimed to improve the health of the consumer (human beings) as well as poultry bird itself. Hence, the present study was undertaken to enrich poultry meat with specified PUFA; so that the changes in the types of PUFA in the tissues of bird may

offer potential benefits to the chicks by modulating eicosanoid production which would help them to be in a **"well being state"** and so also the human beings.

In the present research work, the emphases were laid on the quality of lipid in a diet and efforts were taken to see the effect of dietary lipids on health status of the poultry bird during post hatching development.

MATERIALS AND METHODS

After obtaining the approval of animal ethics committee of Goa University, the day old broiler chicks (Vencobb broiler), *Gallus domesticus* were obtained from a local hatchery (Mandovi Hatcheries, Goa, India). The chicks were acclimatized to laboratory conditions before feeding experimental diet. Based on the fatty acid composition, three commercial oils viz., coconut oil, sunflower oil and fish oil were selected for the study (Table 1). The proximate composition of commercial feed and experimental feed as well as their fatty acid composition were analyzed in the laboratory (Table 2 & 3). The seven days old chicks were divided into four groups. Group I chicks were maintained with the commercial feed (which served as control), remaining groups were fed with a diet containing 5% of coconut oil, sunflower oil and fish oil along with the commercial feed for a period of 30 days. The coconut oil and sunflower oil were procured from the local market. The fish (Sardine) oil was obtained from M/s. Sigma Chemical Co. USA. The chicks were sacrificed after 30 days (when they were grown to 38 day old) of feeding. Since our aim was to find out the effect of the supplemented lipid sources on the health status of the birds, the chicks were fed with grower feed only throughout the experiment.

Total erythrocytes and leucocytes were counted using Neubauer chamber. The blood hemoglobin was estimated by using Sahli's hemoglobinometer. The fatty acid profiles of liver and serum analysed using Gas Chromatogram (Chemito make model 8610) equipped with flame ionizing detector and 10% DEG (Di ethylene glycol) packed column. The identification of the obtained peeks was done with the prepared standard chromatogram of the known fatty acids.

Serum lipid profiles including total cholesterol, total triglycerol, HDL cholesterol, LDL-cholesterol, VLDL – cholesterol were also recorded using the diagnostic kits M/s. Crest Biosystems, Goa India). The liver function test and the cardiac function test were also performed by measuring the activity of serum and hepatic Alkaline Phosphatase [EC 3.1.3.1], Lactate Dehydrogenase [EC 1.1.1.27], Glutamate Oxaloacetate Transaminase [EC2.6.1.1], Glutamate Pyruvate Transaminase [EC 2.6.1.2].[10]

RESULTS AND DISCUSSION

PUFA in the cell is required for the production of chemical messengers that initiate or control wide range of physiological functions including cell growth and divisions, control of blood pressure, coagulation of blood, immunosensitive reaction, tissue inflammation etc.[11] PUFA reduces the incidence of narcotizing enterocolitis by modulating platelet activating factor and endotoxin translocation[12]. No statistically significant changes were observed in the level of hemoglobin concentration after 30 days **of feeding the diet supplemented with coconut oil and sunflower oil (Figure 1). It was also observed that there was a significant decrease in the count of total erythrocytes of the chicks supplemented with coconut oil (62%, $p<0.005$)** and sunflower oil (70%, $p<0.005$) along with the commercial feed. The leucocyte count was decreased significantly (40%, $p<0.005$) in the chicks supplemented with coconut oil, while the changes are insignificant in the chicks supplemented with sunflower oil for 30 days. This indicates an anaemic condition and defective immunoprotective mechanisms in the chicks. The 26%, ($p<0.005$) increase in leucocyte count with fish oil supplementation without altering hemoglobin concentration and total erythrocyte count (Figure I) confirms earlier observation of Klinger.[3] Enhanced leukocyte count in the blood may be correlated with increased immunoprotective condition with the supplementation of fish oil. The role of dietary fatty acids mainly ω6 and ω3 individually or jointly plays a significant role in the action of different antigen to produce antibodies, which ultimately affect the immuno response mechanism of a growing layer hen.[13]

Table 1: Relative composition of fatty acid profiles of different lipid sources used inthe experiment (Mean values of three estimates)

Fatty Acid	Relative Composition		
	Coconut oil	Sunflower oil	Fish oil
14:0	14.78	2.51	7.23
16:0	28.32	15.23	16.55
16:1	16.27	2.61	6.32
18:0	18.37	8.26	17.38
18:1	10.27	6.26	14.25
18:2	8.35	58.38	9.07
18:3	------	1.08	2.56
Others	3.64$	5.67$	26.64*

***- n3 and n6 polyunsaturated fatty acids of C-20 C-22 series**
$ - Unidentified fatty acids of C-16 and C-14 series

Table 2: Proximate composition of feeds used in the experiment (Mean values of three estimates)

Parameters	Dry matter	Crude fat	Ash	Fiber	% crude protein
Control feed (commercial)	92.09	6.50	11.48	2.83	36.28
Feed + 5% coconut oil	92.91	9.50	9.54	3.18	35.70
Feed + 5% sunflower oil	93.25	10.60	10.48	2.54	36.53
Feed + 5% fish oil	93.70	10.50	10.13	2.68	35.75

As far as human nutrition is concerned, not the dietary cholesterol, but the intake of fat in terms of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids is to be looked forward. Both dietary cholesterol[1-4] and fatty acid pattern with regards to total n3/n6 ration PUFA. the dietary lipid fractions are in a close relationship to serious chronic diseases in humans. [2]

About 10% - 30% increase in the relative concentration of saturated fatty acid and arachidonic acid along with 30% - 55% decrease in monounsaturated fatty acid and long chain PUFA in liver and serum of coconut oil supplemented chicks; or 10% - 33% increase in linoleic acid and arachidonic acid content of live and serum at the expense of saturated, monounsaturated and ω3 fatty acids of sunflower oil supplemented chicks or the tremendous increase (91% to almost 5 fold) in ω3 fatty acids viz., linolenic, eicosapentaenoic

and docosahexaenoic acids at the cost of linoleic acid and arachidonic acid along with the saturated and monounsaturated fatty acids in liver and serum of fish oil supplemented birds (as summarized in tables 4 & 5), are in accordance with the earlier observation of Byong[15]; Al Athari and Watkins[16]; Phetteplace and Watkins[17]; Hargis[18]; Cherian and Sim[19]; Manilla[20]; Mieczowska[21]; Schiavone[22]. Shift in saturated fatty acids and monounsaturated fatty acids towards the production of PUFAs of ω3 series and / or

Table 3: Relative composition of fatty acid profiles of feed supplemented with different lipid sources used in the experiment (Mean values of three estimates).

Fatty Acid	Relative Composition			
	Control feed (commercial)	Feed + 5% Coconut oil	Feed + 5% Sunflower oil	Feed + 5% Fish oil
14:0	7.50	10.87	5.00	7.26
16:0	16.00	22.36	15.50	15.26
16:1	2.30	14.37	2.20	4.32
18:0	8.30	12.32	8.00	6.36
18:1	2.50	8.37	4.74	10.26
18:2	55.50	35.37	56.78	22.36
18:3	0.50	---	0.98	1.86
Others	7.40$	5.24	6.80	32.32*

*- n3 and n6 polyunsaturated fatty acids of C-20 C-22 series

$ - Unidentified fatty acids of C-16 and C-14 series

Table 4: Fatty acid profiles (relative percent composition) of Liver total lipid of chicks supplemented with 5% dose of different oils along with the commercial feed for 30 days during post hatching development (Mean values of 3 set of samples are presented)

Fatty acid	Control	Coconut Oil	Sunflower Oil	Fish Oil
16:0	29.80	33.00[a]	30.68	29.90
16:1	4.20	1.80[a]	4.20	3.20[ab]
18:0	18.20	22.70[a]	11.24[a]	19.20[a]
18:1	15.20	8.50[a]	14.37	12.30
18:2(ω6)	15.30	14.44[a]	20.25[ab]	11.30[ab]
18:3(ω3)	1.20	1.25[a]	1.40	4.10[ab]
20:4(ω6)	10.20	13.38[a]	12.40[ab]	7.20[a]
20:5(ω3)	2.40	1.45[a]	1.60[ab]	4.60[ab]
22:6(ω3)	1.40	1.70[a]	1.06[ab]	4.60[a]
Others	2.10	1.78	2.80	3.60
ω3 / ω6	0.19	0.16	0.12	0.72

[a] These values are statistically significant (at $p<0.05$) over the same of control bird

[b] These values are statistically significant (at $p<0.05$) between the two treated groups of chicks

ω6 series in different tissues due to intake of higher amount of linoleic acid (for the sunflower oil supplemented diet) are in accordance with observation of Yau.[23] Dietary supplementation of the fat modulates the desaturation system of fatty acids in birds, which needs to be confirmed in future.

Polyunsaturated fatty acids of ω3 and ω6 series influence the plasma ratio of various lipoproteins viz., Low density lipoprotein (LDL) Cholesterol and High-density lipoprotein (HDL) Cholesterol. The serum lipid profiles in the form of total cholesterol and total triglycerol concentration and in the form of HDL, LDL and VLDL Cholesterol concentration are the key indicators to understand the health condition of animals. Increasing HDL cholesterol and lowering LDL and VLDL cholesterol concentration in serum prevents cardiovascular diseases.[2] High content of ω3 polyunsaturated fatty acid in certain fish oil prevents the rat from cardiovascular diseases like thrombosis and artherosclerosis.[24] Dietary fatty acids influence the production of the polyunsaturated fatty acids (mainly arachidonic acid, eisosapentaenoic acid, docosahexaenoic acid) in various animal tissues as summarized in table 4 & 5 which is in accordance with the reports of earlier workers.[20,21,22]

About two-fold augmentation of LDL-cholesterol along with about 6% & 8 fold increase in HDL and VLDL concentration respectively due to supplementation of coconut oil for 30 days (Table 6) is in accordance with the data of Castillo.[8] Saturated fatty acids which constitute about 60% of total fat in coconut oil raises plasma cholesterol by increasing LDL cholesterol concentration more than the HDL cholesterol.[25] Increase in CH:HDL ratio along with CH: TG ratio is detected due to the coconut oil supplementation (Table 6). This indicates that the addition of coconut oil to the diet might lead to accumulation of liver glycogen rather than accumulating the fat in the tissue.

Around 45% increase (equivocal) in LDL Cholesterol concentration along with 93% decrease in VLDL cholesterol (equivocal) and 33% ($p<0.005$) increase in HDL cholesterol with supplementation of 5% sunflower oil for 30 days leading to reduction of CH: HDL ratio and augmentation of CH: TG ration (Table 6) indicates the metabolization of cholesterol for the production of the lipoprotein by the action of LCAT enzyme.

Table 5: Fatty acid profiles (relative percent composition) of Serum total lipid of chicks supplemented with 5% dose of different oils along with the commercial feed for 30 days during post hatching development (Mean values of 3 set of samples are presented)

Fatty acid	Control	Coconut Oil	Sunflower Oil	Fish Oil
16:0	22.67	25.27[a]	21.26	22.10
16:1	1.20	1.50	1.50	1.20
18:0	23.45	24.27[a]	21.26[ab]	20.26[a]
18:1	11.35	8.86[a]	9.25[ab]	10.50[a]
18:2(ω6)	26.37	26.16	29.35[ab]	22.40[a]
18:3(ω3)	0.50	0.45	1.45	2.50[ab]
20:4(ω6)	10.34	10.37	12.34[ab]	8.20[ab]
20:5(ω3)	1.06	0.75[a]	1.10	4.34[a]
22:6(ω3)	1.30	1.35	1.20	4.30[ab]
Others	1.76	1.02	2.29	4.20
ω3 / ω6	0.08	0. 07	0.07	0.36

[a] These values are statistically significant (at $p<0.05$) over the same of control bird

[b] These values are statistically significant (at $p<0.05$) between the two treated groups of chicks

Table 6 : Changes in the serum lipid profiles of chick (*Gallus domesticus*) supplemented with 5% dose of different oils along with the commercial feed during post hatching development (Mean values of six samples and their standard error)

Lipid profiles	Control	Coconut Oil	Sunflower Oil	Fish Oil
Total CH	146.00 ± 1.09	203.00 ± 3.66	184.00 ± 5.73	149.33 ± 5.32
Serum TG	147.33 ± 2.75	159.33 ± 2.39	125.33 ± 3.85	95.00 ± 9.53
HDL CH	89.67 ± 3.48	95.67 ± 2.15	119.33 ± 2.02	92.00 ± 1.32
LDLCH	26.86 ± 1.83	75.46 ± 5.25	39.6 ±6.74	28.33 ± 6.36
VLDL CH	29.47 ± 1.55	31.87 ± 2.48	25.07 ± 1.77	19.00 ± 1.90
CH:HDL	1.63	2.12	1.54	1.62
CH:TG	0.99	1.27	1.47	1.57

Around 35% - 40% (p<0.005) decrease in LDL and VLDL cholesterol with increase in HDL cholesterol which result in decrease of CH: HDL ratio and increase of CH:TG ratio due to 5% fish oil supplementation for 30 days Table 6) clearly indicates that chicks do not have any severe health hazards. It is evident that even 5% supplementation of sunflower oil for a period of 30 days does not have any severe health hazards on birds but at the same time, 5% supplementation of fish oil might be more beneficial to the birds to maintain themselves in physiologically well being state. Daggy[26] have already observed that long chain PUFA helps in lowering the production rate of VLDL Cholesterol in rooster.

It is reported that dietary fish oil reduces plasma TG levels in normal and hyper triglyceredemic individuals[27] especially in VLDL fractions. The protective effects of fish intake could be caused by n3 PUFA. It is proposed that n3 PUFA may alter the lipoprotein metabolism.[28]

About 27% - 97% (p<0.005) increase in the activity of ALP in liver and serum (Figure II) and 12% - 36% (equivocal – p<0.005) depletion in liver GOT activity along with 3 fold (p<0.005) increase in liver LDH activity with the supplementation of 5% coconut oil for 30 days (Figure II) indicates the poor health status of the bird which might lead to necrosis of liver and cardiac tissues. About 18% (p<0.005) decrease in the activity of ALP in liver and 36% (p<0.01) increase in the serum (Figure II) with 50% (p<0.005) decrease in liver GOT activity and about 3.6 fold (p<0.005) increase in serum GOT activity along with 2 fold (p<0.005) increase in liver LDH activity with sunflower oil supplementation indicates necrosis of cardiac tissue. On the other hand 16% - 17% (p<0.005) decrease in liver and serum ALP activity (Figure II), 18% - 56% (p<0.005) decrease in liver and serum GOT activity and 16% (p<0.005) decrease in serum LDH activity (Figure III) due to fish oil supplementation indicate the well being state of the bird without any necrosis of liver and cardiac tissue. The decreased activity of some liver and cardiac function enzymes may be correlated with shifting of metabolic pathways, which needs to be confirmed in future. Dietary supplementation with palm oil, lowered creatine concentration in serum and activity of GPT in broiler chicken.

The available evidence indicates that ω3 PUFA have distinct physiological functions. [29,30] From the present study it is recommended that the exogenous supplementation of 5% fish oil is better to maintain the chicks in a healthy state. The amount and type of fat consumed is the focus of much interest in maintaining the good health. **It is not the quantity, rather the quality of the fat intake that determines the "well being state".**

REFERENCES

1. **Henderson RJ & Tocher DF** (1987), *Prog. Lipid Res.* 26: 281-348.
2. **Lands WEM** (1987), In, *"Fish and Human Health"*.
3. **Klinger RC, Blazer VS & Echeuarria C**(1996), *Aquaculture*, 147: 225-233.
4. **Farndale BM, Bell JG, Bruce MP, Bromage NR, Oyen F, Zanvy S, Sargent JR, Wilson RP (Ed) & Izqierdo MS**(1999), *Special issue: Fish Nutrition and Feeding. Proceedings of VII International Symposium on Feeding and Nutrition in Fish (recent advances in finfish and crustacean nutrition)*, 179: 335-350.
5. **Szymczyk B, Pisuleroski P, Szczurek W & Hankzakowski P** (2000), *Journal of the science of Food and Agriculture*, 80: 1553-1558.
6. **Lopez FS, Baucells ME, Barroeta AC & Grashorn MA** (2001), *Poltry Sci.*, 80: 741-752.
7. **Yamada N, Takita T, Wada M, Kannke Y & Innami S**(1996), *Journal of Nutritional Science and Vitaminology*, 2: 423-434.
8. **Castilio M, Amalik F, Linares A & Garcia P** (2000), *Mol.Cell. Biochem.*, 210: 121-130.
9. **Quoc KP, Pascaud M & Quoc Kietpham** (1996), *Annals of Nutrition and Metabolism.*, 20: 99-108.
10. **Godkar PB**(1994), *In: "Clinical Biochemistry Principle and Practice"*.
11. **Lands, WEM** (2000), *Biochemica et Biophysica Acta*, 1483: 1-15.
12. **Caplan MS & Jilling T** (2001) 26: 1053-1057.
13. **Sijben JWC, Nieucoland MGB, Kemp B, Parmentier HK & Schrama JW** (2001), *Poultry Science*, 80: 885-893.
14. **Rudel LL, Parks JS, Hedrick CC, Thomas M & Williford K** (1998) *Prog. Lipid. Res.*, 37: 353-370.
15. **Byoung KA, Chizuko B, Zhong SX, Tanaka K & Ohtani S**(1987), *Comp. Biochem. Physiol.*, 116B: 119-125.
16. **Al Athari AK & Watkins BA**(1988), *Poultry Sci.*, 67: 778-786.
17. **Phetteplace HW & Watkins BA** (1989), *J. Food Compos. Anal.*2: 104-117.
18. **Hargis PS, Van Elswyk ME & Hargis BM** (1991), *Poultry Sci.* 874-883.
19. **Cherian G & Sim JS** (1991), *Poultry Sci.* 70: 917-922.
20. **Manilla HA, Husveth F & Nemeth K** (1999), *Acta Agraria Kaposvariensis*, 3: 47-57.
21. **Mieczkowska A, Nguyen VC & Smulikowska S** (2001), *Journ. Anim. Feed Sci.*, 10: 279-284.
22. **Schiavone A, Romboi I, Chiarini R & Marzoni M** (2004), *J.Anim. Physiol.Anim.Nutr.*, 88:88.
23. **Yau JC, Denton JH, Bailey CA & Sams AR** (1991), *Poultry Sci.*, 70:167-172.
24. **Banerjee I, Saha S & Dutta J** (1992), *Lipids* 27: 425-428.
25. **Hayes KC & Khosla P** (1992), *FASEB J.* 6:2600-2607.
26. **Daggy B, Arost C & Bensadoun A** (1987), *Biochem. Biopys.Acta*, 920: 293-300.
27. **Harris KB, Cross HR, Pond WG & Mersmann HJ** (1993), *J. Anim. Sci.*, 71: 807-810.
28. **Schmidt EB, Kristensen SD, De Caterina R & Illingworth DR** (1993), *Atherosclerosis*, 103: 107-121.
29. **Kobatake Y, Kuroda K, Jinnouchi H, Nishide E & Innami S** (1984), *J. Nutr.Sci. Vit*, 30: 357-372.
30. **Willumsen N, Hexeberg S, Skrove J, Lundquist M & Berge RK** (1993), *J. Lipid Res.*, 34: 13-22.