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SUMMARY - The lipogenic enzyme activity of the following enzymes was studied: Glycerol 3-phosphate dehydrogenase (G3PDH), fatty acids synthetase (FAS), NADP-Malate dehydrogenase (ME) and Glucose 6-phosphate dehydrogenase (G6PDH) in five fat depots (omental, OM; mesenteric, MES; kidney knob and channel, KKC; subcutaneous, SC; intermuscular, IM) in 45 male Rasa Aragonesa lambs divided into three groups of 15 lambs (G12, G24, G36). They were slaughtered respectively at 11.70 ± 0.67; 24.50 ± 0.57 and 35.80 ± 1.74 kg of live weight (LW) and at 32 ± 5; 89 ± 8, and 123 ± 8 days old. The G12 lambs were slaughtered on the day of weaning, having consumed solely their mother's milk; those in the G24 and G36 groups were weaned at 16.20 ± 1.32 and 18.30 ± 2.46 kg LW respectively, were fed from then onward concentrated fodder and barley straw *ad libitum* until they were slaughtered. Between 24 and 36 kg LW an increase in the activity of the G3PDH and FAS lipogenic enzymes was observed. The increase in the *'de novo'* synthesis (FAS) at 36 kg LW was matched by G6PDH activity, which indicates greater use of acetate as a precursor of fatty acids. Furthermore, the maintenance of ME activity during the period studied would indicate that the glucose rate utilization for the fatty acids synthesis was not modified throughout this period.

Key words: Lambs, growth, fattening, lipogenic enzyme activity.

RESUME - *"Activité enzymatique lipogénique chez l'agneau de race Rasa Aragonesa au cours de la croissance". On a étudié l'activité enzymatique lipogénique des enzymes Glycérol 3-phosphate déhydrogénase (G3PDH), Synthétase des acides gras (SAG), NADP-Malate déhydrogénase (EM) et Glucose 6-phosphate déhydrogénase (G6PDH), dans 5 tissus adipeux (omental, OM ; mésentérique, MES ; pelvien-rénal, PVR ; sous-cutané, SC ; et intermusculaire, IM) chez 45 agneaux mâles de race Rasa Aragonesa répartis en trois lots de 15 agneaux (G12, G24, G36), abattus respectivement à 11,70 ± 0,67 ; 24,50 ± 0,57 et 35,80 ± 1,74 kg de poids vif (PV) et 32 ± 5 ; 89 ± 8 et 123 ± 8 jours d'âge. Les agneaux du lot G12 ont été abattus le jour du sevrage et ont ingéré uniquement du lait de la mère ; ceux du lot G24 et du lot G36 ont été sevrés à 16,20 ± 1,32 et 18,30 ± 2,46 kg de PV, et ont ingéré après le sevrage de l'aliment concentré et de la paille d'orge ad libitum jusqu'à l'abattage. Entre 24 et 36 kg de PV, on a observé une augmentation des activités des enzymes G3PDH et SAG. L'augmentation de la synthèse* "de novo" *(SAG) à 36 kg de PV a été accompagnée par une augmentation de l'activité de la G6PDH, qui pourrait indiquer une utilisation plus élevée de l'acétate comme précurseur des acides gras. D'autre part, l'absence de variation dans l'activité de l'EM pourrait indiquer que l'utilisation du glucose pour la synthèse des acides gras n'a pas été modifiée pendant la période étudiée.*

Mots-clés : *Agneaux, croissance, engraissement, activité enzymatique lipogénique.*

Introduction

As animals enter the 'growth' phase and depending on the maturity of each breed, a very high rate of lipid deposition occurs, with a resulting reduction in the efficiency of food utilisation and in the quality of the carcass and the meat. Most Spanish sheep breeds are considered early maturing breeds because fat depots occur prematurely in them. In the Rasa Aragonesa breed the sharp increase in fat depots seems to occur from 24 kg upwards LW in females and from 30 kg upwards in males (Colomer and Espejo, 1973).

The main components of the fat depots are triacylglycerols, whose fatty acids may be obtained from blood plasma or from *'de novo'* synthesis from different cellular precursors through the action of lipogenic enzymes. It has been observed that lipogenic intensity is affected by sex, breed, age, physiological condition and the type and level of feeding, plus the fatty depots in the animal (Vernon, 1981). Few authors, however, have studied the intensity and evolution of lipogenic enzyme activity of adipose tissue in relation to the animal growth and the development of the digestive functions of the rumen (Howarth *et al.*, 1968; Bas *et al.*, 1985).

This study presents initial results of the evolution of lipogenic enzyme activity in different fat depots of lambs during growth and fattening.

Material and methods

Forty-five (45) male Rasa Aragonesa lambs were assigned to 3 weight groups of 15 per group (G12, G24, G36) and slaughtered at 11.70 ± 0.67; 24.50 ± 0.57 and 35.80 ± 1.74 kg of live weight (LW) and at 32 ± 5; 89 ± 8, and 123 ± 8 days old. The G12 lambs were slaughtered on the day of weaning, being on an all milk diet; those in the G24 and G36 groups were weaned at 16.20 ± 1.32 and 18.30 ± 2.46 kg LW respectively, being fed concentrated commercial fodder and barley straw a*d libitum* until they were slaughtered.

Adipose tissue samples (5 g) from the greater omentum, omental (OM); the medium rectus, mesenteric (MES); fat adjacent to the right kidney; kidney knob and channel (KKC); tail head, subcutaneous (SC) and external fat, intermuscular (IM) were obtained immediately after exsanguination which were kept at -40°C until they were analysed.

The activity of the lipogenic enzymes Glycerol 3-phosphate dehydrogenase (G3PDH), fatty acids synthetase (FAS), NADP-Malate dehydrogenase (ME) and Glucose 6-phosphate dehydrogenase (G6PDH) was tested. The enzymatic extracts were obtained by homogenization on a STEG pH 7.6 buffer at 0°C, using an Omni-Mixer microhomogenizer at 50,000 rpm in three 10-second periods, separated by 20-second intervals to avoid overheating. After filtering (20 µm nylon mesh and a 0.40 µm cellulose ester filter) and centrifuged the homogenates at 6,000 rpm for 10 minutes and 20,000 g for 45 minutes at 4°C. The supernatants were stored at -40°C.

The determination of the enzyme activity was made with a spectrometer (340 nm), as described by Glock and McLean (1953) for G6PDH; Ochoa (1955) for ME; Halestrap and Denton (1973) for FAS and Wise and Green (1979) for G3PDH.

The enzyme activity of the three groups in each depot was compared by simple hierarchical variance analysis and, where appropriate, by Duncan's test at 1 and 5%, the results having previously been logarithmically converted.

Results

Table 1 shows the results of average enzyme activity for G3PDH, FAS, ME and G6PDH in the five fat depots studied (OM, MES, KKC, SC and IM) in male Rasa Aragonesa lambs according to their live weight at slaughter (G12, G24, G36).

As the Table shows, G3PDH activity (estimate of the total synthesis of triglycerides) is maintained in the MES, KKC and SC depots between 12 and 24 kg LW, increases in the OM ($P<0.05$) and decreases in the IM between 12 and 24 kg LW ($P<0.01$). However, in the 24-36 kg range the activity of this enzyme increases in all the depots studied ($P<0.01$).

The evolution of FAS activity (estimate of *'de novo'* synthesis) is fairly similar to that of G3PDH. The level of activity is maintained between 12-24 kg LW in all depots except the mesenteric, where it decreases ($P<0.01$), when the LW increases from 24 to 36 kg LW the activity increases in all the depots studied ($P<0.01$).

Table 1. Enzyme activities (nM/min/g) of fat depots omental (OM), mesenteric (MES), kidney knob and channel (KKC), subcutaneous (SC) and intermuscular (IM) in Rasa Aragonesa lambs of 11.7 (G12), 24.5 (G24) and 35.8 (G36) kg live weight at slaughter. Comparison between groups for every tissue[†]

	OM			MES			KKC			SC			IM		
	G12	G24	G36	G12	G24	G36	G12	G24	G36	G12	G24	G36	G12	G24	G36
G3PDH	$10,280^{aA}$	$15,241^{bA}$	$59,402^{C}$	$5,435^{A}$	$6,368^{A}$	$31,326^{B}$	$7,223^{A}$	$9,288^{A}$	$35,359^{B}$	$9,307^{A}$	$12,337^{A}$	$82,376^{B}$	$9,678^{A}$	$5,456^{B}$	$34,135^{C}$
FAS	59^{A}	57^{A}	129^{B}	58^{A}	25^{B}	125^{C}	55^{a}	45^{aA}	86^{bB}	53^{A}	47^{A}	237^{B}	71^{A}	59^{A}	142^{B}
ME	92^{aA}	182^{bB}	121^{a}	128	137	114	91^{aA}	171^{bB}	104^{a}	154	137	118	80	100	92
G6PDH	266^{A}	$1,474^{B}$	870^{C}	163^{A}	997^{B}	626^{B}	156^{A}	$1,431^{B}$	505^{C}	407^{A}	$1,374^{B}$	$1,248^{B}$	337^{A}	759^{B}	665^{B}

[†]Different letters: lower case $P<0.05$; capital letters: $P<0.01$
no letters: non significant

The evolution of ME and G6PDH enzyme activity, which provides the reducing agent for the synthesis of fatty acids, is different in the two enzymes analysed. ME maintains its activity in the MES, SC and IM depots throughout the period under study, while it increases in the OM and KKC between 12-24 kg LW ($P<0.01$) and decreases between 24-36 kg LW ($P<0.05$) to the level it showed at 12 kg LW.

G6PDH increases its activity between 12-24 kg LW in all the depots studied ($P<0.01$). It maintains the same level of activity between 24-36 kg LW in the MES, SC and IM depots, while it decreases in the OM and KKC depots ($P<0.01$), although its level of activity is higher than at 12 kg LW ($P<0.01$).

Table 2 illustrates the results of the activity levels of the four enzymes studied (G3PDH, FAS, ME, G6PDH) for the 3 groups of lambs (G12, G24, G36) according to the fat depot analysed (OM, MES, KKC, SC, IM). The table shows that at 12 kg LW the OM, SC and IM depots show high G3PDH activity values but there are not difference in FAS activity between depots. On the other hand, in SC depot there are high actives for the enzymes G6PDH and ME.

In addition, at 24 kg LW the OM and SC tissues continue to show high levels of G3PDH activity, with differences between depots for FAS and ME being slightly noticeable. As far as the reducing power is concerned, the OM depot is the one which shows the greatest G6PDH activity ($P<0.05$). Finally, at 36 kg LW the SC depot is the one with the highest lipogenic activity (G3PDH, FAS and G6PDH) ($P<0.05$) and the IM depot shows low activity. The activity of ME is similar in the five depots studied.

Discussion and conclusion

The results obtained for the G3PDH enzyme indicate that lipogenic activity increases considerably in the 24-36 kg range. These results agree with those of Smith *et al.* (1987) in Columbia lambs, where it was observed that between four and six months of age there was a high increase in the growth of the animals, in the percentage of carcass fat, in the adipocytes diameter and in lipogenic enzyme activity. This is partly due to the adaptation of the animals to high-energy diets.

The synthesis of *'de novo'* fatty acids also increases considerably between 24-36 kg LW, and is matched by an increase in fat deposition during this period (Purroy *et al.*, 1994). Bas *et al.* (1986) observed that Acetil CoA carboxylase in kids (this enzyme regulates the *'de novo'* synthesis) activity in the omental depot of 112-day-old goats increased considerably in relation to 56 to 70-day-old animals.

The maintenance of ME activity would show that the level of cellular glucose or its gluconeogenic precursors as the main source of Acetyl CoA for the synthesis of fatty acids is the same throughout the period. These results would agree with those of Vézinhet and Nouguès (1977), where the use of glucose as a precursor for fatty acid synthesis in adipocytes in lambs remained steady between 10-100 days after birth. In the present study the same glucose rate seems to be maintained between 24-36 kg LW. Glucose could be supplied from gluconeogenic precursors such as propionate, which would be in agreement with the results of Bas *et al.* (1986), who observed a high ME activity in 112-day-old goats fed concentrated fodder. This suggests that the Malate-Pyruvate-Oxaloacetate cycle activity is more related to the gluconeogenic precursors metabolism than other metabolic pathways which provide reducing agent (NADPH).

The increased G6PDH activity at 36 kg LW would indicate a higher NADPH production to cope with the greater synthesis of *'de novo'* fatty acids, which would occur using acetate as a precursor. Nevertheless, an increase of G6PDH activity occurs between 12-24 kg LW which does not accompany greater *'de novo'* synthesis, because FAS activity has not varied in that range. This increase could be a result of a change in feeding, so the higher acetate production could have an effect on the pentose phosphate shunt which precedes the activation of the FAS, or the possible 'excess' of NADPH which may be required for other cellular functions (Vernon, 1992).

Table 2. Enzyme activities (nM/min/g) of fat depots omental (OM), mesenteric (MES), kidney knob and channel (KKC), subcutaneous (SC) and intermuscular (IM) in Rasa Aragonesa lambs of 11.7 (G12), 24.5 (G24) and 35.8 (G36) kg live weight at slaughter. Comparison between groups for every depot[†]

	G12				G24				G36			
	G3PDH	FAS	ME	G6PDH	G3PDH	FAS	ME	G6PDH	G3PDH	FAS	ME	G6PDH
OM	10,280^{a}	59	92^{a}	266^{ab}	15,241^{a}	57^{a}	182^{a}	1,474^{a}	59,402^{a}	129^{ac}	121	870^{a}
MES	5,435^{b}	58	128^{ab}	163^{a}	6,368^{bd}	25^{b}	137^{a}	997^{b}	31,326^{b}	125^{ac}	114	626^{b}
KKC	7,223^{bc}	55	91^{a}	156^{a}	9,288^{bc}	45^{a}	171^{a}	1,431^{b}	35,359^{b}	86^{a}	104	505^{b}
SC	9,307^{ac}	53	154^{b}	407^{b}	12,337^{ac}	47^{a}	137^{a}	1,374^{b}	82,376^{c}	237^{b}	118	1,248^{c}
IM	9,678^{ac}	71	80^{a}	337^{b}	5,456^{d}	59^{a}	100^{b}	759^{b}	4,135^{b}	142^{c}	92	665^{ab}

[†]Different letters: lower case P<0.05; capital letters P<0.01
no letters: non significant

In conclusion, an increase in the total synthesis of triacylglycerols and *'de novo'* synthesis was observed in the period between 24-36 kg LW. Furthermore, at 36 kg LW the higher level of endogenous fatty acids synthesis would be accompanied by a higher acetate rate as a precursor and the maintenance of the glucose level rate utilization.

References

Bas, P., Rouzeau, A. and Morand-Fehr, P. (1985). Poids et métabolisme des réserves lipidiques au cours de la croissance de chevreau. *Reprod. Nutr. Dev.,* 25 (1B): 275-285.

Bas, P., Rouzeau, A. and Morand-Fehr, P. (1986). Lipogenèse des tissus adipeux de chevreaux sevrés à 4, 6 et 8 semaines. *Reprod. Nutr. Dev.,* 26 (2B): 649-658.

Colomer, F. and Espejo, M. (1973). Influencia del peso al sacrificio y del sexo sobre las características de las canales de cordero de la raza Rasa Aragonesa. *An. INIA, Ser. Prod. Anim.,* 4: 133-150.

Glock, G.E. and McLean, P. (1953). Further studies on the properties and assay of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. *Biochem. J.,* 55: 400-408.

Halestrap, A.P. and Denton, R.M. (1973). Insulin and the regulation of adipose tissue Acetyl-Coenzyme A Carboxilase. *Biochem. J.,* 132: 509-517.

Howarth, R.E., Baldwin, R.L. and Ronnig, M. (1968). Enzyme activities in liver, muscle and adipose tissue of calves and steers. *J. Dairy Sci.*, 51: 1270-1274.

Ochoa, S. (1955). *"Malic" Enzyme. Methods in Enzymology.* Vol. 1. Colowick, S.P. and Kaplan, N.O. (eds). Academic Press, New York, p. 739.

Purroy, A., Mendizabal, J.A., Soret, B., Horcada, A., Lizaso, G., Mendizábal, F.J. and Arana, A. (1994). Size and number variation of adipocytes during growth of Rasa Aragonesa lambs. *Options Méditerranéennes.* (In press).

Smith, S.B., Jenkins, T. and Prior, R.L. (1987). Carcass composition and adipose tissue metabolism in growing sheep. *J. Anim. Sci.,* 65: 1525-1530.

Vernon, R.G. (1981). Lipid metabolism in adipose tissue of ruminants. In: *Lipid Metabolism in Ruminant Animals.* Christie, W.W. (ed.). Pergamon Press.

Vernon, R.G. (1992). Control of lipogenesis and lipolysis. In: *The Control of Fat and Lean Deposition.* Boorman, K.N., Buttely, P.S. and Lindsay, D.B. (eds). Butterworth Heinemann, Oxford, pp. 59-81.

Vézinhet, A. and Nouguès, J. (1977). Evolution postnatale de la lipogenèse dans le tissu adipeux et le foie du mouton et du lapin. *Ann. Biol. Anim. Bioch. Biophys.,* 17(5B): 851-863.

Wise, L.S. and Green, H. (1979). Participation of one isozyme of cytosolic glycerophosphate dehydrogenase in the adipose conversion of 3T3 cells. *J. Biol. Chem.,* 254: 273-275.