

the rate-limiting step of mevalonate and ultimately, cholesterol biosynthesis, accounting for Cu's effect on cholesterol levels in the rat [Valsala and Krump (1987) *J Biosci* 12, 137–142].

In a recent report, we presented data confirming the hypothesis that dietary Cu is inversely related to plasma and muscle cholesterol levels in the chicken [Bakalli *et al* (1994) *Poult Sci* 73 (Suppl 1) (in press)]. This inverse relationship exists in chickens fed nutritionally adequate amounts of Cu. Plasma and breast muscle cholesterol and plasma triglycerides and reduced glutathion (GSH) were inversely related to dietary Cu supplements. The duration of Cu supplementation was also inversely related to tissue cholesterol contents. Cholesterol in the edible muscle of broiler chickens was reduced by about 25% without altering the chicken's performance or substantially increasing the Cu content of the meat. The present experiment was conducted to see if the turkey's response is similar to the chickens'.

The experiment was conducted with female poults in wire-floored battery brooders for 21 d. The basal diet was based on maize and soyabean meal and was formulated to meet all National Research Council (1984) nutrient level recommendations including Cu (8 ppm). Cu supplementation was from feed grade cupric sulfate pentahydrate or cupric carbonate (basic). No significant differences due to Cu source were observed, and only main effects are presented.

Significant effects were found for dietary Cu on gain and plasma copper and cholesterol levels. It appears that the turkey poult responds much like the broiler chick to dietary Cu supplements in large excesses of the nutritional requirements.

The levels of dietary Cu that reduce cholesterol in the birds are often fed as growth promotants and mold inhibitors. Thus, we suspect that the poultry meat supplies are very variable in their cholesterol contents. Dietary Cu supplements in excess of what is nutritionally adequate may provide a safe and economical way to lower dietary cholesterol intakes.

Heat-induced changes in lipid metabolism in broilers. H Aïn Baziz¹, PA Geraert², JCF Padilha³, S Guillaumin² (¹ *Institut Technique des Petits Élevages, Algiers, Algeria*; ² *INRA, Recherches Avicoles, 37380 Nouzilly, France*; ³ *CNPq-Universi-*

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In broilers, the first consequence of heat exposure is a reduction of feed intake resulting in a reduction in metabolic heat production and thus maintaining homeothermy. However, even when compared to pair-fed birds exposed to thermoneutrality, heat-exposed chickens exhibited a lower growth [Geraert *et al* (1993) *Proc Nutr Soc*, 52, 165A] and an enhanced fatness [Aïn Baziz *et al* (1993) *Proc 11th European Symposium on the Quality of Poultry Meat, NPSA*, vol 1, 52-58].

The present study was performed in order to analyze the adipose tissues affected by chronic heat exposure: abdominal, sub-cutaneous, inter- or intramuscular and their lipid and fatty acid compositions. The objective was also to determine the origin of the divergence in fatness as changes in lipogenic or lipolytic activities.

The experimental model was based on 3 treatments: TA22 (22°C, *ad libitum* feeding), TA32 (32°C, *ad libitum* feeding) and TR22 (22°C, pair-feeding on the heat-exposed TA32 birds), 36 chickens per treatment. Ambient temperature was kept constant between 4 and 6 weeks of age.

At 6 weeks of age, TA32 chickens had a lower weight gain (-41%) compared to TA22 and -22% when compared with pair-fed birds. Heat-exposed chickens were also fatter; the abdominal to body-weight ratio reached 3.28% in TA32, 2.85% in TA22 and 1.86% in TR22. The dissection of adipose tissues from the leg revealed increased sub-cutaneous and intermuscular tissues in heat-exposed birds while intramuscular depot remained unchanged.

Lipid content of abdominal fat was not different between TA22 and TA32 but it was significantly decreased in the restricted-fed group (TR22). Fatty-acid profiles were modified under heat exposure. While saturated fatty-acid proportions, particularly palmitic acid (C16:0) were increased, unsaturated fatty acid in per cent of total fatty acids were decreased, especially oleic (C18:1) and linoleic (C18:2) acids. Such changes would suggest a decrease in hepatic desaturase activity in heat-exposed broilers. While plasma triglyceride concentrations were little affected by hot conditions, plasma-free fatty-acid contents were significantly reduced in either fasted or fed TAF32 birds compared to control-exposed chickens. The enhanced fatness observed under hot conditions would thus be due more to a reduction in lipolysis than to an increase in lipogenesis.

This could be related to hormonal changes [Padilha *et al* (1994) *Reprod Nutr Dev* (in press)].

III. Metabolism and endocrinology

Effect of insulin, and oleic acid on triglyceride secretion by chicken hepatocytes in primary culture. Role of the $\Delta 9$ desaturase. P Legrand, E Le Bihan, D Catheline, MC Fichot, P Lemarchal (INRA, Laboratoire de Biochimie, 65, rue de Saint-Brieuc, 35000 Rennes, France)

Our aim is the study of the mechanisms which underlie fattening in the chicken. In this species, the liver is the main site for fatty-acid and triglyceride (TG) biosynthesis. The TG are secreted and transported in the blood in the form of very low density lipoprotein (VLDL) towards tissues for utilization or storage (adipose tissue). We developed a primary culture system of chicken hepatocytes in order to study the TG synthesis and secretion, by incorporation of ^3H -glycerol into intracellular and secreted TG. We first proposed a new method for secretion measurement, since we considered that simple medium removal underestimated TG secretion. Indeed, an important amount of VLDL seemed to be trapped in the unstirred water layer around the cells. Thus, after removing the medium, we incubated the cells briefly with trypsin to obtain a second fraction of medium and the cells. We first demonstrated that 90% of the labelled TG were in the form of VLDL in both medium fractions. We observed that the TG secretion increased at the beginning of the culture and reached a plateau after 1 or 2 d of culture. The secretion rate in the absence of insulin varied between 3 and 20 nmol TG/mg cell protein/h depending on the donor animal. Among regulating factors, insulin has a controversial effect on TG secretion. We report here a clear significant activating effect of insulin. Labelled glycerol incorporation into secreted TG was 2.3-fold higher if insulin was present in the culture medium. Another activating factor is the availability of oleic acid (produced by the $\Delta 9$ desaturase). We proposed this hypothesis since we studied lipid metabolism in the fat and lean lines of chickens (selected by Leclercq, INRA, Tours) and reported that both hepatic $\Delta 9$ desaturase activity and plasma VLDL-TG concentration were higher in the fat animals than in the lean ones. In order to elucidate

the role of oleic acid on TG secretion, we first investigated and identified a regulated $\Delta 9$ desaturase activity in cultured hepatocytes. We then blocked the $\Delta 9$ desaturase activity with a specific inhibitor (sterculic acid), and observed that TG secretion was significantly reduced whereas TG synthesis remained unchanged. Moreover, the addition of oleic acid in the culture medium, in the presence of the $\Delta 9$ desaturase inhibitor, partly restored the TG secretion. Our results demonstrate that oleic acid is a limiting factor for the TG secretion process, and that the $\Delta 9$ desaturase plays an important role in this process.

Influence of amino-acid concentration and insulin in culture media on amino-acid and glucose incorporation into cellular and secreted (VLDL) lipids by chicken hepatocytes in primary culture. JP Caffin ^{1,2} (¹ Université Pierre-et-Marie-Curie; ² INRA, Station de Recherches Avicoles, 37380 Nouzilly, France)

The liver is the main site of lipogenesis in birds. Hence, the hepatocytes are a useful model for studying the mechanisms controlling lipogenesis.

Hepatocytes were isolated from broiler-type chicken (6–7 weeks old) by collagenase dispersion. The cells were plated at a density of $5 \cdot 10^5$ cells/cm² at 37°C under a controlled atmosphere (95% air and 5% CO₂) in a medium containing Earle's balanced salts supplemented with MEM-vitamins, glucose (2 g/l), amino acids (2.38 g/l), fetal calf serum (2.5%), and chicken serum (3.5%) in plastic 6-well plates. After plating, the medium was replaced with medium without fetal calf serum or chicken serum, with variable amino-acid concentrations (0.3–5.96 g/l) and with or without insulin (100 nM). After the time of preincubation the medium was supplemented with ^3H -glycerol (25 nM) and U ^{14}C -protein hydrolysate or U ^{14}C -glucose and the incubation was continued for 2–24 h. The incorporation was stopped by medium aspiration and the cells were lysed by freeze-thawing in water. The chloroform/methanol extracts of medium and cells were used for radioactivity determination.

The increase in amino-acid concentration in culture media increased incorporation of amino acids and glucose into cellular and secreted lipids (VLDL) with a cooperative effect on glucose incorporation into secreted lipids (VLDL). Increases