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Effect of Marine Lipids on Innate Immunity, Represented by NK Cell Activity

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Abstract: **- The objective of the current experiment was to study the effect of long chain omega-3 polyunsaturated fatty acids (PUFA), present in marine algae, on an innate immune response, represented by NK cell activity. One day old Cobb 500 broiler chickens (n=17) were fed on DHA algal biomass-enriched diet until 35 d of age. At slaughter, samples of blood were collected. Natural killer (NK) cell activity was analyzed. It was higher in blood samples of birds fed algal biomass than the control group. This suggests that algal biomass-rich diet may be used to enhance the innate immunity, represented by the NK cell activity.**

***Keywords*: Hygiene; Algal biomass, fatty acids, immunity, innate immunity, NK cell activity.**

I. INTRODUCTION

Some studies in the literature show an immunosuppressive effect of omega-3 PUFA on NK cell cytotoxicity in mice [1, 2], rats [3, 4], and humans [5, 6]. Animal studies tend to suggest that both eicosa pentaenoic acid (EPA) and docosa hexaenoic acid (DHA) have immune modulatory effects. Both EPA and DHA fed to rats at 4.4 g/100 g total fatty acids inhibited lymphocyte proliferation, although only EPA inhibited NK cell activity [7]. In humans, Thies et al [8] compared the effects of supplementation with fish oil, highly purified DHA or a placebo on lymphocyte proliferation in healthy subjects and showed that fish oil suppresses lymphocyte proliferation whereas DHA has no effect. This could be taken to suggest either that EPA is responsible for the inhibitory effect or that both EPA and DHA are required. Based on above, the effect of omega-3 PUFA on NK cell activity in animals and humans is contradictory. The objective of the current experiment was to investigate the effect of omega-3 PUFA in algal biomass-enriched diet on innate immunity, represented by NK cell activity in broiler chickens

II. MATERIALS AND METHODS

One-day-old male Cobb 500 broiler chicks were fed the same basal diet for 21 d. Then, half of the chickens were fed diet rich in DHA algal biomass (n=17). The other half were fed the control diet until slaughter. Table 1 shows the composition of the finisher diets used. The fatty acid profile of the experimental diets is shown in Table 2. At slaughter, blood was collected in heparinized tubes. The blood was then layered onto an equal volume of Lympholyte-H and centrifuged. The interface of the limmune cells was collected and suspended in medium. The blood cells were washed and suspended in medium. Target cells were purchased and serially sub-cultured with complete nutritive medium every two days. Target cells were used within 24-36 hours of subculture. The effector blood cells were prepared and re-suspended in complete medium. The target cells were re-suspended in phosphate buffer saline (PBS) and were labelled with diluted 5(6)-carboxyfluorescein diacetate succinimidyl ester and mixed with the effector cells at different and were incubated for 2 hours at 41oC in an atmosphere containing 5% CO2. After incubation, a red fluorescent DNA dye was added to label the killed target cells. Flow cytometere was used to measure and analyse the NK killer cell activity of the blood cells. Results of the assay were expressed as % cytotoxicity after subtracting % cytotoxicity in the control.

Statistical analysis

The overall differences between dietary treatments were analyzed using one-way analysis of variance (ANOVA) using the general linear model in Minitab. Differences between the treatment groups were considered statistically different at $P \leq 0.05$.

TABLE I
Composition of Finisher Experimental Diets Used (On As-Fed Basis)

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Feed	Diet composition (g/kg fresh weight) Control diet	Algal biomass
Wheat	583	581
Soyabean meal	285	276
CaCO3	13	13
Dicalcium phosphate	10	10
Algae	0	18
Soya oil	32	40
Salt	3.5	3.5
Vitamin/mineral supplement	50	50
DL Methionine	2	2
NaHCO3	2	5
Lysine	1.5	1.5
VitaminE (i.u./kg)	100	100

TABLE II
Fatty Acid Composition of the Diet Mixtures Used

	Fatty acids (wt %) Control diet	Algal biomass
C14:0	0.16	4.07
C16:0	11.16	19.30
C16:1	0.20	0.29
C18:0	3.81	2.91
C18:1n9	18.94	13.56
C18:2n6	42.74	33.38
C18:3n-6	13.26	0.44
C18:3n3	3.05	4.78
C20:1n-9	0.64	0.06
C18:4 n-3	3.45	0.24
C20:4n6	0.25	0.86
C23:0	0.05	0.33
C20:5n3	0.10	0.49
C22:5n3	0.10	0.20
C22:6n3	0.24	17.24
∑SAT[1]	15.17	26.61
∑MUFA[2]	19.78	13.91
∑PUFA[3]	53.27	57.72
∑n-6[4]	56.33	34.77
∑n-3[5]	6.94	22.95
∑n-6:∑n-3[6]	8.11	1.53

1∑SAT = Sum percentage of saturated fatty acids (C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0)
2∑MUFA = Sum percentage of monounsaturated fatty acids (C14:1, C16:1, C17:1, C18:1n-9t C18:1n-9c, C22:1 n-9)
3∑PUFA = Sum percentage of PUFA (C18:2n-6t, C18:2n-6c, C18:3n-6, C18:3n-3, C20:2n-6, C20:3n-6, C20:4n-6, C20:5n-3, C22:2, C22:5n-3, C22:6n-3)
4 ∑n-6 = Sum percentage of n-6 PUFA (C18:2n-6t, C18:2n-6c, C18:3n-6, C20:4n-6, C20:2n-6, C20:3n-6)5∑n-3 = Sum percentage of n-3 PUFA (C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3) 6∑n-6:∑n-3= ratio of ∑n-6 to ∑n-3

TABLE III
Fatty Acid Composition Of Blood of 5-Wk-Old Broilers Fed Algal Biomass Treatment Vs. the Control Diet

	% of total weight Control	Algal biomass	SEM	*p-value*
C18:3n-6	0.82 [a]	0.46 [b]	0.395	0.037
C18:3n3	0.73 [a]	0.28 [b]	0.331	0.014
C18:4n3	0.15	0.12	0.034	0.322
C20:4n6	9.58[a]	16.57[b]	2.557	< 0.001
C20:5n3	0.59 [a]	1.17 [b]	0.353	< 0.001
C22:5n3	2.06 [a]	1.30 [b]	0.161	< 0.001
C22:6n3	1.09 [a]	6.54 [b]	0.110	< 0.001
∑n-6[1]	27.06 [a]	34.51[b]	2.574	< 0.001
∑n-3[2]	4.47[a]	9.30 [b]	0.329	< 0.001
∑n-6:∑n-3[3]	6.27 [a]	3.71 [b]	0.713	< 0.001

∑n-32 4.47a 9.30 b 0.329 < 0.001
∑n-6:∑n-33 6.27 a 3.71 b 0.713 < 0.001
Means within rows are significantly different at p≤ 0.05, Values are expressed as means (n=4 for each dietary treatment), pooled standard error of means (SEM)
1∑n-6 = Sum percentage of n-6 PUFA
2∑n-3 = Sum percentage of n-3 PUFA
3∑n-6:∑n-3= ratio of ∑n-6 to ∑n-3

III. RESULTS

Fatty acid composition of blood

The effect of using marine algal biomass on the fatty acid composition of blood cells is shown in Table 3. The concentration of blood EPA was higher in broilers fed the algal biomass than the control group (Table 3). Also, DHA was higher in the chickens fed the algal biomass diet than those fed the control diet. The level of blood α-linoleic acid was observed to be higher in broilers fed algal biomass (Table 3). However, arachidonic acid (AA) was observed to be lower in the algal biomass group than the control group. The n-6:n-3 ratio was lower in the blood of birds fed the algal biomass than those fed the control diet (Table 3).

Natural killer activity of blood cells

NK activity of blood leukocytes was higher in the algal biomass group than the control group (Table 4).

Table IV
Effect of Feeding Algal Biomass on Natural Killer (NK) Cell Activity of the Blood Cells from 5-Week Old Broilers at

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Different Ratios

	Treatment			
	Control	Algal biomass	SEM	P-value
E:T	% cytotoxicity			
100 :1	2.97[a]	6.55[b]	2.471	0.047
50 :1	4.62[a]	9.93[b]	2.309	0.050
25 :1	5.78	5.49	1.961	0.164
12.5 :1	0.82[a]	2.33[b]	1.000	0.041

E:T, effector cell/target cell ratio
Means within rows with no common superscripts are significantly different ($p \leq 0.05$) Values are expressed as means (n=4 for each dietary treatment), pooled standard error of means (SEM)

IV. DISCUSSION

The objective of the current experiment was to study the effect of long chain omega-3 polyunsaturated fatty acids (PUFA), present in marine algae, on innate immune response, represented by NK cell activity. The n-3 PUFA source was marine algal biomass. Changes in the fatty acid compositions of blood cells were as expected, with algal biomass resulting in enrichment of blood with omega-3 fatty acids, with a lower ration of omega-6 to omega-3 fatty acids. In birds fed algal biomass, levels of DHA were higher than in the control group, demonstrating that small amounts of algal biomass can result in greater incorporation of DHA and about a third of the EPA as a large amount of FO containing an equivalent amount of total n-3 PUFA (Rymer et al. [9]; [10].

V. CONCLUSION

Algal biomass enrichment of broiler meat can be used efficiently to enrich poultry meat with omega-3 PUFA. This enrichment resulted in enhancement of the innate immunity, represented by the NK cell activity.

VI. ACKNOWLEDGMENTS

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