Ann Zootech (1997) 46, 309-319

Original article

Changes in cell number and size and in lipogenic enzyme activity in adipose tissues during growth and fattening of Lacha (Manech) lambs

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(Received 12 February 1996; accepted 15 January 1997)

Summary — Variations during growth and fattening in the number and size of adipocytes and in the activity of the lipogenic enzymes, G3PDH, FAS, EM and G6PDH, were studied in the omental, mesenteric, kidney knob and channel fat, subcutaneous and intermuscular adipose tissues of 57 male Lacha (Manech) lambs. The animals were slaughtered at live weights (LW) of 11.4 (n = 15), 18.1 (n = 15), 24.6 (n = 15) and 35.3 (n = 12) kg. A significant increase in the quantity of fat was observed as the LW of the lambs increased ($P < 0.001$). Fattening was more rapid between 24 and 36 kg LW than between 12 and 24 kg LW. Hyperplasia of adipocytes occurred predominantly between 12 and 24 kg, when changes in size and lipogenic activity were small. Between 24 and 36 kg LW, a marked increase in fat deposition was accompanied by an increase in the size of adipocytes and in lipogenic enzyme activity.

adipocytes / lipogenic enzyme activity / lambs / growth

Résumé — Variation de la cellularité et des activités enzymatiques lipogéniques des tissus adipeux chez l'agneau de race Lacha (Manech) pendant la croissance et l'engraissement. L'évolution du nombre et de la taille des adipocytes ainsi que les activités enzymatiques lipogéniques de différents tissus adipeux ont été étudiés chez 57 agneaux mâles de race Lacha (Manech), pendant les périodes de croissance et d'engraissement. Les agneaux ont été abattus au poids vif (PV) de 11,4 (n = 15) (G12), 18,1 (n = 15) (G18), 24,6 (n = 15) (G24) et 35,3 (n = 12) kg (G36) à l'âge respectif de 25, 69, 87 et 131 j. Les agneaux du groupe G12 ont été abattus le jour du sevrage ; ceux des groupes G18, G24 et G36 ont été sevrés respectivement à 13,3, 14,2 et 14,5 kg de PV soit 36, 36 et 37 j d'âge. Ils ont eu à leur disposition de l'aliment concentré et de la paille d'orge ad libitum jusqu'à l'abattage. Les paramètres étudiés sont la quantité de gras et le nombre des adipocytes dans les dépôts omental (OM), mésentérique (MES) et pélvique-rénal (KKCF), et dans les dépôts OM, MES, KKCF, sous-cutané (SC) et intermusculaire (IM), le diamètre des adipocytes et l'activité des enzymes glycerol 3-

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phosphate déshydrogénase (G3PDH), synthétase des acides gras (FAS), NADP-malate déshydrogenase (EM) et glucose 6-phosphate déshydrogénase (G6PDH) pendant la période correspondant au poids vif de 12–36 kg des animaux. Une augmentation de la quantité de gras avec le PV a été notée à l'abattage des agneaux ($p < 0,001$) ; cette augmentation étant plus élevée entre 24 et 36 kg de PV qu'entre 12 et 24 kg. Le tissu MES a montré une hyperplasie significative des adipocytes pendant toute la période étudiée tandis que les tissus OM et KKCF ont presenté une hyperplasie entre 12 et 24 kg de PV. Les tissus OM, MES, KKCF et SC ont presenté une hypertrophie entre 24 et 36 kg de PV ($p < 0,05$), tandis que pour le dépôt IM la taille des cellules n'a pas varié pendant la période étudiée. L'activité de l'enzyme G3PDH (estimateur de la synthèse totale de triglycérides) a augmenté avec le PV dans les cinq tissus étudiés ($p < 0,001$). La synthèse de novo des acides gras, traduite par l'activité de l'enzyme FAS, a également augmenté avec le PV ($p < 0,001$), cette augmentation étant plus élevée entre 24 et 36 kg de PV. Parmi les enzymes contrôlant la synthèse du NADPH, l'EM a seulement augmenté entre 24 et 36 kg de PV ($p < 0,05$), alors que l'activité de la G6PDH a augmenté régulièrement en fonction de l'augmentation du PV ($p < 0,001$).

adipocytes / activités enzymatiques lipogéniques / agneaux / croissance

INTRODUCTION

The increase in adiposity of the adipose tissues of lambs is due to an increase in the number of adipocytes, hyperplasia, and in their size, hypertrophy (Vernon, 1986). Development of adipose tissues which is related to lipogenic enzyme activity results in the accumulation of triglycerides in the adipocytes. The fatty acids in the triglycerides are derived from plasma or from de novo synthesis. It has been observed that hyperplasia, hypertrophy and lipogenic enzyme activity are all influenced by sex, breed, age, physiological condition, type and level of feeding, and type of adipose tissue in the species (Hood, 1982; Vernon, 1986).

Adiposity increases as the animal grows and becomes more intense in the final phases of development and maturity when muscle growth decreases (Vernon, 1980). The relative contribution of hyperplasia and hypertrophy to the development of adipose tissues varies during growth and fattening (Nouguès and Vézinhet, 1977). Serum hormones are involved in the different rates of both processes and, consequently, in lipid metabolism of adipose tissue (Anderson and Kauffman, 1973). Serum insulin tends to increase with age and is positively correlated with adiposity, while serum growth hormone, which is negatively correlated with adiposity, decreases with age (Flint and Vernon, 1993).

The enzymes glycerol 3-phosphate dehydrogenase (G3PDH), fatty acid synthetase (FAS), NADP-malate dehydrogenase (EM) and glucose 6-phosphate dehydrogenase (G6PDH) are involved in the lipogenic pathways. G3PDH activity is closely correlated with triglyceride synthesis (Shidu et al,1973). Fatty acid synthetase (FAS) is implicated in the conversion of acetate to fatty acids, and EM and G6PDH are two of the main enzymes which catalyze the production of NADPH required for fatty acid synthesis.

This study examined changes in hypertrophy, hyperplasia and lipogenic enzyme activity in adipose tissues of lambs during growth and fattening.

MATERIALS AND METHODS

Animals and diets

Fifty-seven male Lacha (Manech) lambs from a single lambing at the Instituto Técnico de Gestión Ganadero of the Navarra Governement, were distributed randomly in four groups (G12, G18,

G24 and G36). Lambs in group G12 were fed entirely on suckled ewes milk until slaughter. Lambs in groups G18, G24, G36 were fed ewes milk and a commercial concentrate starter, with a composition of OM 92.5% dry matter, CP 17.5%, EE 4.7%, CF 5.1%, until weaning and thereafter on a commercial concentrate, with a composition of OM 92.6% dry matter, CP 16.5%, EE 4.5%, CF 7.1%, and barley straw ad libitum until slaughter. The lambs were not fasted before killing. Growth parameters are shown in table I.

After slaughter, samples (0.5 g for adipocyte preparation and 5 g for enzyme assays) were removed from five adipose tissues: omental (OM; middle area of the greater omentum), mesenteric (MES; middle area of the rectum), kidney knob and channel fat (KKCF: the cephalic part of the left kidney), subcutaneous (SC; base of the tail) and intermuscular (IM; between the sternum and the pectoral muscles). The total weight of the OM and MES was recorded. The digestive tract of the animals was emptied, weighed and the empty live weight (ELW) calculated. The carcasses were held at 4°C for 24 h and the KKCF was removed and weighed. Back fat thickness (BFT) was measured at a point 4 cm from the spine and 4 cm behind the last rib. Slaughter parameters are shown in table I.

Number and size of adipocytes

The number and size of adipocytes were determined on 0.5 g of fresh tissue from each of the five adipose tissues. Immediately after slaughter the samples were placed in Tirode solution (NaCl, 9 g; KCl, 0.42 g; $CaCl_2$, 0.24 g; glucose, 1.0 g; $NaHCO_3$, 0.2 g; distilled H_2O to 1 L) (pH 7.62) at 39°C and transported to the laboratory. Adipocytes were fixed with 2% osmium tetroxide (Hirsch and Gallian, 1968) and treated with 8 M urea to dissolve the connective tissue (Etherton et al, 1977). Adipocytes were filtered twice in succession (filters of 800 and 0.45 mm pore diameter) and collected in the second filter. The average diameter of the adipocytes was obtained by measuring approximately 180 adipocytes per sample (Biocom, 1992).

The number of adipocytes from OM, MES and KKCF adipose tissues was calculated on the basis of chemical fat content (Soxhlet's method),

Table I. Parameters of growth (live weigth and age) and slaughter (weight, age, digestive content, empty live weight, carcass weight and back fat tickness) observed in G12, G18, G24 and G36 Lacha lambs.

	G12	*G18*	*G24*	*G36*
n (animals)	15	15	15	12
Birth weight (kg)	4.9 ± 0.82	4.2 ± 0.70	4.5 ± 0.62	4.5 ± 0.71
Weaning weight (kg)	11.4 ± 0.70	13.3 ± 1.15	14.2 ± 1.51	14.5 ± 1.53
Weaning age (days)	25 ± 8	36 ± 6	36 ± 6	37 ± 5
Mean daily gain (g/day) (birth-weaning)	253 ± 73.1	260 ± 36.9	274 ± 32.7	273 ± 64.7
Mean daily gain (g/day) (weaning-slaughter)	–	145 ± 23.9	220 ± 53.1	225 ± 27.5
Slaughter weight (kg)	11.4 ± 0.70	18.1 ± 0.65	24.6 ± 1.41	35.3 ± 1.67
Slaughter age (days)	25 ± 8	69 ± 7	87 ± 12	131 ± 12
Digestive content (kg)	0.54 ± 0.164	2.62 ± 0.557	3.73 ± 0.472	4.18 ± 0.754
Empty live weight (kg)	10.8 ± 0.64	15.5 ± 0.61	20.8 ± 1.27	31.1 ± 1.65
Carcass weight (kg)	5.9 ± 0.34	7.8 ± 0.33	10.6 ± 0.73	16.5 ± 0.98
Back fat thickness (mm)	1.6 ± 0.47	0.8 ± 0.30	1.2 ± 0.50	3.1 ± 0.92

the chemical fat density (d = 0.915 g/cc; Keys and Brozek, 1953) and mean adipocyte volume, assuming the adipocytes were spherical.

Lipogenic enzyme activity

Tissue homogenates were prepared in STEG pH 7.4 buffer (sucrose, 0.3 M; Trizma base, 30 mM; EDTA, 1 mM; gluthatione (GSH), 1 mM) (1:4 w/v) from the frozen 5 g samples of tissue, using a Sorvall Omni-Mixer homogenizer (10 s at 50 000 rpm, three times) while keeping the tissue ice-cold. Homogenates were filtered (20 and 0.45 mm pore diameter) and centrifuged (6 000 rpm, for 10 min at 4°C). The supernatant was filtered (20 mm pore diameter) and centrifuged (18 000 rpm, for 45 min at 4°C). After filtering (0.45 mm pore diameter) the extracts were assayed at 37°C for glucose 3-phosphate dehydrogenase (G3PDH; EC 1.1.1.8) (Wisse and Green, 1979), synthetase of fatty acids (FAS; EC 2.3.1.85) (Halestrap and Denton, 1973), NADP-malate dehydrogenase (EM; EC 1.1.1.40) (Ochoa, 1955) and glucose 6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) (Glock and McLean, 1953). Reactions were linear over the period of assay and were proportional to the amount of extract added.

Statistical analysis

Results were analyzed statistically using variance analysis (LSMLMW program; Harvey, 1987). Adipocyte diameters were normally distributed. The other measurements had exponential distributions and were therefore converted logarithmically. The model used was the following:

$$y_{ijk} = \mu + LW_i + AT_j + (LW \times AT)_{ij} + e_{ijk}$$

where: y_{ijk}, amount of fat (fresh and chemical), number and diameter of the adipocytes, enzyme activity; μ, least square mean; LW_i, fixed effect due to the LW of the lambs (1 = 12 kg, 2 = 18 kg, 3 = 24 kg, 4 = 36 kg); AT_j, fixed effect due to the type of adipose tissue (1 = OM, 2 = MES, 3 = KKCF, 4 = SC, 5 = IM); $(LW \times AT)_{ij}$, effect due to the interaction between live weight and adipose tissue, and e_{ijk}, random residual effect.

RESULTS

Table II shows that the amount of fat increased significantly ($P < 0.001$) as the LW of the lambs increased from 12 to 36 kg. Fattening was quicker between 24 and 36 kg than between 12 and 24 kg LW (fig 1). The same pattern was also observed if the amount of fat was related to the empty LW and even a decrease in KKCF depot weight was found between 12 and 24 kg LW ($P < 0.05$) in that case. Comparison of total weights of fresh fat from the three internal adipose tissues showed that KKCF was the largest depot in lambs of 12 kg LW, while at 36 kg the OM depot was largest.

The number of adipocytes increased in MES tissue between 12 and 36 kg. In the OM and KKCF tissues, hyperplasia occurred between 12 and 24 kg LW. The small increase in cell number between 24 and 36 kg LW was not significant (fig 2). The number of adipocytes was the highest in KKCF at each live weight.

There were no significant differences in the size of adipocytes between 12 and 24 kg LW, but between 24 and 36 kg LW significant increases occurred in OM, MES,

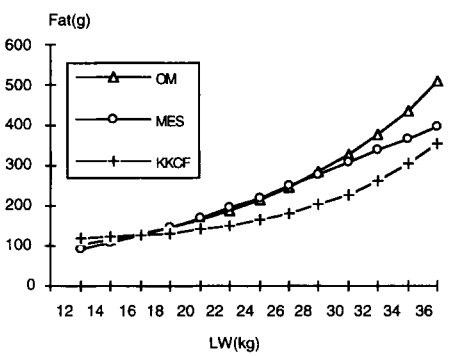

Fig 1. Quantity of fresh fat (g) in omental (OM), mesenteric (MES) and kidney knob and channel fat (KKCF) adipose tissues in male Lacha lambs of 11.4 (G12), 18.1 (G18), 24.6 (G24) and 35.3 (G36) kg of slaughter live weight (LW).

Table II. Quantity of fresh fat (g), fresh fat referring to empty live weight (ELW, g/kg), percentage of chemical fat (%), chemical fat referring to empty live weight (ELW, g/kg), number of adipocytes ($x10^7$) and their diameter (μm) observed in male Lacha lambs of 11.4 (G12), 18.1 (G18), 24.6 (G24) and 35.3 (G36) kg of slaughter weight in the omental (OM), mesenteric (MES), kidney know and channel fat (KKCF), subcutaneous (SC) and intermuscular (IM) adipose tissues.

	G12	*G18*	*G24*	*G36*	*LW*	*AT*	*LW×AT*
Fresh fat (g)					***	***	***
OM	102.2^{a1}	146.5^{b}	215.7^{c1}	506.6^{d1}			
MES	90.6^{a1}	146.5^{b}	220.0^{c1}	396.7^{d2}			
KKCF	118.2^{a2}	$131.7^{a,b}$	163.9^{b2}	354.2^{c2}			
Fresh fat/ELW (g/kg)					***	***	***
OM	9.1^{a}	9.5^{a1}	10.6^{a1}	16.0^{b1}			
MES	7.8^{a1}	$8.7^{a,b}$	9.9^{b1}	13.0^{c2}			
KKCF	10.6^{a2}	$8.5^{a,b2}$	8.0^{b2}	11.2^{a2}			
Chemical fat (%)					***	***	***
OM	75.6^{1}	73.1^{a1}	73.7^{a1}	84.6^{b1}			
MES	47.1^{a2}	47.1^{a2}	49.6^{a2}	61.9^{b2}			
KKCF	84.9^{3}	86.2^{3}	87.2^{3}	87.8^{3}			
Chemical fat/ELW (g/kg)					***	***	***
OM	7.6^{a1}	7.2^{a1}	8.5^{a1}	13.8^{b1}			
MES	2.9^{a2}	5.1^{b2}	5.2^{b2}	7.8^{c2}			
KKCF	10.3^{a3}	$7.8^{b,c1}$	7.2^{b1}	$9.9^{a,c2}$			
Number of adipocytes ($\times 10^7$)					***	***	***
OM	62.7^{a1}	99.1^{b1}	136.6^{c1}	171.4^{c1}			
MES	40.3^{a2}	68.7^{b2}	102.0^{c2}	147.6^{d1}			
KKCF	153.0^{a3}	$170.1^{a,b3}$	$188.5^{b,c3}$	229.6^{c2}			
Diameter of adipocytes (μm)					***	***	***
OM	61.5^{a1}	58.3^{a1}	60.1^{a1}	77.8^{b1}			
MES	$58.0^{a1,2}$	57.1^{a1}	58.3^{a1}	67.0^{b2}			
KKCF	50.2^{a3}	50.5^{a2}	52.8^{a2}	$63.5^{b2,3}$			
SC	56.2^{a2}	52.8^{a2}	55.8^{a}	80.3^{b1}			
IM	$58.2^{1,2}$	57.0^{1}	56.7	58.9^{3}			

Comparison between live weight (LW, in rows with letters) and adipose tissues (AT, in columns with numbers): ***, $P < 0.001$. ^{a,b,c,d} values within a row with different letters differ significantly ($P < 0.05$); same or absence of letters, $P > 0.05$. ^{1,2,3} values within a column with different numbers differ significantly ($P < 0.05$); same or absence of numbers, $P > 0.05$.

KKCF and SC tissues ($P < 0.05$). No significant differences in size were observed in IM tissue. The patterns of change in size of adipocytes are shown in figure 3.

Table III shows the activity of G3PDH, FAS, EM and G6PDH enzymes in each adipose tissue at the four slaughter weights. G3PDH activity increased significantly with LW ($P < 0.001$). The differences between 12 and 24 kg were not significant, with the exception of IM, where a decrease occurred, and KKCF, where a significant increase was

found. Between 24 and 36 kg LW significant increases occurred in all five adipose tissues ($P < 0.05$) (fig 4).

De novo synthesis of fatty acids, as quantified by FAS activity, was significantly affected by weight ($P < 0.001$). FAS activity did not vary significantly with weight in OM tissue. FAS activity increased in all tissues between 24 and 36 kg LW, with significant increases in MES, KKCF and SC (fig 5). At 12 kg LW the lowest FAS activity was observed in OM and IM tissue, and at 36 kg LW the highest activity was observed in the MES and KKCF tissues.

There was no variation in the activity of the EM enzyme as LW increased from 12 to 24 kg, but a significant increase occurred between 24 and 36 kg LW ($P < 0.05$). There were no significant differences in EM activity between the adipose tissues.

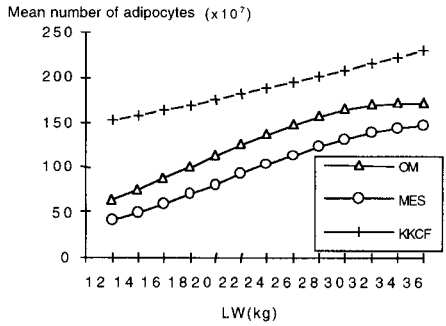

Fig 2. Number of adipocytes ($\times 10^7$) in omental (OM), mesenteric (MES) and kidney knob and channel fat (KKCF) adipose tissues in male Lacha lambs of 11.4 (G12), 18.1 (G18), 24.6 (G24) and 35.3 (G36) kg of slaughter live weight (LW).

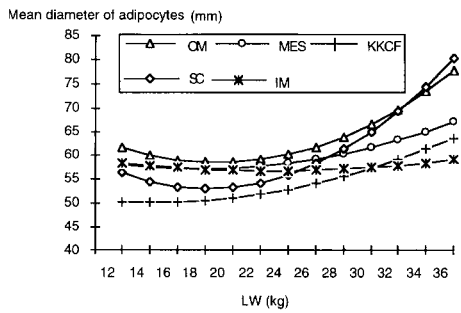

Fig 3. Diameter of adipocytes (μm) in omental (OM), mesenteric (MES), kidney knob and channel fat (KKCF), subcutaneous (SC) and intermuscular (IM) adipose tissues in male Lacha lambs of 11.4 (G12), 18.1 (G18), 24.6 (G24) and 35.3 (G36) kg of slaughter live weight (LW).

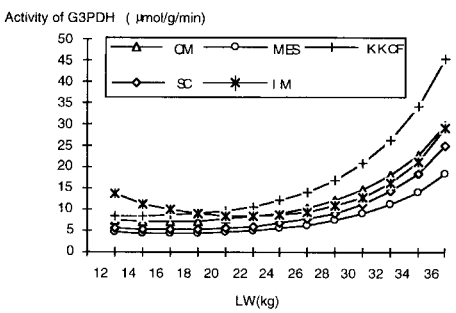

Fig 4. Activity (μmol/g/min) of glycerol 3-phosphate dehydrogenase (G3PDH) in omental (OM), mesenteric (MES), kidney knob and channel fat (KKCF), subcutaneous (SC) and intermuscular (IM) adipose tissues in male Lacha lambs of 11.4 (G12), 18.1 (G18), 24.6 (G24) and 35.3 (G36) kg of slaughter live weight (LW).

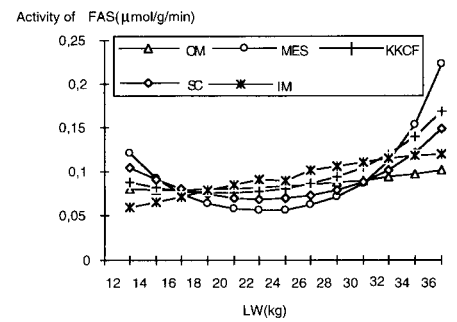

Fig 5. Activity (μmol/g/min) of fatty acid synthesase (FAS) in omental (OM), mesenteric (MES), kidney knob and channel fat (KKCF), subcutaneous (SC) and intermuscular (IM) adipose tissues in male Lacha lambs of 11.4 (G12), 18.1 (G18), 24.6 (G24) and 35.3 (G36) kg of slaughter live weight (LW).

Table III. Changes in activities (μmol/g/min) of glycerol 3-phosphate dehydrogenase (G3PDH), fatty acid synthesase (FAS), glucose 6-phosphate dehydrogenase (G6PDH), and NADP-malate dehydrogenase enzyme (EM) in omental (OM), mesenteric (MES), kidney know and channel fat (KKCF), subcutaneous (SC) and intermuscular (IM) adipose tissues in male Lacha lambs of 11.4 (G12), 18.1 (G18), 24.6 (G24) and 35.3 (G36) kg of slaughter weight.

	G12	*G18*	*G24*	*G36*	*LW*	*AT*	*LW×AT*
G3PDH					***	***	**
OM	$7.305^{a1,2}$	7.237^{a1}	$9.111^{a1,2}$	$29.639^{b1,2}$			
MES	4.542^{a3}	4.415^{a2}	5.528^{a4}	18.514^{b3}			
KKCF	8.392^{a2}	8.958^{a1}	12.131^{b1}	45.422^{c2}			
SC	$5.472^{a1,3}$	5.227^{a2}	$6.635^{a3,4}$	$25.077^{b1,3}$			
IM	13.750^{a4}	8.851^{b1}	$8.657^{b2,3}$	29.030^{c}			
FAS					***	*	*
OM	$0.080^{1,2}$	0.080	0.084^{1}	0.102^{1}			
MES	0.122^{a3}	0.064^{b}	0.057^{b2}	0.223^{c2}			
KKCF	$0.089^{a1,3}$	0.076^{a}	0.081^{a1}	$0.168^{b2,3}$			
SC	$0.106^{a1,3}$	0.074^{b}	0.070^{b}	0.149^{a}			
IM	0.060^{a2}	$0.079^{a,b}$	$0.097^{b,c1}$	$0.120^{c1,3}$			
EM					**	NS	NS
	0.158^{a}	0.144^{a}	0.145^{a}	0.201^{b}			
G6PDH					***	***	***
OM	0.537^{a1}	0.684^{a1}	0.865^{a}	1.352^{b1}			
MES	$0.280^{a2,3}$	0.354^{a2}	0.496^{a1}	1.309^{b1}			
KKCF	0.196^{a3}	0.617^{b1}	1.117^{c2}	$0.695^{b,c2}$			
SC	0.313^{a2}	0.639^{a1}	1.035^{b2}	1.350^{b1}			
IM	0.882^{a4}	0.646^{a1}	0.675^{a1}	2.143^{b1}			

Comparison between live weight (LW, in rows with letters) and adipose tissues (AT, in columns with numbers): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, $P > 0.05$. ^{a,b,c} values within a row with different letters differ significantly ($P < 0.05$); same or absence of letters, $P > 0.05$. ^{1,2,3,4} values within a column with different numbers differ significantly ($P < 0.05$); same or absence of numbers, $P > 0.05$.

G6PDH enzyme activity increased significantly with LW ($P < 0.001$). In OM, MES and IM tissues, there were no differences between 12 and 24 kg LW, and significant increases between 24 and 36 kg LW ($P < 0.05$). In KKCF and SC tissues, significant increases occurred earlier, between 12 and 18 kg LW in KKCF and between 18 and 24 kg LW in SC. There were significant differences in G6PDH activity between adipose tissues. The highest G6PDH activity in IM tissue was at 12 kg while KKCF tissue had the lowest activity at 36 kg LW ($P < 0.05$).

DISCUSSION

Fat accumulation rate increases with live weight during growth and fattening in lambs. Lacha (Manech) is a non-improved rustic breed and it has been observed that fat accretion takes place earlier in this kind of breed than in improved breeds (Kempster, 1980–1981). In this study, development of adipose tissues, expressed both in absolute terms and relative to empty LW, was greater between 24 and 36 kg LW than between 12 and 24 kg. This is consistent with the observation that adipose tissue is the tissue which develops the latest and its allometric coefficient is between 1.3 and 1.6 (Hammond, 1932; Fourie et al, 1970; Kirton et al, 1972; Kempster, 1980–81). Between 12 and 24 kg, fat expressed as g/kg empty LW increased in MES tissue (2.1 g/kg), was unchanged in OM, and decreased in KKCF (2.6 g/kg), while between 24 and 36 kg LW there were significant increases in all adipose tissues (table II).

In the phase of growth between 12 and 24 kg LW (25–87 days) muscle growth predominates (Searle and Griffiths, 1976). In this experiment, fattening between 12 and 24 kg LW could have been influenced by weaning and physiological and metabolic changes in digestion and absorption of nutrients as the lamb changes from a pre-ruminant to a ruminant, with fully developed rumen fermentation. Ørskov et al (1971), Robelin et al (1977), Thériez et al (1981), Walker (1986) and Bocquier et al (1988), have shown that early weaning reduces feed intake and hence growth rate. The decrease in the fat in KKCF tissue, relative to empty LW, suggests that a greater mobilisation of triglycerides occurs after weaning in the earliest developing adipose tissue, which presented in our study an allometric coefficient of 0.98 compared to 1.41 and 1.50 in MES and OM, respectively. This finding agrees with the results of Morand-Fehr et al (1985) founding that in kids the largest relative weight lost after weaning among the depots studied omental, mesenteric, perirenal and pericardic was observed in the perirenal depot.

Haugebak et al (1974), Allen (1976) and Hood (1982) reported an increase in the number of adipocytes (hyperplasia), specially in the early stages of the development of adipose tissues. Similarly, in this study significant increases in the number of adipose cells in OM and KKCF tissues were observed only between 12 and 24 kg LW, which is in agreement with the reduction in hyperplasia with age. In KKCF tissue the increase of number of adipocytes between 12 and 36 kg LW was 50% lower than in the OM and MES tissues, with increases of 173% and 266%, respectively. This confirmed the results of Nouguès and Vézinhet (1977), Broad et al (1980) and Vernon (1986), showing that KKCF tissue is earliest to mature in terms of cell proliferation. MES tissue was the last of the adipose tissues to mature and adipocytes continued to proliferate significantly to 36 kg LW.

There were two phases in the evolution in the size of adipocytes in this study. In the first, between 12 and 24 kg LW, referred to above as the moderate fattening stage, there was no variation in size. In the second phase, between 24 and 36 kg LW, the stage of intense fat accumulation, adipocyte size increased, except in IM tissue. The lack of hypertrophy during the first phase, in which muscle growth predominates, is consistent with studies which found that the initial development of adipose tissue is due to hyperplasia. The results of the present work indicate that this occurs earlier in the Lacha breed than in larger breeds for meat production. Haugebak et al (1974) observed that in growing lambs (cross-breed western wether) fed on a diet ad libitum, hyperplasia occurred up to 24 kg carcass weight (CW), while from 24 kg to 35 kg CW the increase in adipose tissue was mainly due to hypertrophy of adipocytes.

The diameter of the adipocytes did not change between 12 and 36 kg LW in IM depot, which is thought to be the earliest developing depot (Wood et al, 1980; Kempster, 1980–81). These authors also state that SC adipose tissue is the last to mature. This agrees with our findings that, at 36 kg LW, the size of adipocytes in this tissue was larger than those in the MES, KKCF and IM depots (table II). Hypertrophy in SC tissue has an effect on the thickness of back fat, which increased from 1.6 to 3.1 mm ($P < 0.001$) between 12 kg and 36 kg LW.

The moderate increase in fat deposition between 12 and 24 kg LW was not accompanied by a significant increase in the total esterification of triglycerides, estimated by G3PDH enzyme activity, while the intense deposition of fat between 24 and 36 kg LW was accompanied by a significant increase in this activity. This is consistent with the fact that triglyceride synthesis increases with adipocyte volume (Hood, 1982; Rule et al, 1987; Vernon et al, 1987; Gagliostro and Chilliard, 1991). In OM tissue, for example, where adipocytes were generally the largest, a significant correlation between adipocyte size and G3PDH enzyme activity was found over the weight range ($r = 0.38$; $P < 0.01$). The correlation was not significant in KKCF tissue, which had in general small adipocytes.

The considerable increase in triglyceride esterification in lambs of 36 kg LW may be due to changes in the partitioning of nutrients in favour of adipose tissue. Other authors (eg, Vernon, 1981, 1992) have observed an increase in lipogenic enzyme activity and in the quantity of fat deposited in the later stage of lamb development.

FAS enzyme activity, that estimates de novo fatty acid synthesis, generally evolved in the same way as fat accumulation, and was greater between 24 and 36 kg than between 12 and 24 kg LW. De novo synthesis of fatty acids requires hydrogen and carbon that are provided mainly from glucose or acetate. In the preruminal phase glucose is the major carbon precursor, but when rumen is fully developed acetate is the major carbon precursor for de novo fatty acid synthesis. The variations observed in FAS activity as lamb live weight increased were related with differences in the use of different carbon sources for fatty acid synthesis. The level of precursor utilization could have been the same at 12 and 24 kg LW, while at 36 kg LW, when a major increase in FAS activity took place, it could have increased.

Activity of the EM enzyme, which produces NADPH, showed a similar pathway to the FAS enzyme, with no significant variations between 12 and 24 kg LW, but with increases between 24 and 36 kg LW. The EM is important in the production of reducing power when glucose is the precursor of the acetyl CoA needed for de novo synthesis of fatty acids. Until weaning the lambs mainly use glucose from milk lactose as a carbon source. After weaning, EM activity did not change up to 24 kg LW as a result of utilization of gluconeogenic precursors produced by ruminal digestion of high starch food. The rise of EM activity between 24 and 36 kg LW could be due to physiological changes in the partitioning of nutrients responsible for the increase in fat accumulation.

In ruminants, the increase of acetate concentration or availability activates the G6PDH enzyme (Martin et al, 1973; Pothoven and Beitz, 1973; Yang and Baldwin, 1973; Whitehurst et al, 1978). This fact could contribute to explain the increase in G6PDH activity observed in this study from 24 kg LW. The NADPH produced (as in EM activity) was needed for the increased de novo synthesis in the fattening phase.

In conclusion, a moderate increase in fat deposition between 12 and 24 kg was found in the lambs in this study. During this phase an increase in the number of adipocytes was observed with little change in their size, and only small changes in the activity of

lipogenic enzymes. Between 24 and 36 kg there was a large increase in fat accumulation, accompanied by an increase in the size of adipocytes and in the activity of lipogenic enzyme.

ACKNOWLEDGEMENT

The authors thank the Comisión Interministerial de Ciencia y Tecnología (CICYT) for the grant to carry out this work (Project GAN 91/1258).

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