

Effect of dietary oils and conjugated linoleic acid on the growth performance of broilers vaccinated with the La Sota Newcastle vaccine

R. Aydin[#]

Department of Animal Sciences, Kahramanmaraş Sutcu Imam University, 46060 Kahramanmaraş, Turkey

Abstract

The objective of this study was to investigate the effects of dietary oils on growth performance of broiler chicks vaccinated with La Sota vaccine against Newcastle disease. One hundred and seventy five 1-week old Ross PM3 male broiler chicks were randomly allocated to five dietary groups (n = 35 chicks/group) and fed diets supplemented with 0.5% sunflower oil (Group A), 0.5% olive oil (Group B), 0.5% beef tallow (Group C), 0.5% conjugated linoleic acid (CLA, Group D) or 0.5% hazelnut oil (Group E) for five weeks. Diets contained 230 g crude protein/kg and 12.97 MJ metabolisable energy (ME)/kg (starter diet); 220 g crude protein/kg and 12.97 MJ ME/kg (grower diet) or 200 crude protein/kg and 12.97 MJ ME/kg (finisher diet). The broiler chicks were vaccinated with La Sota Newcastle vaccine at 22 days of age. Body weights were measured weekly and carcass characteristics and some organ (liver, heart, gizzard and intestine) weights were determined after slaughter. The growth rates of broilers from the Groups A, C and E were negatively influenced by vaccination, compared to Group D, which maintained its growth rate. There was no difference in the proportions of abdominal fat (%) of the chickens among the groups. The respective relative organ weights did not differ significantly between the respective dietary treatments. This study showed that the broiler chicks fed a diet supplemented with CLA had significantly better performance and carcass weights compared to the other groups. The present study also indicated that CLA included at a level of 0.5% in the diet prevented weight loss due to vaccination.

Keywords: Conjugated linoleic acid, vaccination, broiler, immune system

[#] E-mail: rahimaydin@ksu.edu.tr

Introduction

Specific fatty acids such as omega-3 fatty acids and conjugated linoleic acid (CLA) were shown to improve performance and to decrease indices of inflammatory response in the growing chicks (Hellerstein *et al.*, 1989; Cook *et al.*, 1993; Korver & Klasing, 1997). Omega-3 fatty acids from fish oil were shown to enhance the antibody response of chicks to sheep red blood cells, but suppressed rates of lymphocytes proliferation after mitogen stimulation (Fritsche *et al.*, 1991). Conjugated linoleic acid is a naturally occurring substance in dairy products and meat from ruminant animals, as a result of bacterial biohydrogenation in the rumen (Ha *et al.*, 1989) and was reported to exert a variety of biological properties in several animal models, including anti-carcinogenic (Belury, 2002), anti-atherogenic (Lee *et al.*, 1994) and anti-diabetic (Houseknecht *et al.*, 1998) properties. Dietary CLA was first shown to be effective in the prevention of growth depression induced by immune stimulation in chicks and mice (Cook *et al.*, 1993). Growth suppression induced by the injection of endotoxin was markedly reduced in mice and chickens fed diets supplemented with CLA (Cook *et al.*, 1993). Conjugated linoleic acid also promotes immunoglobulin production and modulates the production of various cytokines in rat and mouse splenocytes (Sugano *et al.*, 1998; Yamasaki *et al.*, 2003). Studies by Yamasaki *et al.* (2003) showed that the t-10, c-12 CLA isomer, but not the c-9, t-11 CLA isomer, was an active isomer to enhance immunoglobulin (Ig) production *in vivo*. Studies involving fish oil and CLA illustrated possible means of modulating the growth suppressive effects resulting from immune induced alterations in the nutrient metabolism (Klasing *et al.*, 1987; Cook *et al.*, 1993).

The immune system in poultry, like that of humans, has developed several levels of defence strategies to cope with a wide spectrum of pathogens, including parasites, bacteria and viruses (Erf, 2004). Newcastle disease is a highly contagious disease of poultry and other bird species caused by specified viruses of the avian paramyxovirus Type I serotype of the genus *Avulavirus*, belonging to the family of Paramyxoviridae (Mayo, 2002). Newcastle disease causes great losses in poultry in production systems where preventive

measures are not taken. In the poultry industry vaccination against Newcastle disease with live vaccines is a common practice and even obligatory in many countries throughout the world. Stimulation of the chicken's immune system by a wide variety of immunogens decreases the rate of weight gain, feed intake and the efficiency of feed utilization (Klasing *et al.*, 1987). Vaccination is reported to be a potent immune stimulant which depresses growth performance in poultry (Chamblee *et al.*, 1992) and causes a decrease in the rate of protein synthesis and accretion in the skeletal muscle (Hentges *et al.*, 1984). Oral vaccination of chicks against Newcastle disease and infectious bronchitis resulted in decreased protein synthesis and protein accretion in the skeletal muscle (Hentges *et al.*, 1984).

In light of these findings, this study investigated the effect of supplementing broiler chick diets with CLA and different oils, and the effect thereof on the growth performance of male broiler chicks vaccinated with La Sota Newcastle vaccine.

Materials and Methods

Four hundred 1-day old Ross PM3 broiler chicks were obtained from a commercial hatchery and were housed in a room with 24-hour constant fluorescent lighting. They were vaccinated against Newcastle disease (HB1) in the hatchery. At the end of one week of feeding, sexes of the broiler chicks were determined. In this study, male broiler chicks were used because uniformity of birds is really important in an experiment. As known, body weights of the male and female birds are pretty similar at the beginning of the hatch. However, there would be a great difference in the body weights between male and female chicks at the end of 6-weeks feeding. For this reason, only male chicks were used to get better uniformity in the study. One hundred and seventy five 1-week old Ross PM3 male chicks were randomly allocated to five dietary groups (n = 35) and fed a commercially prepared diet supplemented with 0.5% sunflower oil (Group A), 0.5% olive oil (Group B), 0.5% beef tallow (Group C), 0.5% CLA (Group D) or 0.5% hazelnut oil (Group E) for five weeks. The objective of the study was to determine the effect of different oils containing markedly different fatty acids and CLA on growth performance of the broilers vaccinated with Newcastle La Sota vaccine. Individual fatty acids have different properties due to the number and configuration of double bonds in the chain. In the current study the oils chosen represent different groups of fatty acids. For example, sunflower oil contains 69% linoleic acid; olive oil contains about 72% oleic acid and beef tallow contains higher levels of saturated fatty acids (mainly C14:0, C16:0 and C18:0). The source of CLA-80 consisted of 35.33% c-9, t-11 and t-9, c-11-CLA, 35.72% t-10, c-12-CLA; 1.11% c-9, c-11-CLA; 1.57% c-10, c-12-CLA; 0.91% t, t-9, 11 and 10, 12-CLA. Other fatty acids in CLA were 6.64% palmitate, 2.39% stearate, 13.77% oleate, and 0.81% linoleate, and 1.75% unknown. Oils and CLA were mixed into the diets later.

Table 1 Chemical composition of the diets¹ commercially prepared (as fed basis)

| | Starter Diet | Grower Diet | Finisher Diet |
|-------------------------|--------------|-------------|---------------|
| Dry matter, g/kg | 880 | 880 | 880 |
| Crude protein, g/kg | 230 | 220 | 200 |
| Metabolic energy, MJ/kg | 12.97 | 12.97 | 12.97 |
| Ash, g/kg | 80 | 80 | 80 |
| NaCL, g/kg | 3.5 | 3.5 | 3.5 |
| Calcium, g/kg | 15 | 15 | 15 |
| Phosphorus, g/kg | 7 | 7 | 6.5 |
| Lysine, g/kg | 12 | 11 | 10 |
| Methionine, g/kg | 5 | 5 | 4 |
| Cysteine, g/kg | 4 | 4 | 3 |

¹Also contained per kilogram of diet: 8000 IU vitamin A; 800 IU vitamin D₃; 15 mg vitamin E; 2 mg vitamin K₃; 4 mg vitamin B₂; 10 mg vitamin B₁₂; 60 mg manganese; 40 mg zinc

Diets were isoenergetic and isonitrogenous. The birds were allowed *ad libitum* access to feed and water and maintained on a 24-hour constant lighting program. When chicks reached an age of 22 days, water was withdrawn for two hours before vaccination to allow them to get thirsty. Broiler chicks were vaccinated with La Sota vaccine (Intervet International B.V. Boxmeer, Holland) reconstituted with unchlorinated water. Ample water space was provided so that all birds could get water easily. Body weights of broiler chicks were recorded weekly and mortality in the groups was recorded daily. At the end of the study the chickens were slaughtered and carcass weight and the weights of some organs (liver, heart, gizzard and intestine) were recorded. In this study, data on broiler performance and organ weights were analyzed using the general linear models procedure and differences were considered significant at a level of 95% (SPSS software 10.0; Chicago, IL). The significance of the differences among the groups has been determined by Duncan's range test (SPSS software 10.0; Chicago, IL).

Results

Table 2 represents the effect of dietary oils and CLA on the performance of the broiler chickens vaccinated against Newcastle disease. There was no significant difference between the body weights in the groups until vaccination. However, after vaccination on day 22 after hatching, body weight performances were significantly influenced in almost all the groups, with the exception of Group D. Group D received the diet supplemented with 0.5% CLA, which prevented ($P < 0.05$) the vaccine induced decrease in performance.

Table 2 The effects of dietary treatments on body weight recorded on a weekly basis in male broiler chickens

| Week | Dietary treatments ¹ | | | | |
|------|---------------------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|
| | Group A | Group B | Group C | Group D | Group E |
| 1 | 191.5 ± 2.4 | 186.5 ± 3.3 | 195.4 ± 3.7 | 192.9 ± 2.7 | 189.9 ± 3.1 |
| 2 | 500.6 ± 8.6 | 507.5 ± 9.2 | 513.3 ± 9.4 | 516.2 ± 7.7 | 499.1 ± 10.2 |
| 3 | 1025.2 ± 23.3 | 1066.5 ± 17.2 | 1051.3 ± 20.4 | 1072.2 ± 22.3 | 1028.2 ± 25.3 |
| 4 | 1520.5 ^b ± 31.9 | 1575.2 ^{ab} ± 25.6 | 1566.2 ^{ab} ± 29.2 | 1608.9 ^a ± 31.4 | 1516.6 ^b ± 36.1 |
| 5 | 2068.2 ^b ± 43.9 | 2128.1 ^{ab} ± 27.7 | 2073.2 ^b ± 33.7 | 2163.3 ^a ± 40.4 | 2037.8 ^b ± 45.6 |

¹ Dietary treatments: Commercial diets supplemented with 0.5% sunflower oil (Group A); 0.5% olive oil (Group B); 0.5% beef tallow (Group C); 0.5% CLA (Group D) or 0.5% hazelnut oil

^{a,b} Means within a row lacking a common superscript differ ($P < 0.05$)

Table 3 shows the effect of the different dietary oils on body performance, feed conversion ratio (FCR) and carcass characteristics in male broiler chickens. Final body weights of broilers from the Groups A, C and E were significantly ($P < 0.05$) lower than those of the CLA-fed broilers. Dietary CLA at an inclusion level of 0.5% reduced ($P < 0.05$) body weight loss. The differences in FCR were not significantly different. Eviscerated carcass weights of Group D birds were higher ($P < 0.05$) compared to the carcass weights of the other three groups. The proportions of body parts and abdominal adipose tissue did not differ among the groups.

The effects of dietary treatments on the percentage of organ weights of male broilers are presented in the Table 4. No significant differences in the proportions of liver, heart and intestines of the male broilers were observed among the groups. However, the proportion of the gizzard was found to be higher ($P < 0.05$) for males from Group C.

Table 3 The effects of dietary treatments on the performance and body parts of male broilers

| | Dietary treatments ¹ | | | | |
|--------------------------------------|---------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | Group A | Group B | Group C | Group D | Group E |
| Initial B.W., g | 191.5 ± 2.4 | 186.5 ± 3.3 | 195.4 ± 3.7 | 192.9 ± 2.7 | 189.9 ± 3.1 |
| Final BW, g | 2068.2 ^b ± 43.9 | 2128 ^{ab} ± 27.7 | 2073.2 ^b ± 33.7 | 2163.3 ^a ± 40.4 | 2037.8 ^b ± 45.6 |
| FCR ² | 1.80 | 1.77 | 1.78 | 1.76 | 1.77 |
| Eviscerated carcass ³ , g | 1548.6 ^b ± 38.3 | 1574.1 ^b ± 33.8 | 1531.5 ^b ± 33.9 | 1648.2 ^a ± 26.8 | 1509.3 ^b ± 40.6 |
| Neck (%) | 4.68 ± 0.2 | 4.65 ± 0.2 | 5.17 ± 0.2 | 5.04 ± 0.1 | 5.05 ± 0.1 |
| Breast ⁴ (%) | 32.41 ± 0.5 | 33.75 ± 0.5 | 32.43 ± 0.5 | 33.24 ± 0.5 | 33.26 ± 0.8 |
| Leg ⁴ (%) | 28.97 ± 0.4 | 27.99 ± 0.3 | 29.15 ± 0.3 | 28.55 ± 0.3 | 28.19 ± 0.3 |
| Wing ⁴ (%) | 10.71 ± 0.2 | 10.49 ± 0.2 | 10.76 ± 0.2 | 10.26 ± 0.2 | 10.76 ± 0.1 |
| Back (%) | 23.24 ± 0.3 | 23.11 ± 0.2 | 22.49 ± 0.4 | 22.92 ± 0.4 | 22.75 ± 0.3 |
| Abdominal fat (%) | 1.36 ± 0.09 | 1.53 ± 0.06 | 1.46 ± 0.13 | 1.42 ± 0.11 | 1.56 ± 0.08 |

¹Commercial diets supplemented with 0.5% sunflower oil (Group A); 0.5% olive oil (Group B); 0.5% beef tallow (Group C); 0.5% CLA (Group D) or 0.5% hazelnut oil

²FCR = feed intake / body weight gain

³Eviscerated carcass = carcass without head, internal organs, abdominal fat and feet

⁴Percentage of eviscerated carcass

^{a,b}Means within a row lacking a common superscript differ (P < 0.05)

Table 4 ¹Percentage of organ weights of male broilers fed diets supplemented with different oils for 5 weeks

| | Dietary treatments ² | | | | |
|---------------|---------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | Group A | Group B | Group C | Group D | Group E |
| Liver (%) | 2.70 ± 0.09 | 2.55 ± 0.07 | 2.60 ± 0.07 | 2.52 ± 0.13 | 2.57 ± 0.06 |
| Heart (%) | 0.81 ± 0.04 | 0.79 ± 0.03 | 0.83 ± 0.04 | 0.79 ± 0.02 | 0.79 ± 0.03 |
| Gizzard (%) | 1.87 ^b ± 0.06 | 1.84 ^b ± 0.07 | 2.17 ^a ± 0.11 | 1.82 ^b ± 0.07 | 1.87 ^b ± 0.06 |
| Intestine (%) | 5.81 ± 0.34 | 5.56 ± 0.18 | 5.61 ± 0.20 | 5.48 ± 0.10 | 5.50 ± 0.16 |

¹% of carcass weight

² Commercial diets supplemented with 0.5% sunflower oil (Group A); 0.5% olive oil (Group B); 0.5% beef tallow (Group C); 0.5% CLA (Group D) or 0.5% hazelnut oil

^{a,b}Means within a row lacking a common superscript differ (P < 0.05)

Discussion

The possibilities of immune stimulants in growing poultry chicks are numerous and could induce antigen exposure from cuts, management procedures (toe and beak trimming) and stimulants in the air, water and feed (Cook *et al.*, 1993). One important immune stimulant was reported to be vaccination procedure against disease outbreaks (Chamblee *et al.*, 1992). As vaccination against the deadly Newcastle virus prevents mortality, weight loss in chickens is being neglected in the poultry industry. Vaccination of chicks was reported to cause a decrease in the rate of protein synthesis in the skeletal muscle and protein accretion (Hentges *et al.*, 1984). Klasing *et al.* (1987) also reported that stimulation of the immune system by a wide variety of immunogens decreased feed intake, feed conversion rate and weight gain in growing chicks.

CLA was shown to act as a growth factor in rats (Chin *et al.*, 1994) and pigs (Ostrowska *et al.*, 1999). In a study conducted in male broiler chickens, the influence of dietary CLA on early inflammatory responses induced by lipopolysaccharide was studied and found that supplementation of CLA into the diets partially prevented reductions in body weight gain (Takahashi *et al.*, 2002). Another study conducted in laying hens showed that dietary CLA modulated certain aspects of the immune system (Politis *et al.*, 2003). The authors suggested that the increased quantity of urokinase plasminogen activator on the membrane of macrophages and heterophils isolated from hens fed the CLA diet might facilitate the ability of those cells to reach the point of a potential inflammation (pro-inflammatory effect) (Politis *et al.*, 2003). In the present study broilers fed a diet containing 0.5% CLA prevented ($P < 0.05$) vaccine-induced reductions in the body weights. Following immune stimulation, cytokines of interleukin-1 (IL-1) and tumour necrosis factor (TNF- α) released by macrophages induce degradation of skeletal muscle and decrease muscle synthesis (Klasing *et al.*, 1987). It was reported that IL-1 stimulated the production of muscle prostaglandin E₂ (PGE₂) (Hellerstein *et al.*, 1989) which is an elongated desaturated product of linoleic acid. Interleukin-1 is a cytokine produced by macrophages following immune stimulation and is capable of decreasing rate of gain and feed intake (Klasing *et al.*, 1987). In a study conducted in rats, it was shown that rats fed a diet containing fish oil did not exhibit a characteristic depression in feed intake when injected with IL-1 (Hellerstein *et al.*, 1989). Application of PGE₂ directly into muscle also caused a wasting process (Rodemann & Goldberg, 1982). Two eicosanoids important in the inflammatory response are PGE series and leukotrienes of the B series (Korver & Klasing, 1997). After feeding diets containing 0% (control), 0.5% or 1% CLA for three weeks, there was a trend toward a reduction in the release of leukotriene B₄ (LTB₄) from the exudate cells in response to the dietary CLA levels (Sugano *et al.*, 1998).

Studies involving fish oil and CLA illustrated possible means of modulating the growth suppressive effects resulting from immune induced alterations in the nutrient metabolism. Omega-3 fatty acids are generally known to decrease the levels of pro-inflammatory cytokines. i.e. IL-1, IL-6 and TNF- α and increase the levels of anti-inflammatory cytokines e.g. IL-2 (Chandrasekar & Fernandes, 1994). In a study conducted on mice, it was reported that CLA supplementation resulted in increased T-cell proliferation and enhanced IL-2 by splenocytes (Hayek *et al.*, 1999). Previously, dietary CLA was reported to decrease the production of prostaglandins in a number of tissues (Cunningham *et al.*, 1997; Whigham *et al.*, 1998). Another study conducted in early weaning pigs suggested that dietary CLA enhances cellular immunity by modulating phenotype and effector functions of CD8⁺ cells involved in both adaptive and innate immunity (Bassaganya-Riera *et al.*, 2001). It was reported that t-10, c-12 CLA, but not c-9, t-11 CLA, was an active isomer of CLA to enhance immunoglobulin (Ig) production *in vivo* (Yamasaki *et al.*, 2003). (Sugano *et al.*, 1998) found that splenic levels of IgA, IgG, and IgM increased while those of IgE decreased significantly in rats fed 1% CLA diet.

In the present study, dietary oils except CLA did not prevent vaccine-induced loss in the body weights. Broiler chickens fed a diet supplemented at the level of 0.5% gained significantly ($P < 0.05$) more in body weight than the other groups. This may be due to prevention of the catabolic activity of immune stimulation by feeding CLA in the broilers (Cook *et al.*, 1993). Zhang *et al.* (2005) showed that dietary CLA at the level of 1% elevated antibody production in male broiler chickens. In the present study, feeding 0.5% hazelnut oil (high in C18:3, n-3) was not effective to prevent vaccine-induced body weight loss. Cook *et al.* (1993) found that growth suppression induced by the injection of endotoxin was markedly reduced in mice and chickens fed diets supplemented with CLA. CLA, which is a group of positional and geometrical isomers of linoleic acid (C18:2, n-6), was found to be more effective to alleviate the effects of endotoxin injection on the growth rate compared to the fish oil. In a study of Miller *et al.* (1994) it was shown that mice fed a basal diet or diet with 0.5% fish oil lost twice as much in body weight after endotoxin injection than mice fed CLA. Cook *et al.* (1993) hypothesized that dietary CLA protected against cytokine-induced muscle wasting by altering the eicosanoid metabolism pathway.

Conclusions

The growth rate in the broilers fed diets supplemented with 0.5% sunflower oil or hazelnut oil was negatively influenced after vaccination, when compared to the other treatment groups. The relative organ weights of the broilers did not differ significantly between dietary treatments. This study showed that the broiler chicks fed a diet supplemented with CLA had better ($P < 0.05$) performance and carcass weights. The

present study also indicated that dietary CLA at a level of 0.5% protected weight loss due to vaccination against Newcastle disease. The actual mechanism of how dietary CLA modulates the effects of vaccination or any antigen is not fully understood. This study might help the poultry industry to prevent the adverse effects of vaccination against Newcastle disease.

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