CHAPTER 5

THE INFLUENCE OF RESTRICTED FEEDING DURING REARING ON BODY COMPOSITION IN POULTRY

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5.1 INTRODUCTION

The magnitude of the effects on body composition of poultry at the cessation of rearing feed restriction depends primarily on the extent of liveweight reduction, and therefore *inter alia*, in the degree of reduction in cumulative nutrient intake (see Section 1.5.1.3, Figure 1.4, Chapter 1). However, a major confounding factor in the assessment of such alterations is the time or age at which body composition is determined. Many studies showed marked differences in body composition between birds restricted in feed intake compared to birds allowed *ad libitum* intake when measured immediately at cessation of the restriction programmes (Lee *et al.* 1971b; Gous and Stielau 1976; Maclachlan *et al.* 1977a). Although some studies found alterations at sexual maturity (Fuller *et al.* 1969; Connor *et al.* 1977b), the magnitude of these effects will clearly be influenced by factors which are without influence when body composition is determined at the same chronological age. For example, the amount of time between cessation of feed restriction and attainment of sexual maturity may have a large influence on body composition at sexual maturity. Body composition at sexual maturity is clearly a good indication of fundamental physiological alterations due to restricted feeding. This is particularly important for investigations in egg producing birds because of special difficulties in the determination of continued or subsequent changes in body composition. These difficulties are apparent because the production of eggs *per se* can cause changes in body composition which are unrelated to rearing treatment, particularly when external (e.g., temperature) or internal (e.g., behavioural stress) factors cause nutrient inadequacies in dietary intake.

Lee *et al.* (1971a) concluded that there was a need for more information on the effects of restricted feeding on body composition of poultry, but little work has yet been carried out (see Table 1.5, Chapter 1). There is no information on body composition after realimentation and proceeding sexual maturity, particularly with regard to the influence of type of feed restriction. These considerations are important not only because they represent unknown effects, but because there were suggestions that body composition *per se* may be a determinant of egg production (Fuller *et al.* 1969; Gous 1972; Greenberg 1976; Neil *et al.* 1977). In

addition, there is little information on the effects of normal feed restriction programmes on liver composition and liver lipogenesis. Studies on other animal species, particularly on the mouse and rat, showed that certain feeding regimens, similar to the time-limitation methods used in poultry (e.g. meal feeding), caused a marked liver hyperlipogenesis (Tepperman and Tepperman 1958; Tepperman and Tepperman 1964). Studies on young birds confirmed this effect (Leveille 1966; Yeh and Leveille 1970; Leveille and Yeh 1972; Simon and Brisson 1972), and Balnave *et al.* (1979) found alterations in liver weight and lipid content with an indication of concommitant changes in the specific enzyme activities of important lipogenic enzymes (ATP citrate lyase and NADP-Malate dehydrogenase) in layer type birds on limited-time restricted feeding at approximately 13 weeks of age.

This chapter therefore presents the second part of an integrated investigation of body composition in poultry, namely, the effects of rearing nutrition. The equations derived in Chapter 4 were used to predict body composition at certain chronological and physiological ages; detailed studies on liver composition and a single study on liver lipogenesis were also carried out.

5.2 MATERIALS AND METHODS

Birds were derived from the two experiments (Experiments 1 and 2) presented in Chapter 3. Management procedures and details of the three rearing treatments are given in Section 3.2, Chapter 3. As in that chapter, these treatments will be referred to as *ad libitum* (A), limited-time restriction (TR) and quantitative restriction (QR). In Experiment 1, six birds from each treatment were randomly selected at 39 d (*ad libitum* (A) only), 70 and 101 d (*ad libitum* (A) and limited-time (TR)), 162 d, 218 d of 337 d of age. In Experiment 2 four birds were similarly selected from each treatment at 280 d and 476 d of age for body composition determination. Prior to slaughter birds were deprived of feed and water for 2 h and injected with water isotope(s) as described in Section 4.2, Chapter 4. Methods used to slaughter birds, for maceration and carcass preparation, sampling procedures and to determine the chemical composition of the carcasses, are given in Chapter 2. Livers were removed for separate analyses as described in Chapter 2, Section 2.4.1. All carcass composition results reported were corrected to include the determined liver composition.

Body composition was also predicted at different physiological and chronological ages in Experiment 2 using deuterium oxide (D_2O) and the equations derived in Chapter 4. The equations used were the following:

(1) Total body water (TBW, g)

$TBW = 51.3 + 0.315W + 0.361D$ ..... Equation 16, Table 4.10

(2) Protein (P, g)

$P = 47.4 + 0.093W + 0.137D$ ..... Equation 44, Table 4.16

(3) Fat (F, g)

$F = -109.1 + 0.484W - 0.403D$ ..... Equation 49, Table 4.16

Appropriate statistics for these equations are given in the relevant tables in Chapter 4. Using these equations body composition was predicted at sexual maturity (first oviposition) for the majority of the birds (50 per treatment), after production of an equivalent number of eggs (180) for twenty birds from each treatment, at 364 d of age for six birds per treatment and at the same time (220 d) after sexual maturity for six birds per treatment.

Liver lipogenesis was estimated *in vivo* by U-C^{14}-acetate injections during Experiment 2 when birds were 120 d of age. Nine birds were randomly selected from each treatment, and incorporation studies carried out at three different times (periods) on three birds from each treatment at each time to determine changes which occur during each of the feeding cycles. For the *ad libitum* (A) treatment, feed was continually available and birds (N = 3/time) were injected at 0830 h, 1030 h and 1430 h. Birds on the limited-time restriction treatment (TR) were on *ad libitum* feed intake up to 1030 h when feed was removed and three birds were injected at 1430 h. The following day another three birds were injected at 1430 h (after a total feed deprivation of 28 h), and the next day feed was offered at 0815 h and the remaining birds injected at 1430 h. Birds (N = 3/time) on the quantitative feed restriction treatment (QR) were injected prior to the morning feed at 0815 h, at 1030 h after receiving their feed allowance at 0830 h, and at 1430 h.

Injections were given via the wing vein and feed, if available, was not removed after injection. The U-C^{14}-acetate in normal saline contained 2.5 μc/ml and birds were injected with between 1 and 1.5 ml. The

three birds per treatment at each time period were weighed and slaughtered 1 h after injection. The liver was removed rapidly and the procedures given in Section 2.4.1, Chapter 2 were carried out. Livers were placed in small plastic bags and kept on ice until they were taken to the laboratory and frozen with liquid nitrogen (N_2).

Extracted lipid samples (Folch *et al.* 1957) were redissolved in chloroform and small samples were placed in tared scintillation vials and dried in a dessicator. Samples were reweighed to determine the quantity of lipid present (*c.* 30 mg), 10 ml scintillation liquid was added and radioactivity (SR) determined (see Section 4.2.6, Chapter 4 for details of scintillation liquid and counting procedures). The efficiency of counting was determined by using a C^{14}-toluene reference standard (Radiochemical Centre, Amersham).

5.3 RESULTS

5.3.1 Body composition

Summaries of the body composition measurements are given in Tables 4.14 and 4.15 in Chapter 4. These results were directly relevant to the understanding of body composition relationships in poultry and to the derivation of suitable prediction equations. Results on the influence of the rearing feed restriction treatments are given in Tables 5.1 and 5.2 for experiments 1 and 2 respectively. In Experiment 1 there were no major alterations in body composition determined by slaughter up to 162 d of age, but at 162 d of age, immediately prior to cessation of feed restriction, liveweight (W, g) was decreased ($P < 0.001$), total body water (TBW, g/kg W) was increased ($P < 0.01$), fat content (FW, g/kg W) was decreased ($P < 0.05$) and water content of the fat-free mass (WFFM, g/100 g) was increased ($P < 0.001$) due to either limited-time or quanitative feed restriction from 42 d of age compared to birds allowed *ad libitum* feed intake. There were no differences due to type of feed restriction (TR or QR) *per se*. At 218 d of age there were no treatment differences, but at 337 d of age liveweight was reduced ($P < 0.05$) and protein content (PW, g/kg W) increased ($P < 0.05$) for birds sampled from the two rearing restriction treatments (TR and QR). However this was not associated with an increased protein content of the fat-free dry matter (PFFDM, g/100 g) indicating that it was due to a slight but non-significant decrease in fat content (FW, g/kg W) at this age (337 d).

TABLE 5.1 The effect of feeding regimen during rearing on determined body composition of layer-type pullets and hens (6 birds per treatment at each age) at different ages (Experiment 1).

Rearing Treatment[1]	Age (d)	Liveweight (W)		Total body water (TBW)		Protein (PW)		Fat (FW)		Ash (ASW)		WFFM[2]		PFFDM[3]	
		(g/kgW)	(±SD)	(g/kgW)	(±SD)	(g/kgW)	(±SD)	(g/kW)	(±SD)	(g/kgW)	(±SD)	(g/100g)	(±SD)	(g/100g)	(±SD)
Ad libitum	70	849.5	75.0	643.1	12.3	217.3	8.2	96.2	21.0	43.4	5.8	71.2	0.5	83.4	2.0
Limited-time	70	795.5	61.6	654.3	5.5	213.4	15.3	86.5	7.8	43.4	6.3	71.6	0.5	82.3	5.2
Significance[5]		NS		NS		NS		NS		NS		NS		NS	
Ad libitum	101	1175.7	78.9	653.1	22.8	227.5	5.5	77.3	22.0	41.4	2.0	70.8	0.9	84.4	2.5
Limited-time	101	1080.8	58.7	656.0	21.0	214.7	10.0	85.5	15.2	37.8	4.9	71.8	1.3	83.0	1.4
Significance		*		NS		*		NS		NS		NS		NS	
Ad libitum	162	1817.0[a][4]	123.0	560.2[a]	5.7	210.0	9.0	155.3[a]	10.7	41.4	7.9	66.3[a]	0.5	73.8	2.6
Limited-time	162	1540.2[b]	145.0	589.9[b]	14.1	209.5	17.9	129.1[b]	18.5	37.0	2.0	67.8[b]	0.4	74.5	5.8
Quantitative	162	1404.2[b]	86.0	584.3[b]	15.7	211.4	12.9	130.0[b]	15.8	38.5	4.6	67.2[b]	0.7	74.0	4.4
Significance		***		**		NS		*		NS		***		NS	
Ad libitum	218	1842.3	234.3	552.1	13.6	202.3	12.7	205.4	27.0	31.0	6.4	69.5	1.6	83.5	2.9
Limited-time	218	1743.2	208.3	545.0	19.7	211.2	13.8	198.7	29.8	36.8	5.2	68.1	0.7	82.4	2.9
Quantitative	218	1757.0	117.4	564.1	21.3	209.1	9.0	183.7	13.6	32.3	5.2	69.1	1.6	82.9	2.6
Significance		NS		NS		NS		NS		NS		NS		NS	
Ad libitum	337	2151.5[a]	220.0	553.2	26.6	185.2[a]	12.2	209.4	25.3	30.1	2.7	70.0	2.0	78.0	3.3
Limited-time	337	1811.8[b]	184.6	560.7	23.7	199.6[b]	11.9	191.7	19.1	26.8	4.5	69.4	1.7	80.6	2.2
Quantitative	337	1891.8[b]	204.0	560.5	13.8	200.9[b]	10.0	184.1	17.8	30.4	7.3	68.7	1.8	78.8	4.6
Significance		*		NS		-		NS		NS		NS		NS	

1. Details of rearing treatments are given in Chapter 3, Section 3.2.
2. Water content of the fat-free mass.
3. Protein content of the fat-free dry matter.
4. Means within each age with superscripts not containing the same letter are significantly different.
5. See Table 2.6, Chapter 2 for significance levels.

TABLE 5.2 The effect of feeding regimen during rearing on determined body composition of layer-type pullets and hens (4 birds per treatment at each age) at different ages (Experiment 2).+

Rearing Treatment[1]	Age (d)	Liveweight (W) (g/kgW)	(±SD)	Total body water (TBW) (g/kgW)	(±SD)	Protein (PW) (g/kgW)	(±SD)	Fat (FW) (g/kW)	(±SD)	Ash (ASW) (g/kgW)	(±SD)	WFFM[2] (g/100g)	(±SD)	PFFDM[3] (g/100g)	(±SD)
Ad libitum	120	1493.6^{a}[4]	77.7	608.7^{a}	17.4	233.5	11.0	99.5^{a}	15.0	33.5	4.4	67.6^{a}	2.1	80.2^{a}	3.8
Limited-time	120	1309.1^{b}	140.7	648.2^{b}	12.4	222.9	18.4	70.4^{b}	14.0	36.5	3.8	69.8^{b}	1.9	79.4^{a}	5.6
Quantitative	120	1234.0^{b}	152.5	662.5^{c}	12.8	220.4	12.4	41.5^{c}	8.3	34.9	4.6	69.1^{ab}	0.9	74.5^{b}	3.9
Significance[5]		**		***		NS		***		NS		*		*	
Ad libitum	280	1930.8	185.2	553.6^{a}	23.2	210.7	20.2	189.6	34.7	33.1^{ab}	4.6	68.3^{ab}	0.3	81.9	4.1
Limited-time	280	1720.5	147.8	569.3^{ab}	8.4	221.4	10.5	158.1	12.3	31.0^{a}	2.6	67.6^{a}	1.2	81.2	1.4
Quantitative	280	1803.0	83.8	581.1^{b}	13.8	212.9	5.4	156.2	21.1	37.5^{b}	3.8	68.9^{b}	0.4	81.1	1.7
Significance		NS		-		NS		NS		-		-		NS	
Ad libitum	476	1939.8	209.1	555.5	15.6	198.9	13.6	192.9	16.4	40.6	8.5	68.8	1.5	79.0	1.4
Limited-time	476	1791.8	143.4	542.8	8.8	199.2	14.5	201.9	16.5	36.2	8.8	68.0	2.1	78.1	1.9
Quantitative	476	2268.3	295.0	556.7	21.6	186.4	16.7	209.6	30.4	35.7	8.2	70.5	2.1	79.8	1.8
Significance		*		NS		NS		NS		NS		NS		NS	

+ Results presented at 120 d of age are for the birds slaughtered (9 birds per treatment) for the liver lipogenesis study (see Section 5.2).

Notes 1, 2, 3, 4 and 5. See Table 5.1.

Factorial analyses of variance which examined the influence of age (162 d, 218 d and 337 d), treatment and the interaction between age and treatment are given in Appendix Table A5.1. These analyses were carried out in such a way as to partition the variation between rearing (162 d) and laying (218 d and 337 d) effects, and confirmed that the influence of age on determined total body water (TBW, g/kg W), body fat (FW, g/kg W) and water of the fat-free mass (WFFM, g/100 g) for birds in Experiment 1 was primarily due to rearing treatment *per se*. However for protein content (PW, g/kg W) there were significant effects due to rearing treatment and during the laying (egg production) period. There were no significant interaction effects between treatment and age except for water content of the fat-free mass (WFFM, g/100 g) which was increased for birds on the *ad libitum* (A) rather than on the restriction (TR or QR) treatments during the laying period (218 and 337 d of age) as distinct from the rearing period (162 d) in which this effect was reversed.

In Experiment 2 the body composition data obtained from birds slaughtered at 120 d of age for determination of incorporation of radioactive carbon (C^{14}) into liver lipids were used to provide information on the effects of the restriction treatments (TR and QR) during rearing. The effects were similar to those found in Experiment 1, namely at 120 d of age there was a decreased ($P < 0.01$) liveweight (W, g) and a decreased ($P < 0.001$) body fat content (FW, g/kg W), an increased ($P < 0.001$) total body water (TBW, g/kg W) and ($P < 0.05$) water content of the fat-free mass (WFFM, g/100 g). At this age there was also a decreased ($P < 0.05$) protein content of the fat-free dry matter for birds on the quantitative feed restriction treatment compared to birds allowed *ad libitum* feed intake. These differences did not remain significant ($0.05 < P < 0.10$ or $P > 0.10$) after realimentation (280 d of age). Predicted body composition (Table 5.3) showed that at sexual maturity (first oviposition) there were no differences in liveweight between the rearing treatments but that body fat (FW, g/kg W) was reduced ($P < 0.01$) and total body water (TBW, g/kg W) and protein (PW, g/kg W) were increased ($P < 0.01$) in birds on the two rearing restriction treatments (TR and QR) compared to birds on the *ad libitum* treatment. After the production of equal egg numbers (180) there were similar differences between the treatments in predicted body composition as given above for sexual maturity. At the same age (364 d) or same time after sexual maturity (220 d) there were no differences between treatments in predicted body composition.

TABLE 5.3 The effect of feeding regimen during rearing on the predicted body composition of layer-type birds at different physiological and chronological ages (Experiment 2).

Stage	Number of birds	Rearing treatment[1]	Predicted body composition[2] Liveweight (W) (g)	(±SD)	Total body water (TBW) (g/kgW)	(±SD)	Protein (PW) (g/kgW)	(±SD)	Fat (FW) (g/kW)	(±SD)
Sexual maturity	50	*Ad libitum*	1782.1	174.0	585.8^{a}[4]	17.4	211.6^{a}	7.2	152.3^{a}	20.5
	50	Limited-time	1748.2	170.7	597.3^{b}	20.6	216.2^{b}	8.5	139.0^{b}	24.1
	50	Quantitative	1762.7	164.5	595.8^{b}	15.2	215.5^{b}	6.5	140.9^{b}	18.1
Significance[5]			NS		**		**		**	
Production of 180 eggs	20	*Ad libitum*	2076.3	246.0	558.0^{a}	16.2	198.8^{a}	7.3	189.5^{a}	20.1
	20	Limited-time	2013.5	176.0	569.5^{b}	13.7	203.6^{b}	5.9	174.0^{b}	16.6
	19+	Quantitative	2014.7	212.6	578.6^{c}	19.5	207.0^{b}	8.1	163.7^{b}	22.9
Significance			NS		**		**		**	
Aged 364 d	6	*Ad libitum*	2069.8	124.5	559.3	12.5	199.3	5.4	186.1	15.2
	6	Limited-time	1929.8	179.4	559.8	5.1	200.5	3.2	183.7	7.8
	6	Quantitative	2047.2	266.4	564.9	19.7	201.6	8.4	179.4	23.6
Significance			NS		NS		NS		NS	
Day number 220 after sexual maturity	6	*Ad libitum*	1907.3	95.9	573.0	18.2	205.6	7.1	168.8	20.7
	6	Limited-time	1942.8	324.4	567.7	22.0	203.6	10.3	174.7	28.2
	6	Quantitative	1862.5	183.0	571.6	17.2	205.5	7.9	169.4	21.6
Significance			NS		NS		NS		NS	

Notes 1, 4 and 5. See Table 5.1.

2. Body composition predicted using equations given in Section 5.2.

\+ One bird omitted due to insufficient body water sample.

The relationship between egg output (Y, g/d) over the egg production period (to 477 d of age) from sexual maturity for individual birds and predicted body fat (X, g/kg W) at sexual maturity for birds in Experiment 2 for each treatment were

Treatment 1: *ad libitum*

$$Y = 50.2 - 0.015\ X \qquad (5.1)$$

N = 46; R^2 = -0.021; RSD = 6.8; NS$^+$ (F = 0.09)

Treatment 2: limited-time

$$Y = 51.2 + 0.017\ X \qquad (5.2)$$

N = 47; R^2 = -0.018; RSD = 5.8; NS$^+$ (F = 0.20)

Treatment 3: quantitative

$$Y = 49.7 + 0.026\ X \qquad (5.3)$$

N = 44; R^2 = -0.015; RSD = 5.0; NS$^+$ (F = 0.38)

Combined

$$Y = 54.5 - 0.020\ X \qquad (5.4)$$

N = 137; R^2 = -0.003; RSD = 6.4; NS$^+$ (F = 0.58)

+ NS, Not Significant

All equations, irrespective of treatment, were non-significant, including the combined equation.

5.3.2 Liver composition and lipogenesis

The effects of age and rearing feeding regimen on liver weight and composition are given in Tables 5.4 and 5.5 for Experiments 1 and 2 respectively. Liver weights, composition and the radioactivity determinations at 120 d of age are given in Table 5.6. In Experiment 1, liver weight (g/kg W) was increased ($P < 0.05$) and water, protein and ash contents (g/100 g) decreased (see Table 5.4 for significance levels) for birds on the limited-time restriction treatment at 162 d of age compared to these parameters for birds on the *ad libitum* or quantitative treatments. Lipid content (g/100 g) was increased for birds on the limited-time treatment, but this was only significant ($P < 0.05$) when compared to birds on the quantitative feed restriction treatment. There were no major differences between treatments after the cessation of feed restriction.

In Experiment 2 there were similar effects between treatments for liver composition determined at 120 d of age due to limited-time feed restriction. Ignoring period effects, mean liver weights (g/kg W) were

TABLE 5.4 The effects of age and feeding regimen during rearing on liver weight and liver composition in layer-type birds (Experiment 1).

Treatment	Age (d)	Liver Weight				Liver Components							
						Water		Lipid		Protein		Ash	
		(g)	(±SD)	(g/kgW)	(±SD)	(g/100g)	(±SD)	(g/100g)	(±SD)	(g/100g)	(±SD)	(g/100g)	(±SD)
Ad libitum	39	11.3	0.7	26.3	1.7	73.7	0.8	3.4	0.3	19.6	0.3	3.4	0.7
Ad libitum	70	16.8	1.1	19.8	1.4	73.0	0.5	3.7	0.8	19.7	0.9	3.6	1.2
Limited-time	70	18.0	0.8	22.7	1.5	73.1	0.6	3.1	0.4	20.7	0.8	3.1	0.6
Significance≠		-		**		NS		NS		NS		NS	
Ad libitum	101	21.0	2.2	18.0	2.2	72.9	0.7	4.7	0.7	18.7	0.9	2.3	0.6
Limited-time	101	21.6	1.6	20.1	1.2	74.2	1.4	4.6	0.2	17.3	1.2	2.0	0.6
Significance		NS		NS		NS		NS		*		NS	
Ad libitum	162	34.1^{a+}	5.8	18.8^{a}	3.2	67.9^{a}	5.8	8.9^{ab}	5.4	18.5^{a}	1.1	2.0^{a}	0.3
Limited-time	162	39.1^{a}	8.6	25.4^{b}	5.0	62.9^{b}	1.4	10.5^{a}	1.5	14.7^{b}	1.6	1.0^{b}	0.2
Quantitative	162	25.4^{b}	6.2	18.0^{a}	3.8	70.3^{a}	3.2	5.5^{b}	1.9	18.2^{a}	2.7	1.4^{c}	0.1
Significance		*		*		*		-		**		***	
Ad libitum	218	41.0	11.7	22.0	3.8	68.5	6.4	8.6	5.4	16.7	1.5	2.7	1.3
Limited-time	218	40.1	5.0	23.2	3.1	71.8	1.7	6.1	1.5	16.8	1.0	2.7	0.6
Quantitative	218	41.7	6.7	23.9	4.3	70.5	3.2	7.1	2.9	17.3	0.9	3.8	0.9
Significance		NS		NS		NS		NS		NS		NS	
Ad libitum	337	47.1	11.5	21.7	3.9	68.7	2.7	5.5^{a}	1.8	18.7	1.6	2.3	0.3
Limited-time	337	37.1	4.4	20.5	1.4	70.8	0.9	4.2^{ab}	1.0	19.2	0.5	2.3	0.3
Quantitative	337	38.9	4.9	20.6	2.1	70.5	1.2	3.8^{b}	0.5	19.5	0.8	2.0	0.1
Significance		NS		NS		NS		-		NS		NS	

+ Means within age groups with superscripts not containing the same letter are significantly different.

≠ See Table 2.6, Chapter 2 for significance levels.

TABLE 5.5 The effects of age and feeding regimen during rearing on liver weight and liver composition in layer-type birds (Experiment 2).

Treatment	Age (d)	Liver Weight				Liver Components							
						Water		Lipid		Protein		Ash	
		(g)	(±SD)	(g/kgW)	(±SD)	(g/100g)	(±SD)	(g/100g)	(±SD)	(g/100g)	(±SD)	(g/100g)	(±SD)
Ad libitum	280	37.4	4.4	19.4^{a+}	1.0	69.6	3.6	6.0	1.7	17.4^{ab}	0.7	1.4	0.1
Limited-time	280	36.9	11.5	26.4^{b}	5.4	70.2	1.8	6.5	1.5	17.7^{a}	0.9	1.4	0.1
Quantitative	280	43.9	4.5	24.3^{a}	1.6	67.6	2.8	8.5	2.1	16.1^{b}	1.1	1.3	0.1
Significance≠		NS		*		NS		NS		-		NS	
Ad libitum	476	38.5^{a}	4.0	19.9	1.2	68.6	3.4	7.5	2.7	17.5	1.4	1.5	0.1
Limited-time	476	43.3^{ab}	10.5	24.1	5.2	68.8	4.6	7.7	4.5	17.4	1.2	1.4	0.2
Quantitative	476	54.7^{b}	12.4	24.1	4.1	65.0	5.0	12.1	5.6	14.4	3.9	1.3	0.1
Significance		-		NS		NS		NS		NS		NS	

+ Means within each age group with superscripts not containing the same letter are significantly different.

≠ See Table2.6, Chapter 2 for significance levels.

greater ($P < 0.001$), lipid content (g/100 g) higher ($P < 0.001$) and protein and ash (g/100 g) lower ($P < 0.01$) for birds on the limited-time rather than the other two treatments (A or QR) at 120 d of age. There remained an increased ($P < 0.05$) liver weight (g/kg W) for birds on the limited-time treatment at 280 d of age compared to the other two treatments (A and QR) but, similar to Experiment 1, there were no large differences between treatments after cessation of feed restriction.

In both experiments the recovery of the dry matter (sum of fat, protein and ash components, g/100 g) of the liver of birds near or at cessation of the feed restriction treatments (162 d in Experiment 1; 120 d in Experiment 2) was markedly reduced. Mean (±SD) recovery of the major chemical constituents (g/100 g DM) at 162 d of age in Experiment 1 was 91.8 (±3.2), 70.7 (±4.8) and 85.3 (±8.8) for birds on the *ad libitum*, limited-time and quantitative treatments respectively. All treatments differed significantly ($P < 0.001$). In Experiment 2, ignoring period effects, the mean (±SD) recovery (g/100 g DM) was 84.1 (±3.8), 70.6 (±12.3) and 80.6 (±7.9) for the three treatments respectively in the order given above ($P < 0.001$). These differences on the basis of the first, second and third periods (N = 3 birds/period) respectively were 87.1, 85.2 and 80.1 for the *ad libitum* treatment, 70.1, 84.0 and 57.6 for the limited-time treatment and 88.7, 78.7 and 74.3 for the quantitative treatment. There was no significant effect of period on recovered dry matter (g/100 g DM) for birds on the *ad libitum* treatment, but there was an increased ($P < 0.001$) recovery (g/100 g DM) from period 1 to 2 and a decrease ($P < 0.001$) from period 2 to 3 for birds on the limited-time treatment. For birds on the quantitative treatment, recovery (g/100 g DM) decreased ($P < 0.05$) from period 1 to period 2 with no significant change thereafter.

Radioactive carbon (C^{14}) incorporation (% of injected dose) was lower for birds on the quantitative treatment than either of the other two treatments (A and QR) ($P < 0.001$ and $P < 0.05$ respectively). For birds on the *ad libitum* treatment, approximately 0.38 μc of C^{14} was recovered in liver lipid, which represented 11.4% of the injected dose with no differences due to period. In birds on the limited-time treatment, incorporation of C^{14} was initially high but decreased ($P < 0.001$) from period 1 to period 2 and then increased ($P < 0.05$) from period 2 to period 3, while for birds on the quantitative treatment incorporation of C^{14} was initially very low but subsequently increased ($P < 0.05$) to the second and third periods.

TABLE 5.6 Liver weight and composition and the incorporation of radioactive acetate carbon (U-C^{14}-acetate) into liver lipids as influenced by feeding regimen during rearing and period of measurement in layer-type pullets at 120 d of age.

Treatment	Period	Liver Weight (g)	(±SD)	(g/kgW)	(±SD)	Liver Component: Water (g/100g)	(±SD)	Lipid (g/100g)	(±SD)	Protein (g/100g)	(±SD)	Ash (g/100g)	(±SD)	C^{14} recovered in liver lipid (µc)	Incorporation of C^{14} as a % of C^{14} injected
Ad libitum	1	27.4	1.7	18.2	0.8	68.7	1.0	4.3	0.4	21.5	0.8	1.5	0.1	0.33	10.2
	2	28.2	2.5	19.6	1.8	69.3	1.2	4.2	0.3	20.5	0.3	1.4	0.2	0.41	11.2
	3	30.2^{a+}	3.1	19.8^{a}	1.6	67.3	1.1	4.2	0.4	20.0	0.6	2.0	0.4	0.39	12.7
	Mean	28.6^{a+}		19.2^{a}		68.4		4.2^{a}		20.7^{a}		1.6^{a}			11.3^{a}
Limited-time	1	47.4^{a*}	3.5	33.2^{a}	3.5	65.4^{a}	2.4	8.3^{a}	1.6	14.8^{a}	0.3	1.1	0.0	0.44	15.5^{a}
	2	26.7^{b}	5.9	22.2^{b}	5.9	69.9^{b}	1.0	3.9^{b}	0.7	19.9^{b}	1.3	1.4	0.2	0.25	6.6^{b}
	3	33.2^{b}	2.2	26.0^{b}	0.6	66.7^{a}	0.7	3.9^{b}	0.2	14.2^{a}	0.2	1.1_{b}	0.1	0.49	19.4^{a}
	Mean	35.8^{b}		27.1^{b}		67.3		5.4^{b}		16.3^{b}		1.2^{b}			13.8^{a}
Quantitative	1	20.1	2.0	16.2	1.1	67.7	0.8	3.7	0.3	22.9^{a}	1.0	2.0^{a}	0.4	0.09	2.2^{a}
	2	23.7	3.5	18.1	0.9	69.0	1.0	3.5	0.4	19.4^{b}	0.9	1.4^{b}	0.0	0.27	8.7^{b}
	3	20.5	2.4	17.8	0.6	67.6	2.2	4.1	0.7	18.4^{b}	1.3	1.5^{b}	0.1	0.32	11.7^{b}
	Mean	21.4^{c}		17.4^{a}		68.1		3.8^{a}		20.2^{a}		1.6^{a}			7.5^{c}
Significance of differences between treatments		***		***		NS		***		***		***			***

+ Treatment means with superscripts not containing the same letter are significantly different.

* Period means within treatments with superscripts not containing the same letter are significantly different.

≠ See Table 2.6, Chapter 2 for significance levels.

5.4 DISCUSSION

Body composition determined at about 140 d of age for birds allowed *ad libitum* feed intake during rearing in the present study differed from values reported or derived from the literature (see Table 5.7). For example, when expressed on the more exact basis of fat-free mass (Moulton 1923), values previously reported for water content were higher than those found in the present study, although protein contents of the fat-free dry matter were similar. As far as can be determined, the body composition data given in Table 1.5 (Chapter 1) and Table 5.7 were obtained after carcasses were defeathered. Certainly the majority of studies adopted this procedure prior to chemical analysis (Fuller *et al.* 1969; Lee *et al.* 1971b; Fuller and Chaney 1974; Powell and Gehle 1977; Connor *et al.* 1977b). This is the major procedural difference between previously reported values on poultry body composition and those of the present study. There is little justification for feather removal prior to carcass analysis. Edwards *et al.* (1973) found that feathers have a low water content (*c.* 42%) and a high protein content (*c.* 58%) compared to the carcass. Therefore defeathered carcass composition would be higher in water and fat and lower in protein than that determined on the whole body. The magnitude of the differences between literature values and those of the present study support this explanation. The extensive results reported by Cunningham and Morrison (1977) on whole body composition of White Leghorn laying hens are in excellent agreement with those reported in the present study.

Body fat contents determined for birds in the present study are within the range found for similar types of birds (Fuller and Chaney 1974; Gous and Stielau 1976; Maclachlan *et al.* 1977a) at approximately 140 d of age, but those reported by Connor *et al.* (1977b) with an Australian White Leghorn crossbred (WL X A) very similar to the birds used in the present study were much greater (See Table 5.1, Chapter 1), even for birds which were severely restricted during rearing. The pattern of development of body fat for birds in Experiment 1 indicates that a period of stasis occurred at about 100 d of age. In terms of adipose tissue development this may indicate a plateau in hyperplasia (increase in cell number) at this age. Similar patterns of hyperplasia and hypertrophy were shown in broiler breeder birds (R.L. Hodd, pers. comm.). Interestingly, Wood and Grooves (1963) found a somewhat similar pattern of development in pigs. Pfaff and Austic (1976) found that the abdominal fat pad of White Leghorn pullets developed by hyperplasia until about 90 d of age and thereafter by cellular hypertrophy. These workers (Pfaff and Austic 1976) found an excellent

TABLE 5.7 Water and protein contents of the fat-free mass, and protein content of the fat-free dry matter (g/100 g), derived from results given in the literature for birds allowed *ad libitum* feed intake during rearing.

Reference	Strain of bird*	Age at slaughter (wks)	Water (g/100 g fat-free mass)	Protein (g/100 g fat-free mass)	(g/100 g FFDM)
Fuller *et al.* 1969	White Rock	21	74.5	20.2	79.3
Doornenbal *et al.* 1970	WL1	24	73.3	22.6	84.7
	WL2	22	73.3	22.5	83.2
	WL3	25	73.3	22.2	83.1
Lee *et al.* 1971b	White Rock	20	72.7	23.1	84.3
Maclachlan *et al.* 1977a	WL X A	20	70.2	24.2	81.1
Blair *et al.* 1976	Ross	22	72.8	23.1	84.7
Gous and Stielau 1976	NA+	20	72.2	-	-
Powell and Gehle 1976	Cobb	22	68.6	20.4	64.8
Connor *et al.* 1977b	WL X A	22	71.9	-	-
Present study (Exp. 1)	WL X A	23	66.3	24.8	73.8
Present study (Exp. 2)	WL X NH	17	67.6	-	80.2

+ Not available from reference, however a light hybrid strain was used.

* WL is White Leghorn, A is Australorp, NH is New Hampshire.

relationship between fat pad weight and body fat content (on a liveweight basis). The present results on the development of body fat therefore reflect directly the timing of changes in cellularity and cell size which are known to occur in poultry.

The present results indicate that hyperplasia was not influenced by the feed restriction programmes but that there was retardation in cellular hypertrophy. Pfaff and Austic (1976) found similar results with pullets fed low energy diets. The influence of nutrition during growth on subsequent development of adipose tissue is unclear (cf. Hood 1977; Searle 1977) although most reports, especially on cattle, sheep and pigs, showed no permanent effect on fat content in animals subjected to undernutrition during growth and which were subsequently realimentated (e.g., Lee *et al.* 1973). As expected (see Table 1.5) the major alteration in determined body composition at the cessation of feed restriction was a marked reduction in body fat content, but more importantly it was found that body composition predicted (Experiment 2) from deuterium oxide space and liveweight remained altered at sexual maturity (first oviposition), which substantiates previous findings (Fuller *et al.* 1969; Connor *et al.* 1977b). Predicted body composition was in agreement with determined body composition at the same age during egg production in that there were no differences due to rearing treatment. However after individual birds had produced 180 eggs there remained significant differences in body composition, particularly water and fat, between birds which were allowed *ad libitum* feed intake and birds which were restricted in feed intake during rearing; there were no apparent differences due to method of feed restriction. Calculation of total egg mass output in the production of 180 eggs for each treatment (see Chapter 3, Table 3.5) gave expected outputs of 10.71 kg, 11.45 kg and 11.32 kg for birds on the *ad libitum*, limited-time and quantitative treatments respectively, whereas the average periods of time to produce 180 eggs were 236 d, 224 d and 222 d, and the cumulative feed intakes were 28.44 kg, 28.40 kg and 28.86 kg for the three treatments respectively. These calculations indicate a greater efficiency of feed utilization for the birds previously on the restriction treatments compared to birds allowed *ad libitum* feed intake during rearing and may explain their continued lower body fat contents. Differences between body composition predicted after production of 180 eggs and the same number of days after sexual maturity probably reflect the small numbers of birds sampled at the latter time.

Ballam and March (1979) found that restricted feeding of broiler type birds from 7 d of age to 98 d of age resulted in a decreased cellularity and cell size in some adipose tissue sites at 294 d of age. However the severity of feed restriction was such that at 98 d of age, when restriction was terminated and birds were allowed *ad libitum* feed intake, liveweights of the two restriction treatments were 47% and 27% of the birds allowed *ad libitum* feed intake. These treatments apparently resulted in permanent stunting of the birds, and unfortunately no information is presented on important production characteristics such as rate of egg production or feed intake. That study (Ballam and March 1979) is therefore not directly comparable to normal feed restriction studies where there is usually little or no evidence of permanent stunting of liveweight, eg. present study (see Chapter 3), although conclusions concerning liveweight at the end of the egg production period are complicated by a range of factors such as total egg production, feed intake and physiological age. An interesting and unexpected finding was that the predicted protein content of birds from the two restriction treatments was increased on a liveweight basis relative to birds on the *ad libitum* treatment at both sexual maturity and after the production of 180 eggs. The significance of this increased protein content was such that at sexual maturity the total protein contents (in Experiment 2) were 377 g, 378 g and 380 g for birds on the *ad libitum*, limited-time and quantitative treatments respectively, while after the production of 180 eggs the values were 413 g, 410 g and 417 g respectively.

The attainment of the predicted body composition at sexual maturity, on the assumption that body composition for birds in Experiment 1 at cessation of restriction (Table 5.1) would have been similar for birds in Experiment 2, would have involved the following gross chemical gains: limited-time treatment, water 239 g, protein 92 g, fat 67 g; quantitative treatment, water 210 g, protein 76 g, fat 61 g. The finding that protein deposition during the period of compensatory growth for birds on the restriction treatments substantially exceeded fat deposition confirms the importance of maintenance of protein composition in animals (Bailey and Zobrisky 1968) and indicates that this might be a necessary prerequisite for commencement of egg production. The lower body fat contents of the birds from the restriction treatments at sexual maturity confirms the findings (Neil *et al*, 1977; Brody *et al*. 1980) that body fat content is relatively unimportant in the determination of commencement of egg production. The major reason which probably explains the large

quantities of water deposited in the bodies of birds on the restriction treatments during the period of compensatory growth is that protein deposition occurs with a deposition of water because muscles, the major component of protein deposition, have a protein/water ratio of 0.33-0.25 (van Es 1977); the protein/water ratios were 0.32 and 0.36 for birds on the limited-time and quantitative treatments respectively during this period.

Similar to the results obtained for birds slaughtered in Experiment 1 at 162 d of age, there was a lack of recovered dry matter (summation of protein, fat and ash on a dry matter basis) in the carcasses of birds slaughtered in Experiment 2 at 120 d of age. Mean (%, ±SD) values were 93.7 (±3.2), 93.7 (±5.3) and 87.9 (±4.5) for birds (N = 9/treatment) on the *ad libitum*, limited-time and quantitative treatments respectively at 120 d of age, but more importantly there was an effect of time of slaughter of recovered dry matter for birds on the two restriction treatments. Recovered dry matter for birds on the limited-time treatment was 93.6 (±5.4), 98.6 (±0.4) and 88.9 (±3.6), and for birds on the quantitative treatment was 93.5 (±2.0), 84.0 (±0.4) and 86.2 (±1.6) for periods 1, 2 and 3 respectively. There is the possibility that muscle glycogen synthesis may be increased during periods of hyperphagia (see Chapter 3 for details of feed intake). Alleyne and Scullard (1969) showed in malnourished children that muscle glycogen levels in the immediate recovery period were two to three times the levels of full recovery (levels were 0.2, 1.7 and 0.7 mg glycogen/100 mg wet tissue in the malnourished, recovering and recovered periods). In poultry, insulin injections result in markedly increased liver glycogen levels, and insulin is apparently very important for glucose uptake by skeletal muscle (Sturkie 1976). Furthermore the hyperphagia associated with intermittent feeding was shown to cause high plasma insulin levels with a consequent greater glucose tolerance (Simon and Rosselin 1979). Interestingly, Hollands *et al.* (1965) found that layer-type birds on restricted feeding regimes had a greater pancreas size at the cessation of feed restriction than birds allowed *ad libitum* feed intake and that this effect apparently remained throughout the laying period. Watson (1976) also found that broiler breeders restricted during rearing had a significantly greater pancreas size in relation to liveweight at 30 weeks of age compared with birds allowed *ad libitum* feed intake during rearing.

Water content of the fat-free mass was increased in the latter stages of feed restriction irrespective of the type of feed restriction. Reports on other animal species subjected to moderate to severe undernutrition also

showed an increased water content of the fat-free mass (*sheep*: Farrell and Reardon 1972; Searle *et al.* 1979; *mice*: Robinson *et al.* 1975; *children*: Alleyne 1968). Recalculation of the available data on poultry (see Table 1.5, Chapter 1) showed that the majority of reports on poultry also found this effect (Fuller *et al.* 1969; Powell and Gehle 1976; Gous and Stielau 1976; Connor *et al.* 1977b), but some reports found no differences due to restricted feeding during rearing (Lee *et al.* 1971b; Maclachlan *et al.* 1977a). In mature poultry the intracellular and extracellular water contributes approximately 54 and 46% respectively to the total body water (Freeman 1971; Sturkie 1976), and although the nature of the effect of undernutrition on the water content of the fat-free mass would require detailed studies to determine, it may be due either to hydration of the body cells or maintenance of the extracellular water while body solids decrease; it was apparent from the present study that realimentation reversed these effects.

The influence of body composition *per se* on egg production has been the subject of considerable conjecture in the field of poultry research in recent years. Scott *et al.* (1969) stated the following:

> 'If pullets are allowed to become too fat, the layers of adipose tissue enveloping the vital organs may interfere with optimum egg production.'

Although this statement refers to a direct physical effect of obesity on egg production, some workers have interpreted it to imply that there is a direct relationship between body fat content at sexual maturity and subsequent rate of egg production (e.g. Gous 1972). The finding that egg production is inversely related to body fat content during egg production (Greenberg 1976) does not substantiate this assertion, as inherently poor egg producers may deposit greater quantities of fat, rather than *vice versa*. Additionally, Gous (1972) concluded that Fuller *et al.* (1969) found evidence that "reproductive fittness appears to be related to body composition". The present author has interpreted the results of Fuller *et al.* (1969) in direct contrast to this conclusion. For example in the second trial reported by these workers (Fuller *et al.* 1969) the birds reared on full-feed with decreasing light rather than increasing light had a greater egg production over 336 d but a higher body fat content at sexual maturity (30.1% versus 25.4%). Similarly the birds on the restricted energy, decreasing light treatment had a greater egg production than birds on the restricted energy increasing light treatment although fat content at sexual maturity was greater (23.8% versus 19.9%). Other comparisons give

similar conclusions, and this lack of effect of body fat content at sexual maturity was substantiated in further studies (Fuller *et al.* 1973; Chaney and Fuller 1975). There was some evidence in these studies (Fuller *et al.* 1969, 1973; Chaney and Fuller 1975) that birds with higher fat contents suffered greater mortality during periods of high temperature but this could be expected on the basis of the greater liveweights of these birds, as discussed in Chapter 1, Section 1.5.1.4.

However, previous studies on the direct influence of body composition on egg production have by necessity involved the slaughter of birds whereby direct relationships on subsequent egg production cannot be derived. The present study in which body composition was predicted at sexual maturity for individual birds allowed these direct relationships to be derived with rate of subsequent egg production. These relationships (Equations 5.1, 5.2 and 5.3) showed that body fat content at sexual maturity exerted no influence on subsequent egg production *per se*. Combination of all the data (Equation 5.4) again showed the same lack of effect. The present study therefore confirms (Fuller *et al.* 1969, 1973; Fuller and Chaney 1975) that body fat content at sexual maturity is without direct influence on subsequent rate of egg production. Studies to be presented in Chapter 8 on broiler breeder birds further substantiate the present findings and show that grossly over-fat birds, certainly classified as obese, can have equivalent rates of egg production under controlled environment conditions as birds with markedly lower body fat contents. However the suggestion by Scott *et al.* (1969) may still have applicability in situations where the normal limits of fat deposition are greatly exceeded, although this is unlikely with current genetic and nutritional controls.

The changes which occurred in liver metabolism in birds aged 120 d (Experiment 2) showed the importance of time after feeding in the interpretation of such studies, particularly for birds on limited-time feeding schedules. Balnave *et al.* (1979) found that liver weight (g/kg W) was increased for birds on limited-time feeding schedules at 140 d of age relative to *ad libitum* feed controls, but found no increase or a decline in liver lipid levels, while at 91 d liver weight and lipid content were increased only for the most severe restriction treatment. In limited-time feeding schedules feed is offered *ad libitum* for approximately 24 h on day 1, is removed usually in the morning of day 2 and birds are without feed for the remainder of day 2 and also day 3. Birds in the study of Balnave *et al.* (1979) received feed at 0800 h and were slaughtered between

1000 h and 1130 h, but there was no indication given as to which stage of the feeding schedule birds were at for the two slaughter times (91 d and 140 d) during rearing. Indeed, with the different severity of restrictions it is possible that birds were on completely different stages of the feeding schedules at slaughter. On the basis of the results of the present study (Table 5.6) this could explain the variable results obtained by Balnave *et al.* (1979). In the present study, birds in Experiment 1 on the limited-time treatment which were sampled at 70 d of age were on day 2 of the feeding schedule at time of slaughter, and feed and water were removed for 10 h previously; at 101 d of age birds were on day 2 at time of slaughter and feed and water were removed 8 h previously; at 162 d of age birds were on day 2 and feed and water were removed 6 h previously. The extended starvation times were necessary because birds were also used for the body composition prediction studies for which a minimum period of 2 h for feed and water deprivation was used prior to injection of water isotope and thereafter 3 h for equilibration (see Section 4.2, Chapter 4). Changes in liver metabolism are rapid due to starvation (Leveille 1966; Yeh and Leveille 1970) which would clearly have influenced the values for liver weight and components in the present studies.

Birds on the quantitative feed restriction programme at 162 d of age (Experiment 1) had not received feed prior to slaughter which probably accounts for the slightly lower liver lipid levels relative to birds on the *ad libitum* treatment, but the results at 120 d of age (Experiment 2) in which liver parameters were measured prior to and subsequent to feeding indicated that liver weight and lipid levels in birds on this treatment (quantitative) were not largely influenced by the feeding schedule despite the ingestion of feed within a period of approximately 15 minutes (personal observation). This confirms (Simon and Brisson 1972; Simon and Rosselin 1979) that the changes in liver weight and lipid content were not dependent on feed restriction *per se* but on the type of feeding schedule, and indicates that many of the changes observed in the present study for birds on the limited-time treatments were due directly to the hyperphagia of these birds when feed was allowed. For example, birds on the limited-time treatment in Experiment 1 at 162 d of age consumed 163 g/bird in the 24 h prior to the day on which sampled birds were slaughtered. Importantly in this regard, digestive enzyme secretion would be unlikely to be a limiting factor in such birds (Nir and Nitsan 1979).

The present studies explain the basis of the differences between previous reports on the effects of feed restriction on liver metabolism (see Chapter 1, Section 1.5.2.2) as Lee *et al.* (1971b) and Ballam and March (1979), who found no effect on relative liver weight (g/kg W) at 140 d of age due to feed restriction; both used quantitative restriction techniques. Birds on the quantitative feed restriction treatment in Experiment 2 at 120 d of age had only a transient increase in liver weight (g/kg W) with a slight increase in liver lipid content. However liver protein levels were substantially reduced after feeding in these birds, and this was also observed for birds on the limited-time treatment. The physiological significance of the variation in protein levels in the liver is difficult to determine, but in conjunction with the observed variation in the summation of the components of the determined dry matter (*viz.*: protein + lipid + ash), a tentative hypothesis can be advanced on the basis of increased glycogen synthesis. The recovered dry matter for birds on the *ad libitum* treatment at 162 d of age (Experiment 1) and at 120 d of age (Experiment 2) were 92 g/100 g dry matter and 84 g/100 g dry matter respectively. The range of liver glycogen levels reported for poultry varied between 3.2 and 5.3 g/100 g of wet liver, or assuming a dry matter content of 28 g/100 g liver, between 12 to 20 g/100 g dry matter (Pearce and Brown 1971; Neil *et al.* 1977; Sturkie 1976). These values could certainly account for the range of recovered dry matter which was found for birds on the *ad libitum* treatments in the present study.

Many reports found that liver glycogen levels in young chickens (Leveille 1966; Simon and Blum 1972) and mice and rats (Wertheimer and Ben-Tor 1950; Tepperman and Tepperman 1958) on intermittant starvation and repletion feeding schedules were increased. More importantly however with respect to the present studies, Leveille (1966) showed that, in chickens (liveweight 600 g), after 28 d on meal-feeding schedules, liver glycogen levels could be over twice those found in normally fed chickens. The significant effects of period of slaughter on recovered dry matter relative to feeding times found in the present study (Experiment 2) for birds on both the feed restriction treatments at 120 d of age coincide with expected variations in glycogen synthesis, particularly for birds on the limited-time treatment. Considered over both the restriction treatments (120 d of age), the results may indicate that glycogen synthesis occurs more rapidly than *de novo* lipid synthesis. For birds on the limited-time treatment in period 1, lipid level was high and recovered dry matter low (5 h starvation), but after a 28 h starvation (period 2) recovered

dry matter was similar to birds on the *ad libitum* feed treatment; Leveille (1966) showed that liver glycogen levels are rapidly depleted due to starvation. However, in period 3, where birds were allowed *ad libitum* feed intake for 6 h, glycogen levels would be expected to be highest; on the assumption that all the unrecovered dry matter was glycogen this would amount to a glycogen level of 14.1 g/100 g wet liver, three times the expected liver glycogen level for birds on the *ad libitum* treatment. Such a level is not unrealistic (Leveille 1966). An interesting corollary to this is that liver lipid levels in birds on the limited-time treatment (120 d of age) remained unaltered from pre-feeding levels despite an extremely high rate of incorporation of acetate carbon. The reason for the progressive decline in recovered dry matter for birds on the quantitative restriction treatment is more difficult to speculate, although, as for birds on the limited-time treatment, there was clearly a relationship between protein content and recovered dry matter, and here again if glycogen synthesis was responsible then it occurred at an apparent greater rate than lipid synthesis.

However conclusions regarding the rate of liver lipid synthesis must be cautious since corresponding blood parameters (e.g. triglycerides, free fatty acids) were not monitored in conjunction with liver analyses. Accumulation of liver lipid due to meal-feeding schedules indicates that the transfer of synthesized lipid to the blood for consequent utilization or storage is delayed (Simon and Brisson 1972; Shapira *et al.* 1979), and the extent of acetate carbon incorporation found in the present study was not necessarily correlated with increased liver lipid. This may indicate that lipid transfer from the liver for birds on limited-time feeding treatments initially parallels lipid synthesis during immediate refeeding but that with the continued massive glucose load presented to the liver, and given that plasma glucose levels remain moderately stable in chickens due to starvation and refeeding (Yeh and Leveille 1970) probably due to increased insulin secretion (Simon and Rosselin 1979), lipid synthesis may eventually exceed transfer. The relatively stable liver lipid levels found for birds on the quantitative restriction treatment (Table 5.6) may be explained on this basis, although other factors such as rate of passage of feed from the crop to the intestines could also be important.

Directly comparable studies on acetate carbon incorporation into liver lipids in chickens are few, but Yeh and Leveille (1970) showed in male crossbred chickens (liveweight 500 g) that after 1 h of fasting the incorporation of acetate carbon was only 36% of the normally fed levels;

the fed level of incorporation was between 6-7% of the injected dose after refeeding. However, Husband and Brown (1965) found an acetate carbon incorporation of only 1.6% after 30 minutes and 6.8% after 2 h in laying hens, and 0.6% and 1.9% respectively for cockerels. The values found for the birds in the present study should have approximated those found for the cockerels of Husbands and Brown (1965) since sexual maturity was not imminent at 120 d of age. The reasons for the differences between the two studies are not apparent although in the present study, if available, feed was not removed prior to slaughter. The procedure used by Husbands and Brown (1965) was to starve birds overnight (14 h), to allocate feed for 2 h in the morning and then their removal. Birds were then injected and slaughtered between 0.5 to 10 h subsequently.

Summary

The influence of two methods of feed restriction during rearing on the body and liver composition of layer-type birds was investigated at different ages by both slaughter and prediction techniques. Body composition alterations due to feed restriction were related to the length of time which birds were on the restriction programmes. At or near cessation of restriction the major alterations relative to birds allowed *ad libitum* feed intake during rearing were reduced body fat and increased total body water and water of the fat-free mass. There were no major differences in body composition due to method of feed restriction *per se* (limited-time or quantitative). The degree of feed restriction imposed during rearing did not result in permanent alterations in gross body composition, although at sexual maturity predicted body fat content was lower and protein content higher than for birds allowed *ad libitum* feed intake during rearing. It was concluded that these changes may have physiological significance, but there was no demonstrable relationship between predicted body fat content at sexual maturity and subsequent rate of egg production. Changes in liver weight and composition were related to the type of feed restriction, being generally more marked for birds on the limited-time restriction programme. A detailed study on liver composition and lipogenesis showed the importance of the stage of the feeding schedules on these parameters. There were indications that liver glycogen synthesis was substantial during the time feed was available when birds were on the restriction programmes.

CHAPTER 6

STARVATION HEAT PRODUCTION AND REGRESSION ENERGY PARTITION IN LAYER-TYPE STRAINS OF POULTRY AS INFLUENCED BY RESTRICTED FEEDING DURING REARING

Chapter 6

Starvation Heat Production and Regression Energy Partition in Layer-type Strains of Poultry as Influenced by Restricted Feeding during Rearing

6.1 INTRODUCTION

The amount of energy required to keep an animal in liveweight stasis (i.e. zero energy balance) is the maintenance metabolisable energy requirement (ME_m). The contribution which this component makes to the total energy requirement depends primarily on the feeding level. For poultry in egg production allowed *ad libitum* feed intake it is a major energetic cost (cf. Farrell 1975; MacLeod and Shannon 1978; MacLeod *et al.* 1979). The basal metabolic rate (BMR) is defined as the net energy required for maintenance (NE_m) and, based on values determined for the partial efficiency of utilization of metabolisable energy for maintenance (km) in poultry (see De Groote 1974; Farrell 1975), it may represent between 70 and 90% of the maintenance energy requirement. The importance of the basal metabolic rate to the total energy requirements of poultry is therefore considerable.

Measurement of the basal metabolic rate is exacting and must be carried out under standardised conditions (see Blaxter 1962). Since some of these conditions cannot be attained in egg producing poultry and in many other animal species, various terms were generated to describe the determination of a parameter which closely approximates the basal metabolic rate. Examples of these include the fasting heat production (Farrell 1975), the starving heat production (MacLeod and Shannon 1978) and the starvation heat production (Farrell and Swain 1977). The latter term is used throughout this thesis. The duration of starvation required to establish the post-absorptive state in poultry was found to be approximately 24 h in birds with a liveweight below 2.5 kg (Misson 1974).

Starvation heat production (SHP) of poultry, even measured under standard conditions, is influenced by a number of factors. These include surround adjustment (Mission 1974); sex of the bird (MacLeod *et al.* 1979); reproductive state (Waring and Brown 1965; Tasaki and Sasa 1970; Balnave *et al.* 1978); age (cf. Balnave 1974; Lundy 1978; MacLeod *et al.* 1980); feather cover (O'Neil *et al.* 1971; Johnson *et al.* 1978; Tullett *et al.*

1980); strain (Farrell 1975; Kuenzel and Kuenzel 1977; MacLeod and Shannon 1978); season (Tasaki and Sakurai 1969); and nutrition (cf. Lundy 1978). One report also found that stage of egg production influenced starvation heat production (Leeson and Porter-Smith 1970). Starvation heat production (kJ/kgW d^{-1}) and the metabolisable energy required for maintenance (kJ/kgW d^{-1}) of hens and cockerels decreased due to prolonged undernutrition (MacLeod and Shannon 1978; MacLeod *et al.* 1979). Studies on other animal species have either directly or indirectly shown similar effects due to undernutrition (*sheep:* Marston 1948; Graham and Searle 1975, 1979; Gingins 1978; Thomson *et al.* 1980; *calves:* Blaxter and Wood 1951; *rats:* Quimby 1948; Lee and Lucia 1961; Walker and Garrett 1970; *humans:* Keys *et al.* 1950). The contribution of these changes to the often found compensatory growth following realimentation is far from clear; however, the persistence of such alterations due to undernutrition appears to be of a limited duration after adequate realimentation (Keys *et al.* 1950; Graham and Searle 1975, 1979).

Two studies have sought to directly determine the influence of total feed restriction during rearing on the starvation heat production of poultry, both during the period of undernutrition and also, subsequent to realimentation, in the egg production period (Fuller and Dunahoo 1962; Balnave *et al.* 1979). The results obtained in these studies provide equivocal evidence as to the alteration in starvation heat production due to restricted feeding. Fuller and Dunahoo (1962) found a considerable reduction in the rate of oxygen consumption of birds at 126 d of age after varied durations of feed restriction. Some effects were also evident at 364 d of age in this (Fuller and Dunahoo 1962) study. However, in a study which used an essentially similar strain of bird and severity of feed restriction, Balnave *et al.* (1979) concluded that there were no significant effects of rearing undernutrition on starvation heat production measured at different ages. Interestingly, these workers (Balnave *et al.* 1979) found no effect due to prolonged feed restriction during the egg production period on starvation heat production. This apparently directly contradicts the work of MacLeod and associates (MacLeod and Shannon 1978; MacLeod *et al.* 1979).

Both these studies (Fuller and Dunahoo 1962; Balnave *et al.* 1979) had major deficiencies in either technique or experimental planning (see Chapter 1, Section 1.5.3.1). The importance of basal metabolic rate to the energy requirements, and ultimately to the dietary energy

available for production (ME_p), necessitated that a more accurate appraisal of the effects of undernutrition be undertaken. Closed-circuit, indirect calorimetry was therefore used to determine the starvation heat production of representative birds from each of the three treatments during the rearing and egg production periods of Experiment 1 (see Chapter 3, Section 3.2.2). During this study special cognisance was taken of the different rate of physiological development between treatments due to feed restriction. In conjunction with the calorimetric determination of starvation heat production, other techniques (see Chapter 1, Section 1.7.4) were used to provide estimates of the partition of dietary energy between the processes of maintenance and production in layer-type strains of poultry. Such techniques, commonly referred to as regression analyses, can give good estimates of the energetic requirements and efficiencies under actual production conditions (e.g. Brody 1945; Grimbergn 1974). The aim was to use these estimates to account for the observed differences in gross efficiency of energy utilization during the egg production period between treatments which were allowed *ad libitum* feed intake during rearing or which were subjected to undernutrition during rearing.

6.2 MATERIALS AND METHODS

6.2.1 Measurement of starvation heat production

6.2.1.1 Birds and management

The birds used and their management were described in Chapter 3, Section 3.2 (Experiment 1). Six birds from each treatment were randomly selected at 126 d of age. These birds remained in the usual housing facility with uncontrolled environment in their usual cage allocations. Diet, feeding levels and housing conditions were as described for birds in Experiment 1 (Chapter 3).

6.2.1.2 Equipment and calculations

Four closed-circuit indirect respiration chambers, located in a completely darkened room, were used for the determination of gaseous exchange. These chambers were described by Farrell (1972) with modifications as given by Pym and Farrell (1977). The formulae given by Brouwer (1965), without correction for nitrogen (N) metabolism, was used to calculate heat production (HE). Starvation heat production values were expressed on a metabolic liveweight basis ($W^{0.75}$) for comparison with published data.

6.2.1.3 Procedure

After birds were initially selected for this study they were placed in the respiration chambers with feed provided for 24 h for chamber familiarisation. Thereafter at regular intervals during the experiment these birds, apart from the normal scheduled measurement periods in the chambers, were placed in the chambers with feed provided for periods of 12 h to maintain acceptance of the chamber conditions. Measurement of starvation heat production was commenced routinely at 1100 h. Feed was removed at 0900 h the previous day. Great care was taken to ensure that all traces of feed dust was removed from feeders, and waterers were emptied, thoroughly cleaned and replenished with fresh water to ensure the absence of feed particles. To offset the disparity in feed intake which would have occurred prior to measurement of starvation heat production during feed restriction (up to 163 d of age), birds on the two restriction treatments (TR and QR) (see Chapter 3, Section 3.2.2 for treatment details) were offered 50 g of feed for 30 minutes prior to commencement of starvation. This quantity of feed was invariably consumed during this time. On the measurement day, birds were placed in the chambers at 0900 h, 24 h after feed removal. Chamber hoods were lowered but not sealed and air pumps were started but not connected until 1100 h, at which time the 22 h measurement period commenced. Black polyethylene plastic sheets were placed over the chambers to ensure the absence of light both before and during measurement periods. Chamber temperatures were approximately 20-25°C (see Table 6.1) and relative humidity about 70%. Excreta were not collected. During the period of feed restriction, when birds on the restriction treatments (TR and QR) were removed from the respiration chambers after the completion of measurement of starvation heat production, *ad libitum* feed intake was allowed for the following 24 h period.

6.2.1.4 Chronologic and physiologic ages of measurement

Starvation heat production for each of the selected birds was measured on six separate occasions, three chronologic and three physiologic ages. These, and the designated name for each, were as follows:

6.2.1.4.1 Pre-lay (chronologic)

Birds were aged 140 d, were not in egg production and were randomly allocated to the respiration chambers for measurement of starvation heat production.

6.2.1.4.2 Sexual maturity (physiologic)

Criterion for measurement of starvation heat production was first oviposition.

6.2.1.4.3 Peak production (physiologic)

Arbitrarily birds were considered to be in peak of egg production 28 d after first oviposition. Therefore, on the twenty eighth day after first oviposition feed was removed at 0900 h to commence the period of starvation prior to heat production measurement.

6.2.1.4.4 Post-peak production (physiologic)

Arbitrarily designated as the ninety eighth day after first oviposition.

6.2.1.4.5 Same age (chronologic)

Birds were aged 332 d and were randomly allocated to the respiration chambers for measurement of starvation heat production.

6.2.1.4.6 Declining production (chronologic)

Birds were aged 370 d and were randomly allocated to the respiration chambers for starvation heat production measurement.

6.2.2 Partition of metabolisable energy by the use of regression techniques

The production data of individual birds for the two experiments reported in Chapter 3 (Experiments 1 and 2) were used to partition by multiple linear regression, the dietary metabolisable energy between liveweight, liveweight change and egg output. Metabolisable energy intake and egg output for each bird were determined over 7 d periods. Liveweights were determined by interpolation or extrapolation for these periods from the routine liveweight measurements (see Section 3.3.2, Chapter 3).

The metabolisable content of the diets (ME kJ/kg) was determined by the procedures given in Chapter 2, Section 2.3. Two regression models were used to obtain estimates of the partition of dietary metabolisable energy intake (ME) between the processes of maintenance and production. Multiple linear regression techniques were used for both of these models (see Chapter 2, Section 2.9 for details of regression techniques). The first approach was to derive multiple linear regression equations for the relationship between metabolisable energy intake (ME, kJ/bird d^{-1}) and the independent variables liveweight (W, kg), liveweight change (ΔW, g/bird d^{-1}), egg mas output (E, g/bird d^{-1}), temperature (T, ^{o}C) and age (A, d). Brody (1945) described the application of multiple linear regression for the

partition of metabolisable energy. For the equations derived in the present study it was considered statistically inappropriate to force the regression through the origin (ME = 0); therefore the basic multiple linear regression model was:

$$ME = a + bW + c\Delta W + dE \quad \ldots\ldots\ldots\ldots \quad \text{Model 1}$$

where the variables were as defined above. This basic model was further manipulated to include the effects of temperature and age. Feather cover was not monitored with sufficient frequency for inclusion into the model, so the effect of age *per se* is confounded with the effect of changes in feather cover. Feather cover scores for birds in both experiments are given in Chapter 3, Sections 3.3.1.1.5 and 3.3.1.2.5 for Experiments 1 and 2 respectively.

The second approach was to pre-set the efficiency of utilization of metabolisable energy for production (kp) to obtain estimates of the relative changes in the maintenance energy requirements (ME_m) between treatments. Two efficiency (kp) values were used, 70 and 60%. These efficiencies (kp) depend on the energy content of the substrate produced. The energy content of a whole egg (including shell) was assumed to be 6.7 kJ/g (see Chapter 2, Section 2.6). Therefore values of 9.6 kJ/g egg output and 11.2 kJ/g egg output were assigned to give the above efficiencies of utilization of metabolisable energy for egg production (ke). The energy content of a change in liveweight was shown to depend on the liveweight (or age) of the bird (see Chapter 4, Section 4.4.2.2). However a value of 14 kJ/g liveweight change, derived from the linear regression equation (equation A99) in Chapter 4, was used. Therefore values of 20 and 23 kJ/g liveweight change were assigned. The basic model was therefore

$$\frac{(ME - c\Delta W - dE)}{W} = a\left(\frac{1}{W}\right) + b \quad \ldots\ldots\ldots\ldots \quad \text{Model 2}$$

where the variables were defined above.

6.2.3 Statistical procedures

Treatment comparisons between starvation heat production were carried out at each measurement period (Section 6.2.1.4) by normal analysis of variance procedures (Steel and Torrie 1960). Effects of treatments and measurement periods, and their interaction, were determined by split-plot analyses (Steel and Torrie 1960, p. 232) over each of the three chronologic and physiologic series of measurements. Multiple linear

regression techniques were used to partition dietary metabolisable energy (Steel and Torrie 1960). Partial regression coefficients were compared between treatments by a t-test (Steel and Torrie 1960, p. 297).

6.3 RESULTS

6.3.1 Measurement of starvation heat production

Two birds died from the birds selected for serial measurement of starvation heat production, one from each of the *ad libitum* and limited-time treatments at 276 d and 288 d of age respectively. Details are given in Chapter 3, Table 3.1. Starvation heat production of one bird from the limited-time treatment was accidentally measured at 169 d of age for a sexual maturity determination although egg production had not commenced. For this bird the following observations were recorded: Liveweight 1795 g; Respiratory quotient (RQ), 0.728; Starvation heat production, 435 kJ/d, 242 kJ/kgW d^{-1}, 281 kJ/kg$W^{0.75}$ d^{-1}. These measurements were omitted from the analyses.

Certain variables were considered important for a proper interpretation of the values obtained for starvation heat production; these variables are given in Table 6.1. Mean (±SD) liveweight, respiratory quotient and starvation heat production are given for each treatment at each of the chronologic and physiologic measurement times in Table 6.2. Significance levels obtained for the split-plot analyses of variances carried out over the three chronological and three physiological ages are given in Table 6.3. A more detailed presentation of these analyses is given in Appendix Table A6.1.

6.3.1.1 Liveweight

As expected there was a significant effect of time ($P < 0.001$) and a significant ($P < 0.01$) interaction effect between treatment and time for liveweight over the three chronologic measurements. The latter effect was due to the lower ($P < 0.01$) liveweights for the two restriction treatments (TR and QR) at 140 d of age (pre-lay) (see Table 6.2). During the physiologic measurement periods (sexual maturity, peak production and post-peak production) there were no liveweight differences between treatments. Liveweight tended to decline (*ad libitum* and limited-time treatments) or remain steady (quantitative treatment) from sexual maturity to peak production, but these effects were not significant. However liveweight increased ($P < 0.001$) for all treatments from peak production to

TABLE 6.1 Variables associated with the measurement of starvation heat production (SHP) of layer-type pullets and hens at different chronologic (C) and physiologic (P) stages.

Stage[1]	Time[2]	Treatment[3]	Chronologic age (d)	Ambient temperature (°C)[4]	Chamber temperature (°C)[5]	Number of eggs in 7 d prior to SHP measurement	Number of birds which laid in chamber	Liveweight loss (g) during SHP measurement	Feather score[6]
C	Pre-lay	1	137	24	26.8	0	0	48	NA+
		2	141	24	25.7	0	0	38	NA
		3	140	24	25.4	0	0	45	NA
P	Sexual maturity	1	150	22	25.4	1	5	99	NA
		2	175	21	25.6	1	4	102	NA
		3	173	21	25.4	1	4	85	NA
P	Peak production	1	176	20	24.5	5.8	4	127	NA
		2	201	18	23.9	5.7	6	96	NA
		3	199	18	23.5	6.3	4	92	NA
P	Post-peak production	1	268	11	20.8	5.5	4	103	3.4
		2	297	8	21.0	5.8	2	94	1.5
		3	296	8	21.1	5.2	4	95	1.8
C	Same age	1	332	7	23.2	5.2	3	99	3.5
		2	332	7	22.6	4.6	4	105	1.7
		3	331	7	22.9	5.3	4	87	1.8
C	Declining production	1	369	16	22.6	5.6	4	114	3.5
		2	370	16	22.8	3.6	3	86	2.2
		3	370	16	22.5	4.7	4	84	2.1

1. Stages were either chronologic (C) or physiologic (P).
2. See Section 6.2.2.4 for details of the times at which measurements were taken and the procedures used.
3. Treatments, which were applied during rearing, were: *ad libitum* (A) feed intake; limited-time restriction (TR): quantitative restriction (QR).
4. Average approximate shed temperature in 14 d prior to starvation heat production (SHP) measurement.
5. Average chamber temperature during starvation heat production (SHP) measurement.
6. See Chapter 3, Section 3.2.5 for details.

\+ Feather cover not determined at these times.

post-peak production measurements.

6.3.1.2 Respiratory quotient

There were no treatment effects on the respiratory quotient found during measurement of starvation heat production, either on a chronologic or a physiologic basis. The overall mean (±SD) respiratory quotient was 0.725 (±0.003) for the three chronologic times (N = 54) and 0.726 (±0.002) for the three physiologic times (N = 54). The respiratory quotient declined over each of the chronologic ($P < 0.001$) and physiologic ($P < 0.05$) periods. Although the respiratory quotient tended to decline for each treatment, chronologically this was significant ($P < 0.05$) only for birds on the *ad libitum* treatment, while physiologically this was significant ($P < 0.05$) only for birds on the quantitative restriction treatment.

6.3.1.3 Heat production

There was a significant ($P < 0.01$) interaction between treatment and time for starvation heat production (kJ/d) measured chronologically, caused by the higher ($0.05 < P < 0.10$) starvation heat production (kJ/d) of the birds on the *ad libitum* treatment during the first (pre-lay) chronologic measurement and also the slightly higher starvation heat production (kJ/d) of birds on the limited-time treatment during the second and third chronologic measurements. These effects were due mainly to liveweight differences and were removed when starvation heat production was expressed on a liveweight (W, kg) or metabolic liveweight ($W^{0.75}$, kg) basis. Covariance analysis of starvation heat production (kJ/d) using liveweight (kg) as the covariate confirmed this effect.

At the physiologic period of post-peak production (98 d after first oviposition), birds on the limited-time treatment had a higher starvation heat production than those on the quantitative treatment expressed either on a liveweight ($0.05 < P < 0.10$) or metabolic liveweight basis, and a higher starvation heat production than either of the other treatments (A and QR) on a metabolic liveweight basis. Covariance analysis of starvation heat production (kJ/d) between treatments using liveweight as the covariate at post-peak production showed a significant ($P < 0.001$) treatment effect. This was due to a higher adjusted starvation heat production of the birds on the limited-time treatment than on either the *ad libitum* treatment ($P < 0.05$) or the quantitative treatment ($P < 0.01$). Over each of the chronologic and physiologic measurement periods, starvation heat production increased ($P < 0.001$) irrespective of how it

TABLE 6.2 The influence of feeding regimen during rearing (6-23 weeks of age) on the starvation heat production (SHP) of layer-type pullets and hens at various chronologic (C) and physiologic (P) stages. Standard deviations are given in parentheses below each mean (N = 6/treatment).

Stage[1]	Time[2]	Treatment[3]	Liveweight (W, g)	Respiratory quotient (RQ)	SHP (kJ)[4] per d	per kg W d^{-1}	per kg $W^{0.75}$ d^{-1}
C	Pre-lay	1	1626.9[a#] (224.9)	0.749 (0.010)	440.7[a] (67.4)	270.9 (13.9)	305.4 (19.0)
		2	1337.3[b] (138.7)	0.731 (0.026)	364.4[b] (51.3)	271.8 (15.1)	292.2 (21.0)
		3	1338.1[b] (66.8)	0.732 (0.025)	384.4[ab] (33.5)	288.4 (14.3)	309.8 (14.1)
		Significance[5]	**	NS	-	NS	NS
P	Sexual maturity	1	1732.8 (209.8)	0.734 (0.020)	467.1 (77.9)	269.6 (32.8)	308.9 (38.3)
		2	1743.3 (238.2)	0.726 (0.012)	502.7 (73.1)	288.1 (8.7)	330.6 (17.2)
		3	1638.2 (126.6)	0.742 (0.008)	453.0 (67.9)	275.9 (27.7)	312.1 (34.3)
		Significance	NS	NS	NS	NS	NS
P	Peak production	1	1696.8 (198.9)	0.723 (0.015)	516.9 (69.8)	305.4 (29.5)	348.0 (38.0)
		2	1722.0 (229.0)	0.717 (0.017)	520.8 (79.8)	303.4 (33.8)	346.9 (38.2)
		3	1638.1 (85.1)	0.727 (0.019)	476.1 (28.1)	291.1 (16.5)	329.1 (17.3)
		Significance	NS	NS	NS	NS	NS
P	Post-peak production	1	1797.4 (178.9)	0.722 (0.012)	552.5[ab] (40.6)	308.1[ab] (9.7)	356.3[a] (6.1)
		2+	1894.2 (295.5)	0.718 (0.022)	616.6 (91.7)	326.0[a] (14.2)	381.6[b] (19.6)
		3	1820.2 (111.3)	0.722 (0.008)	538.7[b] (42.0)	296.2[b] (19.5)	343.9[a] (22.0)
		Significance	NS	NS	-	*	**
C	Same age	1	1883.4 (243.2)	0.729 (0.028)	591.1 (85.9)	313.9 (23.1)	367.2 (28.5)
		2+	1944.1 (322.9)	0.713 (0.017)	609.1 (113.8)	312.7 (19.9)	368.7 (31.0)
		3	1827.7 (94.4)	0.726 (0.018)	545.1 (29.6)	298.8 (20.5)	347.2 (21.3)
		Significance	NS	NS	NS	NS	NS
C	Declining production	1+	1949.5 (273.7)	0.707 (0.016)	596.8 (58.5)	307.8 (22.1)	362.8 (19.2)
		2+	2220.4 (430.0)	0.708 (0.014)	679.2 (89.2)	309.3 (28.8)	375.5 (24.3)
		3	2015.0 (210.9)	0.717 (0.025)	631.6 (62.2)	314.1 (19.1)	373.7 (21.4)
		Significance	NS	NS	NS	NS	NS

Notes 1, 2 and 3. See Table 6.1.

4. Starvation heat production.

5. See Chapter 2, Table 2.6 for significance levels.

Means without the same superscript are significantly different.

\+ Five birds instead of six at these times for the designated treatment due to mortality (see Results, Section 6.3, for details).

TABLE 6.3 Significance levels obtained in split-plot analyses of variance for each of the parameters over three chronologic and three physiologic stages+

Parameter		Chronologic (C) stages[1]			Physiologic (P) stages[1]		
	df[2]	Treatment 2	Time 2	Treatment x time 4	Treatment 2	Time 2	Treatment x time 4
Liveweight (W, g)		NS[3]	***	**	NS	***	NS
Respiratory quotient (RQ)		NS	**	NS	NS	*	NS
SHP (kJ)[4]:							
per bird d^{-1}		NS	***	**	NS	***	NS
per kg W d^{-1}		NS	***	NS	NS	***	NS
per kg $W^{0.75}$ d^{-1}		NS	***	NS	NS	***	NS

+ See Section 6.2.3 for statistical procedures used for analyses.

1 See Section 6.2.1.4 for details of stages during which measurements were carried out.

2 Degrees of freedom (df) are given below each source of variation.

3 See Chapter 2, Table 2.6 for significance levels.

4 Starvation heat production.

was expressed. Chronologically, birds on the *ad libitum* and limited-time treatments had an increased ($P < 0.001$) starvation heat production (kJ/kgW d) from the pre-lay (140 d) to the same age (332 d) measurements. On a metabolic liveweight basis ($kJ/kgW^{0.75}\ d^{-1}$) this effect was evident for all treatments. There were no further increases in starvation heat production ($kJ/kgW\ d^{-1}$ or $kJ/kgW^{0.75}\ d^{-1}$) after 332 d of age.

Similarly, starvation heat production ($kJ/kgW\ d^{-1}$ and $kJ/kgW^{0.75}\ d^{-1}$) increased ($P < 0.001$) for all treatments from sexual maturity (first oviposition) to peak production (28 d after first oviposition). This effect was particularly marked for the birds on the *ad libitum* treatment (36 $kJ/kgW\ d^{-1}$ increase versus 15 $kJ/kgW\ d^{-1}$ for the two restriction treatments (TR and QR)).

6.3.2 Partition of metabolisable energy by the use of regression techniques

Mean (±SD) values for metabolisable energy intake (ME, kJ/bird d^{-1}), liveweight (W, g/bird), liveweight change (W, g/bird d^{-1}), egg mass output (E, g/bird d^{-1}), temperature (T, ^{o}C) and age (A, d) of the individual bird data over 7 d periods from 10 eggs/100 hen d for nine (Experiment 1) and ten (Experiment 2) 28 d periods in each treatment are given in Appendix Table A6.2. Further information on these parameters is given in Chapter 3. The gross energetic efficiencies of egg production (kJ egg/kJ ME, %) are also given in Chapter 3, Section 3.3.2, and in Appendix Tables A3.4 (Experiment 1) and A3.5 (Experiment 2). For the periods over which the regression equations were derived in the present chapter, in Experiment 1 the gross energetic efficiencies for the *ad libitum*, limited-time and quantitative treatments were 17.5, 19.5 and 17.9 respectively ($P < 0.001$, limited-time greater than either of the other treatments (A or QR)); in Experiment 2 the values were 21.4, 23.1 and 22.9 respectively ($P < 0.001$, limited-time and quantitative treatments greater than the *ad libitum* treatment).

Multiple linear regression equations derived for the relationship between metabolisable energy intake and combinations of the variables given above are shown in Table 6.4. Regression equations derived after setting the partial efficiencies for production at either 70 or 60% (Model 2) are given in Table 6.5. Estimates of the metabolisable energy required for maintenance were derived from appropriate equations for each of the regression models (model 1 or 2) and are given in Table 6.6.

TABLE 6.4 Multiple linear regression equations for the relationship between metabolisable energy intake (ME, kJ/bird d^{-1}), and liveweight (W, kg), liveweight change (ΔW, g/bird d^{-1}), egg output (g/bird d^{-1}), average temperature (T, ^{o}C) and age (A, g) derived from individual bird data over 7 d periods from 10 eggs/100 hen d for nine (Experiment 1) and ten (Experiment 2) 28 d periods in each treatment (Model 1).

Experiment	Equation (Model 1) and Independent Variables	Treatment[1]	Coefficients in the equation a	b	c	d	e	f	(R^2)[2]	(RSD)[3]	Equation Number
1	a + bW + cΔW + dE + eT	1	389.8	438.1	9.1	8.3	-10.2		0.602	162	1-1
		2	633.2	354.5	14.6	6.5	-9.9		0.609	145	1-2
		3	375.9	431.5	15.5	7.9	-8.9		0.566	174	1-3
		Overall	490.9	390.6	14.4	7.7	-9.8		0.587	163	1-4
	a + (b + eT)W + cΔW + dE	1	251.1	508.0	9.2	8.3	-5.3		0.602	162	1-5
		2	492.7	423.8	14.6	6.6	-5.2		0.609	144	1-6
		3	232.3	498.3	15.4	8.0	-4.3		0.563	174	1-7
		Overall	349.0	460.8	14.4	7.8	-5.1		0.587	162	1-8
	a + (b + eT + fA)W + cΔW + dE	1	310.3	387.4	9.7	8.3	-3.3	0.23	0.614	159	1-9
		2	480.3	439.9	14.5	6.6	-5.2	-0.03	0.609	145	1-10
		3	177.9	551.8	15.2	8.0	-4.6	-0.07	0.564	174	1-11
		Overall	375.5	426.1	14.5	7.8	-4.8	0.06	0.588	162	1-12
2	a + bW + cΔW + dE + eT	1	545.6	365.4	9.5	5.6	-8.7		0.539	140	2-1
		2	712.6	254.8	13.2	7.8	-10.5		0.418	155	2-2
		3	523.6	461.2	14.7	4.2	-10.7		0.383	160	2-3
		Overall	598.1	329.1	14.2	6.8	-9.5		0.435	157	2-4
	a + (b + eT)W + cΔW + dE	1	413.3	430.2	9.4	5.7	-4.5		0.538	141	2-5
		2	548.9	338.9	13.2	7.8	-5.5		0.422	155	2-6
		3	360.6	545.6	14.5	4.3	-5.5		0.387	160	2-7
		Overall	451.8	402.1	14.1	6.9	-4.9		0.436	157	2-8
	a + (b + eT + fA) W + cΔW + dE	1	307.5	522.2	8.6	5.9	-4.3	-0.15	0.547	139	2-9
		2	471.9	399.9	12.7	8.1	-4.3	-0.14	0.426	154	2-10
		3	196.8	488.9	13.1	4.4	-3.3	-0.29	0.408	157	2-11
		Overall	378.4	458.1	13.8	7.1	-4.2	-0.11	0.440	157	2-12

1. For details of treatments see Chapter 3, Section 3.2.2.

2. Correlation coefficient (R^2).

3. Residual standard deviation (RSD).

TABLE 6.5 Multiple linear regression equations for the relationship between metabolisable energy intake (ME, kJ/bird^{-1} d), liveweight (W, kg), liveweight change (ΔW, g bird d^{-1}), egg output (E, g/bird d^{-1}), average temperature (T, ^{o}C) and age (A, d) derived from individual bird data over 7 d periods from 10 eggs/100 hen d for nine (Experiment 1) and ten (Experiment 2) 28 d periods in each treatment assuming either an efficiency of energy utilization (kp) of 0.70 or 0.60 (Model 2).

Experiment	Equation (Model 2) and Independent Variables	Treatment[1]	Coefficients in the equation				Equation Number
			a	b	e	f	
1	a + (b + eT + fA)W + 20ΔW + 9.6E	1	287.4	354.7	-3.1	0.27	1-13
		2	357.4	389.5	-3.6	0.03	1-14
		3	50.5	564.1	-4.0	-0.05	1-15
		Overall	287.3	407.4	-4.0	0.10	1-16
	a + (b + eT + fA)W + 23ΔW + 11.2E	1	210.0	350.4	-2.5	0.27	1-17
		2	313.7	357.1	-2.9	0.05	1-18
		3	30.6	516.5	-3.1	-0.01	1-19
		Overall	231.6	386.2	-3.3	0.11	1-20
2	a + (b + eT + fA)W + 20ΔW + 9.6E	1	143.6	491.5	-3.1	-0.16	2-13
		2	325.2	416.3	-4.2	-0.10	2-14
		3	101.8	568.9	-1.7	-0.29	2-15
		Overall	236.1	455.9	-3.4	-0.12	2-16
	a + (b + eT + fA)W + 23ΔW + 11.2E	1	80.3	477.7	-2.2	-0.18	2-17
		2	223.4	424.3	-3.6	-0.13	2-18
		3	49.1	542.4	-1.5	-0.28	2-19
		Overall	155.2	452.8	-2.8	-0.15	2-20

1. See Table 6.4.

TABLE 6.6 Estimates of the metabolisable energy required for maintenance (ME_m) derived from the equations given in Tables 6.6 and 6.7 for a temperature of 20°C and for birds aged 300 d.

Experiment	Model	Treatment[1]	Metabolisable energy for maintenance (ME_m)[2]		Equation Number[3]
			kJ/kg W d	kJ/kg $W^{0.75}$ d	
1	1	1	545.6	648.8	1-9
		2	567.1	674.3	1-10
		3	527.8	627.6	1-11
		Overall	535.9	637.2	1-12
1	2	1	517.4	615.3	1-13
		2	505.2	600.8	1-14
		3	494.4	587.9	1-15
		Overall	501.1	595.9	1-16
1	2	1	486.4	578.4	1-17
		2	471.0	560.1	1-18
		3	466.8	555.1	1-19
		Overall	469.0	557.7	1-20
2	1	1	545.0	648.1	2-9
		2	507.9	604.0	2-10
		3	434.3	516.5	2-11
		Overall	530.3	630.6	2-12
2	2	1	453.3	539.1	2-13
		2	464.9	552.9	2-14
		3	498.8	593.2	2-15
		Overall	470.0	558.9	2-16
2	2	1	420.0	499.5	2-17
		2	425.0	505.4	2-18
		3	453.0	538.7	2-19
		Overall	429.4	510.7	2-20

1. See Table 6.4.

2. Calculations carried out at a mean liveweight (W) of 2 kg.

3. Equations given in Tables 6.4 and 6.5.

Within the range of liveweights used there was no improvement in the precision of the regression equations by the use of metabolic liveweight ($W^{0.75}$, kg) so these are not given. The derived regression equations (model 1, Table 6.4) were all significant (F ~500; P <0.001) and accounted for between 40 and 60% of the total variation.

Analyses of variance of the regression coefficients over treatments for each of the experiments for the equations given in Table 6.4 were significant (P <0.001). This was due to the following differences. In Experiment 1, for the basic equations (equations 1-1 to 1-8), the regression coefficient for liveweight in the limited-time treatment was lower (P <0.001) than for either of the other treatments (A and QR), but this treatment (TR) had a higher intercept; coefficients for liveweight change were greater (P <0.001) for the two restriction treatments (TR and QR); coefficients for egg output were lower (P <0.001) for the limited-time rather than the *ad libitum* or quanitative treatments. In Experiment 2, for the basic equations (equations 2-1 to 2-8) the same effects were evident for the liveweight coefficients as in Experiment 1 except that in addition the quantitative treatment was greater (P <0.001) than the *ad libitum* treatment but again there were intercept differences; the coefficients of liveweight change were greater (P <0.001) for the two restriction treatments (TR and QR); the limited-time treatment had a higher (P <0.001) coefficient for egg output than either the *ad libitum* or quantitative treatments, and the quantitative treatment had lower (P <0.001) coefficient for egg output than *ad libitum* treatment. There were no significant differences between treatments in either experiment for the rate of change in metabolisable energy intake per unit change in temperature.

In both experiments, for the equations derived using Model 1, the intercept was greater and the coefficient for liveweight lower for the limited-time treatment relative to the other treatments (A and QR). However the magnitude of the comparative changes was different between each of the experiments, such that the estimated maintenance energy requirement was greater for the limited-time treatment in Experiment 1 but less in Experiment 2 compared to the *ad libitum* treatments. For the quantitative treatment in Experiment 2, the lower intercept substantially contributed to the lower estimated maintenance energy requirement (see Table 6.6). Setting the partial efficiencies of production at 70 or 60% and using Model 2 for the multiple regression analysis did not give

markedly different intercepts or coefficients for the remaining variables (see Table 6.5). However this technique either decreased (Experiment 1) or increased (Experiment 2) the estimated maintenance energy requirements (Table 6.6) compared to those values estimated from Model 1, particularly for the two restriction treatments (TR and QR) relative to the *ad libitum* treatment. Lower energetic efficiency for production resulted in lower maintenance energy requirement, as expected for this situation.

6.4 DISCUSSION

6.4.1 Starvation heat production

The present study found that there were no major alterations in the appropriately determined starvation heat production at various physiological or chronological ages of birds due to undernutrition during rearing. Starvation heat production values obtained, in conjunction with the respiratory quotient, were well within the range of those determined under similar conditions for various strains of birds actively in egg production (see Table 6.7). The mean starvation heat production for one of the restriction treatments (limited-time) was elevated 98 d after sexual maturity. The reason for this rise in starvation heat production, without any prior indication of an increase during the two previous measurement periods (peak production and sexual maturity), is not clear. The rate of egg production for the total number of birds on this treatment at approximately 300 d of age was substantially higher than either of the other two treatments (see Chapter 3, Figure 3.3), although the extent to which treatment means can be extrapolated to the birds sampled for the calorimeter study, and the influence of rate of egg production on metabolic rate, are open to question. Unfortunately the temperatures of the respiration chambers dropped during this measurement period (post-peak) due to a malfunction in the room air conditioning.

Fuller and Dunahoo (1962) reported alterations in oxygen consumption rate of birds due to various restriction programmes during rearing. For comparative purposes the results given by Fuller and Dunahoo (1962) were recalculated to give starvation heat production values using an assumed respiratory quotient of 0.71 and the formula of Brouwer (1965). These values are given in Table 6.8. It is clear that certain treatment differences were not consistent. For example, although the starvation heat production for birds in group 2 was not reduced relative to group 1 at either 18 or 24 weeks of age, it was significantly lower at 52 weeks

TABLE 6.7 Starvation heat production (SHP) determined under standard conditions for different types of poultry which were in egg production.

Reference	Type of poultry[1]	Number of birds	Age (d)[2]	Liveweight (W, kg)	Respiratory quotient (RQ)	Starvation heat production (kJ) per d	per W d^{-1}	per $W^{0.75}$ d^{-1}
Waring & Brown (1965)	Thornber 404	6	NA≠	1.93	0.72	589	305	360
Waring & Brown (1967)	WL	4	364	1.62	0.72	625	386	435
Shannon & Brown (1969b)	WL	12	252	1.66	NA	593	357	405
Grimbergen (1970)	WL	12	230	1.68	NA	599	357	406
	WL	12	230	1.68	NA	605	360	410
	A X RIR	10	230	2.43	NA	738	304	379
Tasaki & Sasa (1970)	WL	2	NA	1.80	0.72	535	297	344
Leeson & Porter-Smith (1970)	Warren SSL	4	220+	2.42	NA	708	293	365
Farrell (1975)	WL	3	NA	1.56	NA	541	347	389
	A	4	NA	2.53	NA	619	245	309
	WL X A	5	NA	2.30	NA	736	320	394
Johnson *et al.* (1978)	WL X A	5	560	1.98	0.71	583	294	349
Balnave *et al.* (1978)	WL X A	4	315	1.55	0.71	571	368	411
	Broiler breeders	4	315	3.11	0.74	923	297	394
MacLeod & Shannon (1978)	Warren SSL	5	326*	2.18	NA	534	245	298
	Babcock B300	5	326*	1.35	NA	460	341	367
MacLeod *et al.* (1979)	WL	5	431	1.56	NA	603	387	432
Balnave *et al.* (1979)	WL X A	4	266	1.90	0.72-0.75	672	354	415
Present study	WL X A	6	322	1.88	0.73	591	314	368

1. WL: White Leghorn; A: Australorp; RIR: Rhode Island Red.
2. Age was often only approximate.

≠ NA: Not available.

\+ Post-peak production.

* Six determinations on five birds each over a 175 d period.

of age. Again, although birds in group 5 had a lower starvation heat production than those in group 1 at 18 weeks of age, there was no difference at 24 weeks of age but again a difference at 52 weeks of age. The main factor in this study (Fuller and Dunahoo 1962) which may have contributed to such inconsistencies was the short duration of measurement (cf. Cairnie and Pullar 1959). Additionally, there was no indication that measurements were carried out at the same time of day for all birds; if this was not the case then major errors could have occurred due to the pronounced circadian rhythms of poultry (Berman and Meltzer 1978; MacLeod *et al.* 1980).

TABLE 6.8 Starvation heat production (SHP, kJ/kgW d^{-1}) calculated from the oxygen consumption data given by Fuller and Dunahoo (1962) using the equation of Brouwer (1965) and assuming a respiratory quotient (RQ) of 0.71.

Rearing feed regimen	Duration of restriction (wks)	Starvation heat production (SHP, kJ/kgW d^{-1})		
		18 wks	24 wks	52 wks
Full-fed	-	362	257	237
Restricted	6 - 12	314	247	201
Restricted	6 - 18	302	243	237
Restricted	6 - 24	-	219	209
Restricted	12 - 24	275	247	193

In a more recent study, Balnave *et al.* (1979) used equipment of proven reliability and carried out all measurements over the accepted period of 24 h. However Balnave *et al.* (1979) concluded that "no significant differences in FMR [Fasting Metabolic Rate] due to feeding regimen were observed at any specific age between 13 and 70 weeks". This was surprising since the data provided for birds aged 20 weeks show a significant increase in starvation heat production (kJ/kgW d^{-1}) for the 25% restriction treatments (groups 2 and 3) ($P < 0.05$) and the 40% restriction treatments (groups 4 and 5) ($P < 0.001$). The present author reanalysed the original data from this study (D.J. Farrell, pers. comm.) and these significant differences at 20 weeks of age were confirmed by a one-way analysis of variance ($P < 0.001$, standard error of a mean = 7.59; least significant differences were: $P < 0.05$, 24.0; $P < 0.001$, 34.4). Also apparent in the reanalysis of the data from the study by Balnave *et al.* (1979) was that the initial determination of starvation heat production presented at 13 weeks of age included birds which were aged

from approximately 12 to 17 weeks. All of the birds selected from the full-fed control group were measured at 16 weeks of age. Nevertheless it can be concluded that at 20 weeks of age Balnave *et al.* (1979) found a positive correlation between degree of feed restriction and starvation heat production.

One of the main differences between the present study and that of Balnave *et al.* (1979) was that in the present study starvation heat production was measured with the birds maintained in complete darkness. This practice was adopted for two reasons. The first was to reduce the trauma associated with placement of the bird in the respiration chamber. This was considered important despite the regular familiarization procedures. The second was to minimize differences in activity between treatments. Activity is an extremely important component of the maintenance energy requirement, especially in birds subjected to feed restriction (Wenk and van Es 1980). A change in the pattern of activity could also alter metabolic rate (van Kampen 1976a, b & c). These effects may have contributed to the increased starvation heat production found by Balnave *et al.* (1979) for the birds on the restriction treatments. Also, dietary restriction was more severe than in the present study. The liveweights at 20 weeks of age were reduced by 22% and 34% for the two restriction treatments relative to the full-fed control birds (Balnave *et al.* 1979), whereas in the present study the liveweights at the same age were reduced by only 18% relative to the *ad libitum* treatment.

Although studies have found a decreased basal metabolism during undernutrition in a range of animal species (Marston 1948; Keys *et al.* 1950; Walker and Garrett 1970), some of the best estimations of the magnitude of the effect were from carefully controlled studies on sheep (Graham and Searle 1975, 1979; Thomson *et al.* 1980) and poultry (MacLeod and Shannon 1978; MacLeod *et al.* 1979). These latter studies on poultry were carried out during egg production, and indicate that factors such as duration of restriction and strain of bird are important in the magnitude of the reduction in starvation heat production obtained. For example, MacLeod and Shannon (1978) found a 6% reduction in starvation heat production (kJ/kgW d^{-1}) for one strain of bird (Warren SSL, liveweight reduction 10%) and a 13% reduction for another strain of bird (Babcock B300, liveweight reduction 6%) over a twenty-five week period of undernutrition. Subsequently, in another study (MacLeod *et al.* 1979) which used a different strain of bird (White Leghorn), starvation heat production (kJ/kgW d^{-1}) was reduced by 5% after a period of thirty-seven weeks

of undernutrition (liveweight was reduced by 18% compared to birds allowed *ad libitum* feed intake). However Balnave *et al.* (1979) found no alterations in starvation heat production due to prolonged (48 weeks) and severe undernutrition during the egg production period (liveweight reduced by approximately 25% at 70 weeks of age relative to birds allowed *ad libitum* feed intake). These birds (treatments 3 and 4) were also subjected to severe undernutrition during rearing (6-22 weeks of age). This work (Balnave *et al.* 1979) therefore contradicts most of the comparable work in this area.

The general effect of age on starvation heat production was an interesting aspect of the present study. Starvation heat production increased from the initial measurement period at 140 d up to 332 d of age, and from sexual maturity to peak egg production (28 d after sexual maturity). No further increases were apparent after these times. There was not an increase in starvation heat production due to the attainment of sexual maturity. The magnitude of the increases observed with increased chronological or physiological age in the present study were greater than those observed by Balnave *et al.* (1979). In the present study there was no relationship between feather cover and starvation heat production, probably because of the narrow range of feather scores for these birds (see Table 6.1). The reason for the difference between the two studies, despite similar strains of birds and measuring equipment, was probably the pattern of ambient temperature change. In the study of Balnave *et al.* (1979) birds were hatched in May and would have commenced egg production during a period of increasing ambient temperature. Mean temperatures of the respiration chambers for this study (Balnave *et al.* 1979), recalculated from the original data, were 24, 25 and 28^{o}C at 13, 20 and 38 weeks of age respectively. Metabolic rate declines with increasing temperature (Shannon and Brown 1969b; Johnson *et al.* 1978), and the increased temperature of measurement may have masked effects of age in the study of Balnave *et al.* (1979).

Tasaki and Sakurai (1969) found that season influenced the basal metabolic rate of birds in direct relationship with ambient temperature. In the present study, ambient temperature declined during the initial measurement periods (see Table 6.1 and Chapter 3, Figure 3.2). The influence on starvation heat production measured at 20^{o}C of prior temperature acclimation was investigated by Swain and Farrell (1975). For cross-bred cockerels (53 d of age) which were maintained for 25 d

at either 5°C or 34°C prior to measurement of starvation heat production (kJ/kgW d^{-1}) at 20°C, there was a 10% increase and a 6% decrease respectively relative to those birds maintained at 20°C. These changes are directly attributal to carry-over effects. The amount of time required for adjustment of starvation heat production after a rise in temperature was found to be between 3 and 12 d (Shannon and Brown 1969b). A recent study showed that there was an increase in the thermoneutral metabolism of laying hens due to a decrease in ambient temperature and that seasonal acclimatisation was a continual process during seasonal changes (Arieli *et al.* 1979).

Within the age limits of the birds used in the present study it can be concluded that age *per se* would have little effect on starvation heat production (cf. Balnave 1974). However many studies reported an increase in starvation heat production due to egg production: Waring and Brown (1965) found a 16% difference between the mean starvation heat production (kJ/kgW d^{-1}) of seven laying hens versus two non-laying hens; Tasaki and Sasa (1970) reported a 26% increase between laying hens and non-laying hens; Balnave *et al.* (1978) showed an 11% increase for laying rather than non-laying broiler breeder hens; MacLeod *et al.* (1979) found nearly a 50% increase between laying hens and cockerels of the same strain. With the use of more detailed results from Burlacu and Baltac (1971), Balnave (1974) showed that there was an approximate 25% increase in starvation heat production (kJ/kg$W^{0.75}$ d^{-1}) from 20 weeks of age to 20-60 weeks of age in birds maintained at 25°C from an early age. Balnave *et al.* (1978) found a 26% decrease in starvation heat production (kJ/kgW d^{-1}) due to ovariectomy in layer-type strains of birds previously in egg production (372 versus 275 kJ/kgW d); for broiler breeder birds there was a 20% decrease due to ovariectomy (298 versus 218 kJ/kgW d). Interestingly, in both types of bird oestrogen implantation after ovariectomy caused a further decline in starvation heat production. For the broiler breeder birds in that study (Balnave *et al.* 1978), it is possible to directly compare the effects of ovariectomy with simple cessation of egg production. The starvation heat production (kJ/kgW d^{-1}) after ovariectomy for a single bird was 237 compared with a value of 268 for two non-laying birds. To explain this effect there was a number of possibilities, examples of which are: (1) ovarian tissue *per se* has a high metabolic rate; (2) follicular synthesis continues in non-laying birds but resorption of the yolk material occurs; (3) ovariectomy results in a pronounced change

in the activity patterns of the birds; (4) ovariectomy results in an extreme upset in the complex hormonal inter-relationships which exist in birds (cf. Sturkie 1976).

Therefore the increase in starvation heat production found in the present study was probably due to a combination of two factors:

(1) acclimatization to the increasingly lower ambient temperatures with an elevation of starvation heat production and a consequent carry-over effect of this when starvation heat production was measured at approximately 20°C, and

(2) undetermined causes due to egg production.

Since there was no increase in starvation heat production due to attainment of sexual maturity, it can tentatively be concluded that the hormonal and metabolic changes associated with egg production are not the reasons for the afore-mentioned increases. Indeed there is even the possibility, on the basis of data given by Balnave *et al.* (1978), that oestrogen may cause a decrease in starvation heat production. If this is the case then the factors which not only counter-balance this effect but subsequently increase starvation heat production must be considerable. The possible causes of the observed increase in starvation heat production from this aspect are (a) a decrease in the thermoregulatory ability of the bird after a period in egg production; (b) a substantial but gradual change in the behavioural patterns of birds as egg production continues; (c) an alteration in the body composition of birds during egg production which gives an increase in the more metabolically active constituents.

There were no major differences between treatments in the type of body tissue oxidised during starvation heat production measurement (i.e. 26-48 h of starvation), as indicated by the respiratory quotient. Even during the rearing period, where body fat content was substantially reduced due to feed restriction (see Chapter 5), there were no differences between treatments in the respiratory quotient. However, increased duration of starvation would probably have resulted in a more rapid rise in the respiratory quotient of the restricted birds during the initial measurement period (pre-lay, 140 d of age) due to increased protein catabolism. The decrease which occurred in the respiratory quotient with increased chronological or physiological age was unexpected. However, the respiratory quotient at each measurement period was well within that expected for starved poultry (see Table 6.7). Since excreta were not collected it was not possible to partition the respiratory quotient between protein and non-

protein substrates. There is the possibility that the decrease in the respiratory quotient was related to seasonal, and therefore hormonal, changes.

6.4.2 Partition of metabolisable energy by the use of regression techniques

There are fundamental differences between the various techniques which can be used to obtain information on the partition of dietary metabolisable energy in poultry but which are considered regression techniques. The theoretical partition of metabolisable energy was considered in Chapter 1, Section 1.7.3. Brody (1945, p. 882) showed that if production data in experiments such as those reported in the present chapter were collected with sufficient accuracy then realistic estimates of energy partition could be obtained. Reid and associates (Reid *et al.* 1978; Valencia *et al.* 1980) calculated the quantity of energy retained (RE) by the use of constants for egg output and liveweight change. Metabolisable energy required for maintenance, and the energetic efficiency of production (kp) were then estimated by linear regression techniques in the usual manner. In the first study (Reid *et al.* 1978), with White Leghorn hens kept at 24°C, the metabolisable energy required for maintenance was found to be 464 kJ/kgW$^{0.75}$ d^{-1} with an energetic efficiency for maintenance and production of 62%. In the second study (Valencia *et al.* 1980), with White Leghorn hens kept at either 18.3°C or 35°C (1% fat diets), the estimated maintenance energy requirement was 538 and 426 kJ/kgW$^{0.75}$ d^{-1} respectively and the energetic efficiency 79%; at 22°C, assuming linearity, the maintenance energy requirement was 513 kJ/kgW$^{0.75}$ d^{-1} with a 1.2% increase per unit change in temperature (°C). Both the above studies used graded allocations of feed to obtain the necessary variation in retained energy and metabolisable energy intake.

However Byerly *et al.* (1980), similar to the present study (Model 2), allowed *ad libitum* feed intake but set the efficiency of energy utilization for production (*viz.* growth and egg production) prior to the derivation of the regression equation. The estimates for the maintenance energy requirement for a 2 kg bird kept at a temperature of 20°C calculated from the equations given by Byerly *et al.* (1980) were 447 kJ/kgW$^{0.75}$ d^{-1} and 474 kJ/kgW$^{0.75}$ d^{-1} for efficiency set at either 70 or 60% respectively. Additionally this study (Byerly *et al.* 1980) found a 1.2% increase in the energy required for maintenance per unit change in temperature (°C). Grimbergen (1974) discussed regression techniques in relation to calorimetric techniques and concluded that the latter gave lower estimates of

the energy required for maintenance. The range of values for the metabolisable energy required for maintenance obtained in the present study, especially with the more applicable Model 2, are slightly higher than those found in a range of calorimetric studies (see Table 6.9) when the effect of temperature is taken into consideration. This can be exemplified by comparison with the estimates obtained by Farrell (1975) with similar strains of birds; nevertheless, two factors adequately explain the differences between the techniques used to derive such values:

(1) Calorimetric estimates are obtained at one temperature in the thermoneutral zone, whereas there are usually substantial temperature variation in the production experiments which use regression analysis. Acclimatization may have a large influence on the energy required for maintenance (Arieli *et al.* 1979). The interaction in this regard with respect to the usually observed substantial deterioration in feather cover in laying hens (see Chapter 3) is extremely important. Feed intake, maintenance energy requirement and starvation heat production are increased due to poor feather cover (O'Neil *et al.* 1971; Johnson *et al.* 1978; Tauson and Svensson 1980; Hughes 1980; Tullet *et al.* 1980), particularly at low temperatures.

(2) The fundamental assumption in regression techniques is that energy intake is determined directly by energy requirements. Many reports showed that under certain conditions and with certain diets that this may not be the case (cf. De Groote 1974; Vohra *et al.* 1979). Therefore, as Sykes (1972) discussed, the energy requirements estimated by the use of regression techniques do not represent the minimum requirements.

As shown in Table 6.9, the available data from the literature were recalculated to clarify the values obtained for the maintenance energy requirement and the efficiency of utilization of metabolisable energy for production. These are extremely important tabulations because of their direct relevance to the studies reported in this thesis and because this is the first time that such a detailed evaluation of the literature has been undertaken. There is clearly a wide range in the calculated energy requirement for maintenance ($kJ/kgW^{0.75}\ d^{-1}$) for the various strains of poultry on which calorimetric studies were carried out. Many reports indicated a lower maintenance requirement ($kJ/kgW^{0.75}\ d^{-1}$) for the heavier strains of birds studied (Waring and Brown 1965, 1967; Farrell 1975; Grossu *et al.* 1976; Balnave *et al.* 1978; MacLeod and Shannon 1978). This implies *inter alia* two effects. The first is that conversion of the maintenance requirements to a metabolic liveweight ($W^{0.75}$, kg)

TABLE 6.9: Calorimetric determinations of energetic efficiency (km + p and kp) and maintenance metabolisable energy requirements (ME_m) for laying hens with or without inclusion of starvation heat production (SHP)

Type*	Reference	Type of bird	Range of observations			Temperature of determination (°C)	Energetic efficiency and ME_m (kJ/d)				
			SHP	SHP<RE≠<0	RE>0		SHP included		SHP excluded		
							k_{m+p}	ME_m (per kgW)	kp	ME_m (per kgW)	per $kgW^{0.75}$
A	Waring and Brown (1965)	Thornber 404	3	0	6	23	81.5	373.5	75.4	362.3	431.9
	Waring and Brown (1967)	WL	16	3	15	22	84.5	456.0	65.8	414.6	472.5
	Shannon and Brown (1969a)	Light Sussex+	4	5	5	22	70.6	286.8	65.0	280.9	400.8
	Shannon and Brown (1970)	Broilers+	4	2	8	22	85.8	449.9	65.5	415.6	519.9
	Grimbergen (1970)	WL	2	0	21	20	79.8	407.6	62.1	357.3	424.9
	Farrell (1975)	A	4	5	3	22	78.6	347.8	77.8	345.6	441.4
		WL	3	4	4	22	84.2	440.6	81.8	439.6	506.0
		WL x A	6	7	4	22	92.5	371.5	88.4	371.3	463.6
	Balnave *et al.* (1978)	WL x A	4	2	6	22	77.3	514.8	74.4	512.1	584.3
		Broiler breeders	5	5	6	22	78.9	401.8	65.2	394.9	533.9
B	Siregar and Farrell (1980)	Broilers+	4	0	12	25-28	77.0	853.2	50.0	456.0	468.1
	Grossu *et al.* (1976)	Rock	0	0	77	20	-	-	75.8	293.0	379.1
	van Es *et al.* (1970)	WL	0	(48	)[2]	NA	-	-	83.0	451.9	472.8
	Burlacu *et al.* (1974)	WL	0	(80	193)[1]	25	-	-	76.6	413.8	492.0
	MacLeod and Shannon (1978)	Warren SSL	30	(30	)[2]	20	87.1	258.0	-	-	321.1[3]
		Babcock B300	30	(30	)[2]	20	88.9	343.0	-	-	379.6[3]
	MacLeod *et al.* (1979)	WL	12	(12	)[2]	20	69.5	557.0	-	-	626.0[3]

* Type A values were calculated by the present author from data given in text; Type B values were taken from text.
\+ Not laying hens but were included for the effect of SHP on estimation of energetic efficiencies.
≠ RE is retained energy.
1. Approximate only.
2. Range of observation indeterminable.
3. SHP included.
NA is not available.

basis (Kleiber 1961) did not remove inter-strain differences; the second is that it illustrates the peculiar nature of poultry energetics in that substantial alterations can be effected by genetic manipulation (e.g. Pym and Farrell 1977). These effects accordingly mean that a single estimate of the maintenance energy requirement for the domestic fowl without cognisance of detailed strain characteristics is of doubtful application. Grouping the maintenance requirements determined by calorimetric studies for White Leghorn strains within similar liveweights (Waring and Brown 1967; Grimbergen 1970; van Es *et al.* 1970; Burlacu *et al.* 1974; Farrell 1975) gave estimates in the range 470 to 500 $kJ/kgW^{0.75}\ d^{-1}$. The estimates obtained by regression techniques (Reid *et al.* 1978; Valencia *et al.* 1980a,b; Byerley *et al.* 1980) agree well with these values. Values given by MacLeod *et al.* (1979) were not included because the White Leghorn strain used (H & N Chick) were extremely light-bodied at maturity (1.6 kg at 34 weeks of age); values given by Balnave *et al.* (1978) were omitted because the present author was unable to explain the extremely high values obtained. In that study (Balnave *et al.*1978) no temperature acclimatization was allowed prior to calorimetric observations. Although this was similar to the procedure used by Farrell (1975), the study of Balnave *et al.* (1978) was carried out after a period of some months of very cold temperatures. Carry-over effects of acclimatization (Swain and Farrell 1977; Arieli *et al.* 1979) may account for these effects; another factor may be that all birds were sham-operated, but no unoperated birds were included for comparison. The values found in that study (Balnave *et al.* 1978) for broiler breeders are also extremely high, but this will be further discussed in a later chapter (Chapter 7).

The influence of inclusion of starvation heat production on the estimates obtained by calorimetric studies is particularly important (Table 6.9). Recalculation of the literature data but without inclusion of starvation heat production substantially lowered the energetic efficiencies derived. Grimbergen (1970, 1974) previously questioned the high energetic efficiencies found in poultry metabolism studies, and attributed them to the contribution of body tissue reserves (and subsequent heat loss) to egg synthesis. The recalculations shown in Table 6.9 indicate that the disproportionate influence of the inclusion of starvation heat production on the energetic efficiency obtained may account for the high values found in some calorimetric studies. Reanalysis of the data provided by Grimbergen (1970) (not presented) showed that neither the maintenance requirement nor the energetic efficiency for production were

greatly influenced by the proposed correction factor. However, for calorimetric studies in which the majority of the observations approximate the maintenance energy requirement the argument proposed by Grimbergen (1970, 1974) would have greater applicability. On the basis of the values for energetic efficiency calculated from previously published reports in Table 6.9, it is reasonable to conclude that the efficiency of utilization of metabolisable energy for production in poultry is within the range 60 to 90%. Similar to the maintenance energy requirement, the choice of a single energetic efficiency is impossible and without great application.

In the present study, birds in Experiment 1 had a higher maintenance energy requirement and a lower efficiency of utilization of metabolisable energy for production than for birds in Experiment 2. These differences were probably due to a strain effect and explain the lower gross efficiency of egg production found in the first experiment. Application of the basic regression equations in Model 1 (equations 1-1 to 1-8 and 2-1 to 2-8) showed that there was a fundamental change in the partition of dietary metabolisable energy between treatments. In both experiments the birds which were restricted during rearing by limitation of feeding time had a lower regression coefficient for liveweight but a higher intercept. In Experiment 1 this resulted in a higher maintenance requirement compared to the other two treatments, but a higher efficiency of utilization of energy for egg production; in Experiment 2 these relative differences were reversed for the limited-time birds. In Experiment 2, both the restriction treatments had a lower estimated maintenance requirement than for birds which were allowed *ad libitum* feed intake during rearing, as calculated by Model 1. The variability in the regression coefficients for egg output, and particularly the very low coefficients obtained in Experiment 2 (e.g. equation 2-3) which often implied an efficiency of energy utilization for egg synthesis of greater than 100%, indicated that the ability of Model 1 to partition dietary energy was limited; this may be due to the low amount of the total variation (R^2) accounted for by the regression analysis. Nevertheless the relative differences between treatments may be valid indications of fundamental alterations in energy partition.

Setting the efficiency for production prior to regression analysis (Model 2) does not allow for differences in efficiency between treatments. However the technique indicated important differences between treatments. In Experiment 1 there was a lower maintenance requirement for the two

restriction treatments by the use of Model 2. This indicates that if the efficiencies of production are similar between treatments then the maintenance requirement may be lower; this could account for the observed differences in the gross energetic efficiency of egg production. In Experiment 2 the application of Model 2 indicated an increase in the estimated maintenance requirements for the restriction treatments, particularly for the birds which were quantitatively restricted during rearing. Taken in conjunction with the observed higher gross efficiencies for the restriction treatments this may indicate that efficiency of production was, in fact, higher. The reported effects of undernutrition on laying hens with respect to the maintenance energy requirement and the efficiency of utilization of energy are equivocal. Certainly, it appears that during undernutrition there is a reduction in the maintenance energy requirement (MacLeod and Shannon 1978; MacLeod *et al.* 1979). However the total effect of this on gross energetic efficiency was marginal in the experiments of MacLeod and coworkers. Short-term energy restriction can result in substantial increases in gross energetic efficiency (Sykes 1972), but the counter-balance between the magnitude of the decrease in the maintenance requirement and the change in energetic efficiency in long-term feed or energy restriction may result in only slight increases in overall gross efficiency (cf. MacLeod and Shannon 1978; MacLeod 1979).

However the applicability of the above studies to the present study is limited because the carry-over effects of undernutrition on energy metabolism after realimentation were not investigated. Currently there are no calorimetric studies available on poultry which were previously subjected to feed restriction during rearing for comparison with the present study. Walker and Garrett (1970) found an increased energetic efficiency in rats which persisted after realimentation, although the results presented are difficult to interpret. Graham and Searle (1975, 1979) found a reduction in starvation heat production in sheep during undernutrition, but this did not persist after subsequent realimentation. In the present experiment there was no effect either during feed restriction or after realimentation on starvation heat production (see Section 6.3.1), at least for birds in Experiment 1, but this does not eliminate the possibility of changes in the maintenance energy requirement. Starvation heat production plus the endogenous energy output is the net energy required for maintenance. The metabolisable energy

requirement for maintenance is therefore dependent on the efficiency of utilization of energy for maintenance (km).

Summary

The energy metabolism of layer-type birds was investigated both by measurement of starvation heat production and by partition of dietary metabolisable energy between the processes of maintenance and production with regression techniques. Starvation heat production was measured in closed-circuit respiration chambers on six birds from each of the three treatments described for Experiment 1 in Chapter 3 at three chronologic and three physiologic ages. Chronological age determinations were at 140 d, 330 d and 370 d of age, while physiological age determinations were at sexual maturity (first oviposition), peak of egg production and post-peak of egg production. Starvation heat production expressed on a live-weight or metabolic liveweight basis was not influenced by either limited-time or quantitative feed restriction methods compared to birds allowed *ad libitum* feed intake during rearing. Techniques used during measurement partially excluded changes due to differences between treatments in activity. The application of two linear regression models to partition the dietary metabolisable energy intake of birds in two experiments (Experiments 1 and 2 in Chapter 3) during egg production gave disappointing results, mainly because of the lack of variation accounted by technique. Nevertheless there were some indications that significant alterations in energy metabolism occurred during egg production in the birds previously on the feed restriction programmes during rearing. These effects were discussed in relation to the reported alterations in energy metabolism due to undernutrition.