

PLASMA LIPOPROTEIN PROFILES IN BROILER CHICKS (*GALLUS DOMESTICUS*): EFFECTS OF EARLY OVERFEEDING

Abstract—1. Twenty-eight day old broiler chicks gavage fed for 22 days consumed 40% more feed and gained 100–110 g more than control birds.

2. Normal feeding resumed on day 41, by day 63 feed intakes and body weights of overfed and control birds were equal.

3. Moderate differences in lipid compositions of LDL and VLDL were observed at 41 and 50 but not at 63 days.

4. In contrast to mammals where early over feeding predisposes to adult obesity, lipoprotein profiles and *ad lib* feeding patterns of chicks are not readily altered by dietary measures.

INTRODUCTION

The chicken, *Gallus domesticus*, has long been considered a suitable animal model for comparative studies of lipid metabolism because the liver is the main site of *denovo* fatty acid synthesis (Leveille *et al.*, 1975) as it is for man (Luskey *et al.*, 1974). However, in general mammalian adipose tissue is more active in lipid biosynthesis than is the adipose tissue of birds (Pearce, 1977). Lipid transport also differs in that portomicrons transport dietary lipids from the gut directly to the liver (Bensadoun and Rothfeld, 1972) and high density lipoproteins (HDL) predominate in the plasma of chickens other than the laying hen (Yu *et al.*, 1976). Lipid metabolism in avian species is not well understood, and information accumulated from studies of mammals may not be applicable.

Both genetic and nutritional factors affect adult body weight, tissue biochemistry and plasma lipids. Differences in fat deposition among breeds and strains of chickens demonstrate the effects of genetic factors on body composition (Edwards and Denman, 1975; Richard, 1975; Van Middlekoop *et al.*, 1977; Cherry *et al.*, 1978). Lipogenic and lipolytic enzyme activities are correlated with the genetic factors favoring high or low body weight (Hood and Pym, 1982; Lilburn *et al.*, 1982; Calabotta *et al.*, 1983) and the sex-linked gene, *dw*, has been shown to influence lipid metabolism (Granhdi *et al.*, 1975; Guillaume, 1976). Plasma lipoprotein profiles and particularly the concentration of very low density lipoprotein (VLDL) were used as selection criteria for the development of strains of lean or fat broiler chicks (Griffin *et al.*,

1982; Whitehead and Griffin, 1984). Nutritional factors and age of the birds also affect the enzymatic control of lipid metabolism and patterns of fat deposition (Shapira *et al.*, 1978; McMurtry *et al.*, 1988; Deaton and Lott, 1985). Pronounced changes in plasma lipid levels and composition occur in response to changes in diet (Leveille *et al.*, 1967; Beynen *et al.*, 1984). The objective of this study is to evaluate the effects of early feeding patterns on lipid metabolism in the broiler chick and specifically on the lipoprotein profile.

MATERIALS AND METHODS

Animals and diets

Forty-two male Shaver broiler chickens were randomly assigned to either a control or overfed treatment group. Each treatment group was fed a commercial starter diet composed of 24% protein, 4% crude fat, 4% crude fiber, 3042 kcal/kg metabolizable energy. Both the control group and the overfed group (21 birds) had free access to the diet. Feed consumption and body weight were recorded at least every other day from 28 days of age until death. To study the effects of overfeeding, a water slurry of the starter diet (35% feed, by weight) was administered by gavage four times daily from day 29 to 40 to the 21 birds in the overfed treatment group. On day 41, blood was obtained from seven birds of each group, these birds were then killed and their fat pads harvested (McMurtry *et al.*, 1988). *Ad lib* feeding was continued for all remaining birds until days 50 and 63 when plasma and abdominal fat pads were collected from seven birds of each group (only six birds were available for the overfed group at 63 days of age).

Lipoprotein preparation

Blood from the brachial vein was collected into an anticoagulant (sodium citrate) solution. Plasma was prepared by centrifugation at 1000 g for 10 min and was stored at -60°C until analyses for lipoproteins could be begun. To prevent microbial growth, EDTA, sodium azide, and

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thimerosal, at final concentrations of 0.04, 0.01 and 0.001% (w/v) were added to the plasma as it thawed (Mills *et al.*, 1984). Lipoprotein fractions were isolated from plasma by density gradient ultracentrifugation as described by Hermier *et al.* (1985). Plasma density was adjusted to 1.21 g/ml by the addition of 0.275 g NaBr/ml. Solutions of other densities were prepared by adding solid NaBr to aliquots of 0.15 M NaCl at pH 7.4 containing EDTA and azide as described above. A stepwise density gradient consisting of (2 ml of 1.27 g/ml, 3 ml of plasma adjusted to 1.21 g/ml, 2 ml of 1.06 g/ml, 2.5 ml of 1.019 g/ml, 2.5 ml of 1.006 g/ml) was prepared as described by Chapman *et al.* (1981) and layered into 13 ml Ultraclear (Beckman) tubes using a peristaltic pump. Ultracentrifugation was performed at 15°C in a Model L8-70 (Spinco Div., Beckman Inst., Palo Alto, CA) preparative ultracentrifuge in the slow acceleration mode using the SW-40 Ti swinging bucket rotor for 48 hr at 285,000 g. After completion of the centrifugation, a Pasteur pipet was used to remove a 2 ml fraction containing the VLDL from the top of each tube, a peristaltic pump was then used to remove the remaining contents from the top of each tube in 11 equal fractions. The refractive index of each fraction was measured, and the density calculated. All fractions were dialyzed for 48 hr against decreasing concentrations of NaCl, from 0.15 to 0.075 M, dialyzates (pH 7.4) contained 1 mM EDTA and 0.01% sodium azide.

Analytical methods

Whole plasma and dialyzed lipoprotein fractions were stained for lipid with Sudan Black, and electrophoresed on a discontinuous polyacrylamide-gel gradient (separating gel, 3.6%, spacer gel 2.5%, sample gel 3.3%) constructed in tubes as described by Naito and Wada (1980).

Total lipids from each dialyzed lipoprotein fraction were extracted at 0°C with ethanol/diethyl ether as described by Brown *et al.* (1969). Extracted lipids were dried under N₂ and weighed to give the total lipid concentration. Individual lipid classes were separated by thin layer chromatography (TLC), following Method 1 of the Total Lipid Separation described by Yao and Rastetter (1985). High performance LHP-K TLC plates (10 × 10 cm, Whatman Inc., Hillsboro, OR) were twice washed by predevelopment in methanol and activated at 100°C. Lipid residues dissolved in chloroform-methanol (2:1) and spotted onto the TLC plates were first developed in methyl acetate:1-propanol:chloroform:methanol:0.25% KCl (25:25:25:10:9) to a distance of 4.5 cm above the preadsorbent-sorbent division. After 15 min of drying with hot air, a second development in hexane:diethyl ether:acetic acid (75:23:2) proceeded to 7 cm above the preadsorbent layer. After a second drying period, the plates were again developed to 7 cm in hexane alone. To visualize spots, plates were dipped into a solution of 10% copper sulfate in 8% phosphoric acids, drained for 2 min and charred for 15 min at 150°C. Plates were then scanned at 350 nm on a TLC scanner (Camag Scientific, Inc., Wrightsville Beach, NC) equipped with an integrator. Concentrations of specific lipid types were determined by comparison with standard curves established for individual lipids.

Protein concentrations were estimated from ultraviolet scans of each fraction, and by the Pierce (Rockford, IL) BCA method. Electrophoretic patterns of the apolipoproteins were obtained by SDS-PAGE on 8–25% gradient gels using the Phast-Gel System (Pharmacia, Piscataway, NJ). Gels were stained with Coomassie Blue R, and band intensities quantitated at 600 nm on a BioRad (Richmond, CA) Model 620 Video Densitometer.

RESULTS

Feed consumption and weight gain

Feed consumption and weight gains of the birds in the overfed and control groups are compared in

Fig. 1. During the overfeeding period, days 29–40, birds in the overfed group consumed 2200 ± 57 g of feed compared to 1570 ± 43 g per bird in the control group. The overfeeding period was followed by a period of feed refusal on the first day after gavage, and a continuing level of feed intake lower than that of the control birds (Fig. 1a). On day 41, at the cessation of gavage, the average feed consumption of the overfed group was 68 ± 8 g compared to 133 ± 4 g for the control group. Over the interval between days 42 and 50, feed consumption was 1070 ± 62 g and 1410 ± 55 g per bird in the overfed and control groups respectively. By 63 days, feed consumption of the overfed group was 90% of that of the control group.

The average change in weight over each 2 day interval is plotted in Fig. 1b. At 28 and again at 63 days of age all birds were essentially the same weight

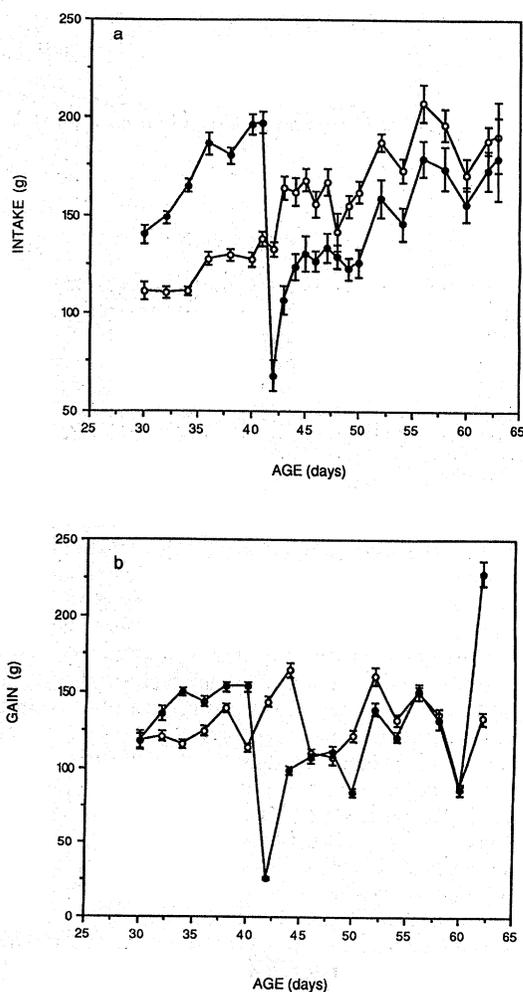


Fig. 1. Feeding patterns and weight gains for birds in the control and overfed groups. Points for days 28–41 are based on 21 birds in each group, points for days 42–50 on 14 birds in each group, and points for days 51–63 on 7 birds in the control group and 6 in the overfed group. Feed intake (a) is in g/day for control birds (○) allowed to feed *ad lib* during the entire time, and overfed birds (●) subjected to gavage 4 × daily on days 29–41 followed by *ad lib* feeding. Weight gains (b) are for 2 day periods. Mean values \pm S.E.M.

Lipoprotein profiles in broiler chicks

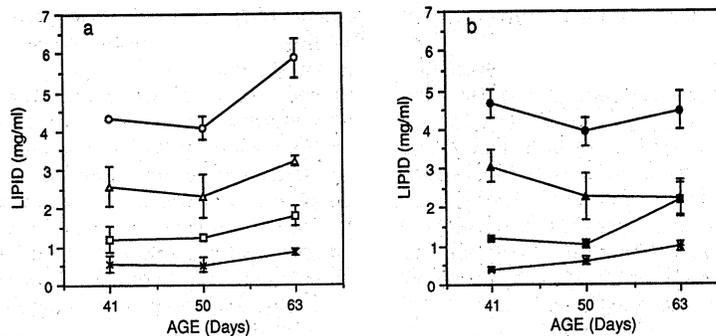


Fig. 2. Lipoprotein concentrations (mg/ml) in the plasma of control (a) and overfed (b) birds at 41, 50 and 63 days of age. In each case the uppermost curve represents total plasma lipid followed by HDL, LDL and VLDL. Mean values \pm S.E.M.

(850 ± 20 g and 3000 ± 50 g, respectively). During the gavage period, the overfed birds gained 10–100 g per day more than the control birds. A portion of this excess weight gain by the chicks during the gavage period was lost during the feed refusal period, and the rest during a period of slower gain. On the first day following the gavage period, the overfed birds lost an average of 15 g each while the control birds gained 81 g. By 46 days of age the rate of weight gain was similar for both groups of birds.

Lipoprotein distribution

The distribution of lipoproteins over the density gradient was similar in all cases. VLDL ($d < 1.016$) formed a thin opalescent film at the top of each centrifuge tube, a layer of clear salt solution separated this film from the low density lipoprotein (LDL) ($d 1.020-1.046$) layer, a pale yellow, slightly turbid band. Below a second clear colorless layer of salt solution, the HDL ($d 1.052-1.130$) fraction formed a clear deep yellow band.

The electrophoretic pattern obtained for prestained whole plasma and dialyzed fractions from the density gradient on discontinuous gels in tubes confirmed the similar distribution of lipoproteins in plasma from

overfed and control birds. Electrophoresis was continued until the HDL fraction reached the bottom of the 3.6% separating gel, at this time the VLDL fraction remained at the interface between the sample gel and the spacer gel (2.5% acrylamide), the LDL fraction had entered the separating gel and moved about 10% of its length. Patterns obtained on discontinuous gel electrophoresis appeared identical for all samples.

In Fig. 2 total plasma lipid and the various lipoprotein fractions from control (Fig. 2a) and overfed (Fig. 2b) birds are compared. For both groups of birds, total lipid was highest at 63 days of age and lowest at 50 days of age. For the control birds, VLDL, LDL, and HDL levels were a constant fraction of total lipid at all ages. In contrast, for birds subjected to gavage VLDL levels increased and HDL levels decreased relative to total plasma lipid as the bird aged.

The concentrations of specific lipids in each class of lipoprotein are listed in Table 1. Patterns of individual lipids in the various fractions were generally similar. For both feeding regimens at all ages the distribution of free cholesterol and cholesterol ester among the lipoprotein classes was essentially the

Table 1. Lipid distribution from control (C) and overfed (OF) birds

Day	Group	FC*	CE	PL	TG	FFA
<i>VLDL</i>						
41	C	0.08 \pm 0.05	0.00	0.02	0.06	0.38 \pm 0.10
41	OF	0.04 \pm 0.01	0.00	0.11 \pm 0.03	0.15 \pm 0.03	0.09 \pm 0.02
50	C	0.17	0.00	0.08 \pm 0.01	0.13 \pm 0.01	0.11 \pm 0.01
50	OF	0.14 \pm 0.01	0.02 \pm 0.01	0.13 \pm 0.01	0.23 \pm 0.01	0.08
63	C	0.02 \pm 0.01	0.00	0.00	0.84 \pm 0.01	0.00
63	OF	0.06 \pm 0.02	0.00	0.05 \pm 0.03	0.89 \pm 0.02	0.00
<i>LDL</i>						
41	C	0.34 \pm 0.04	0.03 \pm 0.00	0.20 \pm 0.01	0.21 \pm 0.04	0.39 \pm 0.02
41	OF	0.26 \pm 0.03	0.01 \pm 0.01	0.32 \pm 0.02	0.25 \pm 0.04	0.31 \pm 0.02
50	C	0.33 \pm 0.03	0.08 \pm 0.06	0.20 \pm 0.06	0.24 \pm 0.02	0.35 \pm 0.05
50	OF	0.33 \pm 0.04	0.03 \pm 0.01	0.21 \pm 0.02	0.23 \pm 0.02	0.19 \pm 0.03
63	C	0.30 \pm 0.04	0.03 \pm 0.01	0.41 \pm 0.06	0.89 \pm 0.15	0.29 \pm 0.05
63	OF	0.24 \pm 0.13	0.03 \pm 0.02	0.29 \pm 0.14	1.08 \pm 0.25	0.54 \pm 0.12
<i>HDL</i>						
41	C	0.22 \pm 0.07	0.61 \pm 0.03	1.34 \pm 0.10	0.18 \pm 0.03	0.18 \pm 0.02
41	OF	0.29 \pm 0.11	0.82 \pm 0.20	1.20 \pm 0.34	0.29 \pm 0.08	0.27 \pm 0.07
50	C	0.23 \pm 0.06	0.26 \pm 0.05	1.42 \pm 0.10	0.18 \pm 0.10	0.07 \pm 0.01
50	OF	0.32 \pm 0.03	0.35 \pm 0.05	1.20 \pm 0.07	0.13 \pm 0.02	0.07
63	C	0.42 \pm 0.08	0.83 \pm 0.15	1.45 \pm 0.23	0.39 \pm 0.11	0.45 \pm 0.08
63	OF	0.30 \pm 0.02	0.60 \pm 0.12	0.67 \pm 0.16	0.20 \pm 0.06	0.36 \pm 0.06

*FC—free cholesterol, CE—cholesterol ester, PL—phospholipid, TG—triglyceride, FFA—free fatty acids. Concentrations are in mg/ml. Mean \pm S.E.M., $n = 4$.

same, as a per cent of total lipid. Phospholipids accounted for 25–30% the VLDL and LDL fractions from overfed birds at 41 days of age, but formed a negligible fraction of the VLDL and 16% of the LDL from control birds at this age. At 50 days of age phospholipid accounted for 16% of both VLDL and LDL lipids from the control birds and 20% in each of these classes from the overfed birds. The fraction of HDL-phospholipid was higher for control birds at all ages than for the overfed birds. For control birds, VLDL-triglyceride levels were 10, 26, 98% at 41, 50, and 63 days of age; whereas the corresponding levels for overfed birds were 38, 38, and 90%. At 41 days of age 70% of VLDL lipid from the control birds was in the form of free fatty acids but only 22% of VLDL lipid from the overfed birds. Free fatty acids made up 32, 28, and 16% of LDL lipids at 41, 50, and 63 days in control birds, the corresponding values for overfed birds were 26, 18, and 24%.

Total apolipoprotein was 40 ± 4 mg/ml for plasma for each age and treatment. For the control birds $95 \pm 0.5\%$ of this protein was HDL and $4.5 \pm 0.3\%$ LDL at all ages, VLDL protein was less than 1% of the total. In plasma from overfed birds there was a small increase in LDL protein relative to HDL protein as the birds aged, from 2% LDL protein at 41 days to 8% at 63 days, with a corresponding decrease in HDL protein while VLDL protein remained less than 1%.

Individual apolipoproteins were examined by SDS-PAGE of delipidated plasma on an 8–25% polyacrylamide gradient. Apoprotein distributions of these samples were indistinguishable. Analysis of these gels using the densitometer showed 50–60% apolipoprotein A1 (MW = 28,000), 18–25% apolipoprotein B and high molecular weight fragments of apoB (MW > 200,000), and 10–20% in two low molecular weight bands (16,000 and 14,000).

DISCUSSION

In mammals, overfeeding during the suckling period induces persistent obesity while underfeeding during this period leads to lower adult body weight in mice (Aubert *et al.*, 1980). In chickens, early feed restriction has been shown to produce adults with lower than normal body weight (McMurtry *et al.*, 1988). In the present study, the overfeeding period was followed by a period of feed refusal on the first day after gavage, and a continuing level of feed intake lower than that of the control birds (Fig. 1a). A portion of the excess weight gain by the chicks during the gavage period was lost during the feed refusal period, and the rest during a period of slower gain. Ultimately, the effects of overfeeding were reversed during an equivalent period of *ad lib* feeding.

Aubert *et al.* (1980) reported a slight variation in lipoprotein profiles, observed as a 55% increase in LDL cholesterol ester, of adult mice subjected to early over feeding when compared to control mice. When 10-week old chickens were force-fed for 7 days a 6 fold increase in VLDL was observed 20 min after the final feeding (Leclercq *et al.*, 1974). VLDL levels remained elevated over those of the control birds during the sampling period in that study, but at three hours had dropped to 80% of the 20 min level. In the

present study, the 12 hr *ad lib* feeding period between the final gavage treatment and the collection of plasma samples was sufficient for total lipid levels to return to near normal. Once this gross normalization had occurred, more subtle effects of overfeeding on the lipoprotein profile could be observed. These effects include the rapid normalization of cholesterol and cholesterol ester levels (complete by day 41) and the slower changes in phospholipid, triglyceride and free fatty acid distributions. All effects were relatively short term, in that by 63 days of age the lipid profiles of the two groups of birds were identical. The only notable difference at 63 days of age was the failure of the overfed birds to maintain as high an HDL level as the control birds.

Both lipid and apolipoprotein compositions of the lipoprotein fractions were in general agreement with avian lipoprotein compositions reviewed by Chapman (1980) except for cholesterol esters which were lacking from the VLDL fractions and present at a low level in LDL fractions from both control and overfed birds.

In conclusion the results of this study show a subtle alteration in lipoprotein profiles as an aftereffect of early overfeeding. The difference between the mammalian system where the distribution of cholesterol ester was altered (Aubert *et al.*, 1980) and the broiler chick where variation in phospholipid and triglyceride levels was greater may imply a difference in regulation of these levels in the different species. The lack of any long term (63 days of age) effects strongly suggests that the broiler chick has genetically determined metabolic controls which determine patterns of *ad lib* feeding and related weight gain as well as lipid and apolipoprotein levels in the plasma lipoproteins, and that these controls are not easily overridden.

REFERENCES

- Aubert R., Suquet J.-P and Lemonnier D. (1980) Long-term morphological and metabolic effects of early under- and over-nutrition in mice. *J. Nutr.* **110**, 649–661.
- Bensadoun A. and Rothfeld A. (1972) The forms of absorption of lipids in the chicken, *Gallus domesticus*. *Proc. Soc. Exp. Biol. Med.* **41**, 814–817.
- Beynen A. C., Katan M. B. and Van Zutphen L. M. F. (1984) Plasma lipoprotein profiles and arylesterase activities in two inbred strains of rabbits with high or low response of plasma cholesterol to dietary cholesterol. *Comp. Biochem. Physiol.* **79B**, 401–406.
- Brown W. V., Levy R. I. and Fredrickson D. S. (1969) Studies of the proteins of human plasma very low density lipoproteins. *J. Biol. Chem.* **244**, 5687–5694.
- Calabotta D. F., Cherry N. A., Siegel P. B. and Gregory E. M. (1983) Lipogenesis and lipolysis in normal and dwarf chickens from lines selected for high and low body weight. *Poult. Sci.* **62**, 1830–1837.
- Chapman M. J. (1980) Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* **21**, 789–853.
- Chapman M. J., Goldstein S., Lagrange D. and Laplaud P. M. (1981) A density gradient ultracentrifugation procedure for the isolation of the major lipoprotein classes from human serum. *J. Lipid Res.* **22**, 339–357.
- Cherry J. A., Ghitelman M. Z. and Siegel P. B. (1978) The relationship between diet and dwarfism in diverse genetic backgrounds on egg parameters. *Poult. Sci.* **57**, 171–179.

- Deaton J. W. and Lott B. D. (1985) Age and dietary energy effect on broiler abdominal fat deposition. *Poult. Sci.* **64**, 2161–2164.
- Edwards H. M. and Denman F. (1975) Carcass composition studies: 2. Influences of breed, sex and diet on gross composition of the adipose tissue. *Poult. Sci.* **54**, 1230–1238.
- Granhdi R., Brown R. G. and Summers J. D. (1975) A study of thyroid activity in dwarf and non-dwarf female chicks during key physiological states of growth and reproduction. *Poult. Sci.* **54**, 47–53.
- Griffin H. D., Whitehead C. C. and Broadbent L. A. (1982) The relationship between plasma triglyceride concentrations and body fat content in male and female broilers—a basis for selection? *Brit. Poult. Sci.* **23**, 15–23.
- Guillaume J. (1976) The dwarfing gene *dw*: Its effects on anatomy, physiology, nutrition, management. Its application in poultry industry. *World's Poult. Sci. J.* **32**, 285–305.
- Hermier D., Forgez P. and Chapman M. J. (1985) A density gradient study of the lipoprotein and apolipoprotein distribution in the chicken, *Gallus domesticus*. *Biochim. Biophys. Acta* **836**, 105–118.
- Hood R. L. and Pym R. A. E. (1982) Correlated responses for lipogenesis and adipose tissue cellularity in chickens selected for body weight gain, food consumption and food conversion efficiency. *Poult. Sci.* **61**, 121–127.
- Leclercq B., Hassan I. and Blum J. C. (1974) The influence of force-feeding on the transport of plasma lipids in the chicken (*Gallus gallus* L.). *Comp. Biochem. Physiol.* **47B**, 289–296.
- Leveille G. A., Pardini R. S. and Tillotson J. A. (1967) Influence of medium-chain triglycerides on lipid metabolism in the chick. *Lipids* **2**, 461–466.
- Leveille G. A., Romsos D. R., Yeh Y-Y. and O'Hea E. (1975) Lipid biosynthesis in the chick. A consideration of the site of synthesis, influence of diet and possible regulating mechanisms. *Poult. Sci.* **54**, 1075–1093.
- Lilburn M. S., Morrow F. D., Leach R. M., Jr Buss E. G. and Martin R. J. (1982) A comparison of the *in vitro* lipogenic rates and other physiological parameters in 2 strains of lean and obese chickens. *Growth* **46**, 163–170.
- Luskey K. L., Brown M. S. and Goldstein J. L. (1974) Stimulation of the synthesis of very low density lipoproteins in rooster liver by estradiol. *J. Biol. Chem.* **249**, 5939–5947.
- McMurtry J. P., Roseborogh R. W., Plavnik I. and Cartwright A. L. (1988) Influence of early plane of nutrition on enzyme systems and subsequent tissue desposition. In *Beltsville Symposium in Agricultural Research 12. Biomechanisms Regulating Growth and Development* (Edited by Steffens G. L. and Rumsey T. S.), pp. 329–341. Kluwer Academic Publishers, Boston.
- Mills G. L., Lane P. A. and Weech P. K. (1984) The collection and preservation of blood plasma. In *A Guidebook to Lipoprotein Technique. Lab. Tech. Biochem. Mol. Biol.* (Edited by Burdon R. H. and van Knippenberg P. H.), Vol 14, pp. 449–459. Elsevier, Amsterdam.
- Naito H. K. and Wada M. (1980) The use of polyacrylamide-gel electrophoresis for the detection of dyslipoproteinemia. In *Handbook of Electrophoresis*, Vol. 1, *Lipoproteins: Basic Principles and Concepts* (Edited by Lewis L. A. and Oppl J. J.), pp. 183–219. CRC Press, Boca Raton, FL.
- Pearce J. (1977) Minireview: Some differences between avian and mammalian biochemistry. *Int. J. Biochem.* **8**, 269–275.
- Richard F. H. (1975) Facteurs genetique influent la qualite des carcasses du poulet. In *The Quality of Poultry Meat* (Edited by Erdtsieck B.), pp. 1–16. Proceedings of the 2nd European Symposium on Poultry Meat Quality. Oosterbeek, The Netherlands.
- Shapira N., Nir I. and Budowski P. (1978) Response of lipogenic enzymes to overfeeding in liver and adipose tissue of light and heavy breeds of chicks. *Brit. J. Nutr.* **39**, 151–158.
- Van Middlekoop J. H., Kuit A. R. and Zegwaard A. (1977) Genetic factors in broiler fat deposition. In *Growth and Poultry Meat Production* (Edited by Boorman K. N. and Wilson J. R.), p. 131. Brit. Poult. Sci., Edinburgh.
- Whitehead C. C. and Griffin H. D. (1984) Development of divergent lines of lean and fat broilers using plasma very low density lipoprotein concentrations as selection criterion: The first three generations. *Brit. Poult. Sci.* **25**, 573–582.
- Yao J. K. and Rastetter G. M. (1985) Microanalysis of complex tissue lipids by high-performance thin-layer chromatography. *Anal. Biochem.* **150**, 111–116.
- Yu J. Y-L. Campbell L. D. and Marquardt R. R. (1976) Immunological and compositional patterns of lipoproteins in chicken (*Gallus domesticus*) plasma. *Poult. Sci.* **55**, 1626–1631.