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IMPACT OF DIFFERENT DIETARY FAT SOURCES ON PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS

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SUMMARY

The effects of two different fat sources: saturated-rich fat source (beef tallow) and n-3 rich PUFA fat source (Linseed oil) as well as a mixture (1:1) of both of them on broilers performance and immune status have been studied. Four groups of broiler chicks (n=40) were fed on concentrate-soybean-corn-basal diet to which either beef tallow or linseed oil or mixture blend of linseed oil and beef tallow was included at level of 3 and 4% (starter and finisher, respectively). The mixture blend of tallow and linseed oil achieved better performance and significantly higher body weight than when each fat source was fed solely. Regarding, immune parameters, the linseed oil feeding had significantly improved several immuno indices (haemagglutinin antibody titers against SRBC and delayed hypersensitivity reaction to phytohemagglutinin-P) in broiler chickens over the control and the other fat sources-fed groups. No significant changes were observed due to the inclusion of different fat sources in the relative weights of spleen and thymus, however, the relative weight of the bursa was significantly increased at age of 3 and 7 weeks in the linseed oil-fed group. It is to be concluded that whereas linsed oil feeding improved several indices of immuno status in broiler chickens which might be useful to fight infections, it was not able to compete with the tallow-linseed oil mixture in respect to performance. Further studies are needed to evaluate such effects and to understand the mechanism underlying the modulation of the immune system by linseed oil feeding.

INTRODUCTION

Immune system can be considered as a good monitor to animal health and resistance. In the past few decades, the important role of dietary factors in modulating immune response had been appreciated. It is generally accepted that protein-calorie malnutrition and certain nutrients deficiencies have an adverse effect on the immune system (Beisel, 1982; Chandra and Chandra, 1986; Mohamed, 1993). Dietary fat providing for the essential fatty acid serving as precursors for eicosanoid such as prostaglandins and leukotrienes are recognized as important modulators for both cell-mediated and humoral immunity (Goldyne and Stobo, 1981; Stenson and Parker, 1982; Rolapleszezynski, 1985). Alteration in the quality and quantity of dietary fat (Meede and Martin, 1978; Chandra and Chandra, 1986) as well as abnormalities in lipid metabolism (Beisel, 1982) had been reported to influence the regulation of the immune system. In mice, it was found that fatty acid deficiency reduces primary and secondary antibody responses and delayed hypersensitivity reaction (Dewille et al., 1979), these parameters were not affected by an increase in polyunsaturated fatty acid (PUFA) content of the diet (Thomas and Erickson, 1985). Recently, much attention has

been focused on the potential health benefitis associated with the consumption of Omega- 3 or n-3 family of PUFA. It was observed that when mice were fed on oil rich in n-3PUFA, antibody production (prickett et al., 1982) and cell mediated cytotoxiciy (Fritsche and Johnston 1989 & 1990) were significantly enhanced.

When chickens were fed on fish oil (n-3 rich PUFA) a significant enhancement in antibody titers was observed compared with other dietary fat sources (Frische et al., 1991 a). Moreover feeding fish oil or linseed oil had a marked influence on the relative amount of eicosanoid precursors present in the immune tissues of chicken (Fritsche et al., 1991 b). In general, studies cocerning the implication of dietary fat in immune response had been found conflicting and inconclusive (Johnston and Marshall, 1984).

The objective of the current study was to examine the ffect of feeding different fat sources: beef tallow (rich in saturated fatty acid); linseed oil (rich in n-3 PUFA) and a mixture blend of both of them (1:1) on performance as well as humoral and cell mediated immunity of broiler chickens.

MATERIALS AND METHODS .

Fat sources:

Two different fat sources, animal fat (beef tallow, BT) vz vegetable oil (Linseed oil, Lo) and a blend mixture (1:1 W/W, TLb) of both were utilized in formulating the experimental diets (table 1). Fatty acid composition of the added fat and oil as well as the blend mixture was measured by BYE Unicam-gas chromotography Model PU 4550, of their methyl esters using a 30-m capillary column fitted with digital integator (table 2).

Chicks and dietary treatment:

One hundred and sixty day-old commercial ma broiler chicks (Cobb) were weighed and equal assigned to one of four treatments. The chick were housed separately in pens fitted with dee litter and supplied by electric heater. Chicks we not vaccinated and none of the antimicrobia agents were used in this study. Chicks had free ac cess to food and water and continuous lightnin programme was emposed. The basal experimenta diets (starter and finisher) included 4 dietary treat ments each, to which the various fat sources were added at level of 3 and 4%, respectively. The diets were formulated to be nearly isonitrogenous and calorie/potein ratio was kept as constant as possible. During the 7 weeks experimental period, body weight development and feed consumption were recorded weekly and subsequently performance parameters were calculated . The diets were freshly prepared and the addition of fat was done every other day to minimize occurrence of rancidity (Fritsche et al., 1991 a) and any feed remaining was discarded.

Hemagglutination Assay .

At age of 3 weeks, 20 chicks from each dietary treatment were marked, blood sampled then injected with 0.5 ml suspension (vol/vol) of 5% thrice washed SRBC in phosphate-buffered saline (PBSS), pH 7.4. Three, 6 and 9 days after inoculation, sera of blood were collected to evaluate the primary hemagglutination antibody response . (Delhanty and Salmon, 1966; Witlin, 1967).

Delayed Hypersensitivity Assay:

At age of 5 weeks, 8 chicks from each dietary treatment were subjected to a delayed type hypersensitivity test (DTH) using-phytohemagglutinin-P (PHA-P, sigma, USA, Lot# 46G. 6820), according to the method of Pimentel et al (1991).

Lymphoid organ weight:

Five chicks from dietary treatment were randomly selected, killed by cervical dislocation and weighed then bursa, thymus and spleen were removed and weighed just before inoculation and at the end of the experiment. Relative lymphoid organ weight was calculated as a percentage of the bird body weight.

Statistical analysis:

Analysis of variance (ANOVA) was applied and when significant differences obtained at P< 0.5, the least significant difference between means was calculated according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The composition of the major fatty acids in the different experimental fat sources is displayed in table 2. Saturated fatty acids predominated in beef tallow while linseed oil was rich in n-3 fatty acid, that is linolenic.

Performance response:

The effect of the various fat sources on body weight development throughout the study is presented in table 3. Irrespective to the various dietary fat sources, the incorporation of fat, had improved significantly (P<0.05) the body weight and resulted in a heavier chickens at age of 7-weeks compared wih the control group. This result is inagreement with the work of Gazia (1971), Donaldson et al. (1985) and Hady et al. (1992) suggesting that chick grows faster and more efficiently at a given C/P ratio when dietary fat increasd in a balanced diet. Comparing the effect of various sources of dietary fat on chicks body weight, it appeared that the feeding of TLb had increased chicks body weight than those fed either fat source and resulted in significant (P<0.05) highest body weight. No significant difference in final weight was observed between BT and Lo-fed groups, however, Lo-fed chickens tended to be lighter. A similar result was also demonstrated by Frische et al. (1991 a) in chicks at day 13 & 17 of age and by Hood (1991) in 8-week Japanese quails fed 8% Lo in commerical turkey diet.

The impact of various dietary fat sources on overall performance of broiler chicks is presented in table 4. Diaetary fat inclusion, regardless to the source had improved gain and Feed/gain over the control. The maximum improvement was in TLb-fed group followed by BT and Lo-fed groups, Feed intake followed the same pattern of gain and the Lo-fed group consumed the least feed and therefore reflected the least gain among all the fat-fed groups. It appeared that the inclusion of fat increased feed intake at different degree regardless to the source. Dale and Fuller (1980) found the same observation at two differet temperature regimens (hot and cool). The slight increase of feed intake by broilers fed TLb and BT over Lo-fed group might suggest a difference in palatability between various fat sources.

The efficiency of feed (feed/gain) and energy utilization was improved by the fat treatment and the TLb-fed group exhibited the maximum improvement over the control (2.15 & 13.85 vs. 2.33 & 14.42, respectively). Interestingly, chicks utilized BT energy more efficiently than Lo (14.08 vs 14.31) which is a result in contrary to Hady et al (1992) who concluded that vegetable oil namely cotton seed oil+ sunflower oil mixture is better utilized than BT by broilers. The discrepancy in this particular point might be attributed to the nature of oil (presence of nonsaponifiables in the expeller fraction, Fritsche et al., 1991 a) , the percentage composition of saturated to unsaturated

Table (1): Composition and calculated analysis of the experimental diets

Ingredients \ Dietary treatment	Starter diet (0-4 wk)				Grower diet (4-7 wk)			
	Control	BT	Lo	TLb	Control	BT	Lo	TLb
- Broiler concentrate mixture[1,2,3]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
- Soybean meal (44%)	24.0	27.0	28.0	27.5	14.0	17.0	18.0	17.5
- Ground yellow corn	66.0	60.0	59.0	59.5	76.0	69.0	68.0	68.5
- Fat	-	3.0	3.0	3.0	-	4.0	4.0	4.0
Calculated analysis[4]								
ME kcal/kg	2869.0	2943.4	2999.0	2966.7	2985.0	3095.8	3161.8	3128.8
CP %	21.44	22.24	22.59	22.42	17.9	18.6	18.9	18.8
Ether extract %	3.29	6.74	6.05	6.07	3.59	7.34	7.31	7.33
Methionine %	0.47	0.47	0.47	0.47	0.4	0.43	0.44	0.43
Calcium %	0.89	0.90	0.9	0.9	0.86	0.87	0.87	0.87
C/P ratio	133.8	132.4	132.4	132.3	166.8	166.4	166.7	166.5
Energy density	100	102.6	104.5	103.4	100	103.7	105.9	104.8

1- Broiler concentrate mixture (Fralex) supplied 52% Cp; 2200 kcal ME/kg; 5% EE; 8% Ca; 1.2 % methionine, 1.6 % methionine + cystine.
2- Mineral & vitamin added to each kg concentrate: vit A, 120,000 IU; vit D 3, 25.000 IU; vit E, 200 mg; vit K3, 30 mg; vit B1, 20 mg; vit B6, 30 mg; Folic acid, 10 mg; Pantothenic acid, 120 mg; niacin, 350 mg; choline, 600 mg; Biotin, 750 µg ; Vit B12, 130 µg ; Fe, 500 µg ; I, 5 µg; Zn, 500 µg; Mn, 750 µg; Cu, 50 µg; Se, 1 µg .
3- BHT at level of 250 mg supplied as antioxident/kg concentrate.
4- Calculated analysis was based on the ingredients analysis tables presented by Scott et al. (1982).

Table (2): Percentage composition of the major fatty acids in the experimental fat sources.

Fatty acid [1]	Dietary fat		
	Beef tallow (BT)	Linseed oil (Lo)	Tallow-linseed oil(TLb)
C 14:0	3.2	-	1.4
C 16:0	25.8	7.1	13.2
C 16:1 n-7	2.8	0.3	1.4
C 18:0	22.1	3.9	12.6
C 18:1	36.9	21.7	26.6
C 18:2 n-6	1.9	17.3	11.8
C 18:3 n-3	0.3	42.9	19.9
C 20:1	0.5	0.1	Tr [4]
[2]Saturates	51.1	11.0	27.2
[3]PUFA	2.2	60.2	31.7

[1] Fatty acids are designated by the carbon length, : number of double bond and the position of the first double bond from the methylene end of the molecule.

[2]Saturates = Sum area percentage of C14:0, C16:0 and C18:0

[3]PUFA = Sum total area percentage of C18:2 n-6, C18:3 n-3, C20:4 n-6, C20:5 n-3, C22:5 n-3 and C22:6 n-3.

[4]Tr = trace, minor fatty acids are not included.

Table (3): Body weight development of broiler chicks fed different fat sources.

age in weeks \ Dietary treatment	Control	BT	LO	TLb
0	40.1±0.7	39.6±0.6	40.9±0.5	38.3±0.6
1	94.6±2.5	100.2±2.4	89.9±2.7	101.4±2.9
2	248.3±4.8^{b}	271.2±6.2^{a}	249.6±5.0^{b}	282.6±6.6^{a}
3	483.7±7.8^{c}	538.3±8.8^{ab}	517.7±8.9^{b}	551.9±8.2^{a}
4	717.5±12.8^{c}	800.9±14.4^{b}	765.3±13.1^{b}	843.5±16.3^{a}
5	1095.3±22.4^{c}	1192.0±21.4^{b}	1165.4±20.7^{b}	1281.7±26.2^{a}
6	1407.7±28.3^{c}	1515.6±32.4^{b}	1491.2±36.5^{b}	1639.6±37.3^{a}
7	1664.4±31.2^{c}	1788.0±35.1^{b}	1763.2±37.4^{b}	1898.3±44.0^{a}

Values are means ± SE

a,b,c values in the same raw with different superscriptes are significantly differed at P < 0.05.

Table (4): Effect of various dietary fat sources on overall performance of broiler chicks

Parameter	Dietary treatment			
	Control	BT	Lo	TLb
Total gain (g)	1624.3	1748.4	1723.3	1860.0
Gain (g)/day/bird	33.15	35.68	35.15	37.96
Total food consumption (g)	3791.5	3861.9	3799.1	3993.5
Overall feed/gain	2.33	2.26	2.20	2.15
Total energy intake (kcal intake/bird)	478.1	502.44	502.97	525.74
Energy efficiency (kcal intake/g gain)	14.42	14.08	14.31	13.85

Table (5): Effect of dietary fat sources on haemagglutinin antibody titers [1] (Log2) and delayed hypersensitivity[2] of broiler chicks.

Time post-treatment	Dietary treatment			
	Control	BT	Lo	TLb
Antibody titers				
0 d	0	0	0	0
3 d	4.0 ± 0.19^{b}	4.33 ± 0.15^{b}	5.8 ± 0.20^{a}	4.27 ± 0.16^{b}
6 d	5.46 ± 0.21^{b}	5.41 ± 0.13^{b}	7.4 ± 0.21^{a}	5.6 ± 0.19^{b}
9 d	4.65 ± 0.18^{b}	4.7 ± 0.11^{b}	6.9 ± 0.22^{a}	5.23 ± 0.17^{b}
DHS				
8 h	0.39 ± 0.02^{b}	0.37 ± 0.02^{b}	0.56 ± 0.03^{a}	0.42 ± 0.01^{b}
16 h	0.35 ± 0.02^{b}	0.36 ± 0.01^{b}	0.46 ± 0.02^{a}	0.39 ± 0.02^{b}
24 h	0.32 ± 0.01^{b}	0.33 ± 0.03^{b}	0.41 ± 0.02^{a}	0.36 ± 0.01^{b}

a, b means within row with no commo superscriptes are singificantly differed at $P < 0.05$.
1 heamagglutinin antibody titers against sheep red blood cells inoculation.
2 Delayed hypersensitivity to phytohemagglutinin-P inoculation.

fatty acids and the position of fatty acid on the triglycride molecule which further complicate fat absorption (Brockerhoff, 1971).

The beneficial synergestic effect observed by feeding TLb mixture on broilers performance was also reported by several investigators but with different types of vegetable oils (Lall and Slinger, 1973; Hulan et al., 1984) which might be attributed to the enhancement occurred in the meabolic process particularly intestinal absorption when saturated and unsaturated fatty acids were combined together (Griffiths et al. 1977; Hulan et al 1984). This suggestion was also supported by the work of Sibbald and Kramer (1977) who postulated that the true metabolizable energy of several mixtures were often greater than the sum of the individual fat.

Immune response:

Results of haemagglutinin titers against SRBC at different intervals postinoculation are presented in table 5. Chicks fed Lo had maintained significant ($P<0.05$) higher mean antibody titers against SRBC, starting 3d postinoculation upto 9d postinoculation, than that group of chicks fed BT, TLb and control diets. The other dietary fat sources did not significantly affect antibody titers, though, the TLb-fed chicks showed a slight increase compared to the control group. These results implyed that saturated fatty acid rich source (BT) neither suppressed nor enhanced antibody production, whereas n-3 PUFA rich source enhanced it . Stimulation of antibody production agaist SRBC by feeding Lo was previously reported by Fritsche et al., (1991a) in 26-d-old chicks and against bovine serum albumin in male Newzeland rabbit by Kelley et al. (1988). The present findings are, however, at variance with the result of Phetteplace et al. (1989) who found no difference in antibody response to SRBC of two different genetic lines of chicken fed either n-3 rich fat source (fish oil) or n-6 rich fat source (Soybean oil). The enhancement effect of n-3 PUFA rich source used in the current study (Lo) might be explainable on view of the work of Fritsche et al., (1991a & b) who demonstrated that feeding chicks on fat source rich in n-3PUFA (e.g Lo) decreased significantly the level of arachidonic acid present in serum and immune tissue by 50 to 75%. Since, prostaglandins derived from arachidonic acid have been reported as important immune modulator, so the ev-

Table (6): Effect of dietary fat sources on relative weight of some lymphoid organs of broilers chicks

Organ	Thymus		Bursa		Spleen	
Dietary treatmnet \ Time in weeks	3	7	3	7	3	7
Control	0.46±0.032[a]	0.62±0.040[a]	0.48±0.028[b]	0.66±0.029[b]	0.156±0.009[a]	0.284±0.021[a]
BT	0.47±0.036[a]	0.64±0.041[a]	0.50±0.031[b]	0.72±0.024[b]	0.126±0.015[a]	0.292±0.018[a]
Lo	0.50±0.038[a]	0.70±0.044[a]	0.69±0.033[a]	0.84±0.039[a]	0.166±0.012[a]	0.305±0.025[a]
TLb	0.49±0.30[a]	0.64±0.039[a]	0.52±0.030[b]	0.71±0.028[b]	0.159±0.011[a]	0.281±0.019[a]

Mean ± SE

a,b values within the same column with no common superscriptes are significantly differed at $P < 0.05$.

idence is mounting that dietary n-3PUFA can enhance immune response by reducing eicosanoid production, particularly, prostaglandin E2 (Johnston, 1988).

The results of delayed-type hypersensitivity test in response to phytohemagglutinin-P inoculation are presented in table 5. The DHS reaction was significantly (P<0.05) increased in Lo-fed group to 143.6, 131.43 and 128.13% of the control reaction at 8,16 and 24 h, respectively to phytohemagglutinin inoculation. The differences in DHS reaction among the fat-fed groups and the control group were modest except for the Lo-fed group which was more responsive. Delayed type hypersensitivity an in vivo index of T-cell function was markedly enhanced in broiler chicks fed Lo. Thomas and Erickson (1985) found that DHS of mice fed a diet deficient in essential fatty acids was less than of mice fed control deit. On the contrary, kelley et al. (1988) found that delayed hypersensitivity response to topically applied T-cell antigen was not affected by feeding male Newzeland rabbit with linseed oil , safflower oil, Menhaaden oil or hydrogenated soybean oil-containing diets. We are not certain of the cause for different effects of n-3 dietary fat sources on DHS reaction, but several factors may be inocrporated including differences in animals studied, diets, length of carbon chain of n-3 fatty acids, n-3 to n-6 fatty acid ratio and the type of immunogens utilized. Nevertheless, the changes might occur in fatty acid composition of the immune tissues due to Lo-feeding especially the affinity of thymus to C18 fatty acids (Fritsche et al. 1991 b), might have importent consequences on eicosanoid production which acts in modulating cell-mediated immune events by influencing cell intercommunication and differentiation (Goodwin and Webb, 1980).

The results of the relative lymphoid organ weight as influenced by different dietary fat sources are presented in table 6. No significant changes in the relative weights of spleen and thymus were observed between treatments. This result is in accordance with Fritsche et al. (1991 b). However, the Lo-fed group maintained significant (P<0.05 higher bursal weight at age of 3 and 7 weeks. The profound increase in the relative weight of bursa in Lo-fed group might indicate bursal activity which was supported by the significant increase in haemagglutinin titers and reflected the stimulatory effect on the humoral branch of immunity (Marsh et al., 1981; Saad et al., 1993).

In conclusion, the current study demonstrated that the inclusion of different dietary fat sources in well balanced startar and finisher broiler's diet had improved overall performance of broiler chicks. The mixture blend (w:w) of animal and vegetable fats surpassed in performance either of them when fed singly. Linseed oil feeding tended to produce lighter chickens at age 7 weeks than other dietary fat-fed groups. However, the increase in immuno-responsiveness in Lo-fed group exhibited by the marked enhancement of hemagglutinin titers and delayed hypersensitivity reaction might useful to defende against infections.

Further studies are needed to evaluate such effects and to uderstand the mechanism underlaying the modulation of the immune system by linseed oil feeding.

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