

The influence of extruding corn grain on glucose metabolism in pregnant ewes

Serge Landau^{a*}, Zafrira Nitsan^b, Zipora Zoref^{fb},
Zecharia Madar^c

^a Department of Natural Resources, Institute of Field and Garden Crops, Agricultural Research Organization, PO Box 6, Bet Dagan 50250, Israel

^b Department of Poultry Science, Institute of Animal Science, Agricultural Research Organization, PO Box 6, Bet Dagan 50250, Israel

^c Department of Biochemistry and Human Nutrition, the Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel

(Received 15 July 1998; accepted 9 February 1999)

Abstract — Glucose metabolism was studied in 31 pregnant ewes fed mixtures (50:50) of chopped alfalfa hay and corn grain given in whole (HWC) or extruded form (HEC). Number of fetuses was 1 ($n = 10$), 2 ($n = 11$) or 3 ($n = 10$). Diets were supplied at three dietary levels: maintenance (1.0 M), 1.25× maintenance (1.25 M) or twice maintenance (2.0 M). Glucose metabolism was estimated by a double isotope dilution procedure at days 97–121 of pregnancy. Glucose entry rate (GER) was higher ($P < 0.04$), and glucose irreversible loss rate (GILR) tended to be higher ($P < 0.07$) in ewes given HEC than HWC. Dietary level ($P < 0.001$) and the number of fetuses ($P < 0.005$) affected GER and GILR positively. These results provide a probable explanation for higher birth weights previously found in lambs born to HEC-, compared with HWC-fed dams. © Inra/Elsevier, Paris.

sheep / ewe / glucose metabolism / pregnancy

Résumé — **Influence de l'extrusion de maïs sur les concentrations de glucose chez la brebis gestante.** Le métabolisme du glucose a été étudié chez 31 brebis gestantes qui ont reçu un mélange (50:50) de foin de luzerne et de grain de maïs distribué entier (HWC) ou extrudé (HEC). Le nombre des fœtus était 1 ($n = 10$), 2 ($n = 11$) ou 3 ($n = 10$). Les rations ont été distribuées à trois niveaux d'alimentation : entretien (1,0 M), 1,25 × entretien (1,25 M) ou 2 × entretien (2,0 M). Le métabolisme du glucose a été évalué après injection de deux isotopes à 97–121 j de gestation. Le taux d'entrée (GER)

* Correspondence and reprints

E-mail: vclandau@volcani.agri.gov.il

Contribution of the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, no. 133/98

a été plus élevé chez les brebis recevant la ration HEC ($p < 0,04$) et une tendance semblable a été notée ($p < 0,07$) pour le taux d'utilisation (GILR). Le niveau d'alimentation ($p < 0,001$) et le nombre de fœtus portés ($p < 0,005$) ont affecté GER et GILR positivement. Ces résultats expliquent probablement le poids à la naissance plus élevé des agneaux nés de mères recevant la ration HEC, ce que nous avions précédemment observé. © Inra/Elsevier, Paris.

ovin / brebis / métabolisme du glucose / gestation

1. INTRODUCTION

The uterus and accompanying fetuses are dependent on glucose as an energy source and utilize a major part of the glucose produced by pregnant ewes [12]. The birth weight of lambs, which largely determines perinatal survival [6], is positively related to the glucose entry rate (GER) of ewes during late pregnancy [1]. Although the glucose metabolism of sheep is related to the energy intake [15], this relationship is not linear in pregnant sheep [7]. The effect of feeding level on glucose metabolism in monocotous and polycotous pregnant sheep has not yet to our knowledge been reported.

Sheep fed maintenance level rations containing whole corn grain, which is moderately degradable in the rumen, had higher GER than sheep fed iso-energetic rations containing highly degradable extruded corn grain [8]. However, quite surprisingly, feeding extruded corn grain – at twice the maintenance level – to ewes bearing twin lambs during late pregnancy resulted in higher lamb birth-weights than feeding whole corn grain [10].

The purpose of the present research was to study the combined effects of starch ruminal degradability, level of intake and the number of fetuses on glucose metabolism. Glucose metabolism was evaluated in pregnant ewes fed diets containing corn given in whole or extruded form at different levels of intake.

2. MATERIALS AND METHODS

2.1. Animals and diets

The experiment was conducted with ten pregnant Finn \times Awassi and 12 pregnant Booroola \times Assaf (F1) ewes. Pregnancies were the result of synchronized estrus followed by assisted mating. The sheep were kept in individual cages and given 1:1 mixtures of alfalfa hay (150 g·kg⁻¹ of crude protein, 411 g·kg⁻¹ of neutral detergent fiber, 326 g·kg⁻¹ of acid detergent fiber, on a dry matter basis) chopped to a mean length of 18 mm and corn grain fed as whole (hay and whole corn (HWC), $n = 11$) or extruded (200 °C, 22 s, 70 bars; hay and extruded corn (HEC), $n = 11$). The daily ration was distributed in two meals at 06.00 and 18.00 hours, as described previously [8]. Vitaminized licking blocks (Koffolk, Tel Aviv, Israel) and water were available at all times. The ewes were fed at maintenance level (1.0 M) or 1.25 M from d 90 to d 107, and 1.25 M or 2.0 M from d 108 to d 121 of pregnancy. The maintenance requirement was derived from body weight [11] and values of digestibility in vivo published previously [8]. Glucose kinetic studies were conducted on d 97–107 (at the 1.0 and 1.25 M levels), and d 114–121 (at the 1.25 and 2.0 M levels) of pregnancy.

2.2. Glucose kinetic studies

Procedures have been fully described previously [8]. In brief, indwelling cannulas (BARD-I-CATH, 14 G, 280 mm) were inserted in the two jugular veins and kept open by flushing every fortnight with heparinized saline (300 IU·mL⁻¹). Glucose kinetic experiments were started at least 7 d after insertion of the cannulas. The procedure was carried out only if two conditions were fulfilled: ewes had fully recovered from cannula implantation and consumed all the diet at least 3 d

before the expected date of glucose dilution procedure. Eight glucose dilution procedures were carried out at 1.0 M in the HEC-fed group, whereas 12 procedure were carried out for HWC; 14 and 17 procedures at 1.25 M, and 11 and 12 procedures at 2.0 M, respectively.

Blood was sampled from each sheep just before the morning meal via one of the permanent cannulas to serve as the baseline for radioactivity. Three hours after the morning meal, doses of 150–200 μCi of D-[U- ^{14}C]-glucose and D-[2- ^3H]-glucose (American Radiolabelled Chemicals, Saint-Louis, MO, USA) were heated to body temperature and injected into the animals through one of the cannulas. Twenty-eight blood samples were collected from the second cannula at different intervals until 470 min post-dosing, according to the following sequence: seven samples were taken every 3 min, then four samples every 10 min, followed by eight samples every 15 min and finally nine samples every 30 min. The samples were kept on ice until deproteinized, and radioactive glucose was separated from radioactive non-glucose compounds by elution through a Dowex-8 (Sigma) resin ion-exchanger filled with 0.1 M glycine buffer. Data on the efficacy of separation have been presented earlier [8]. Water was evaporated from eluents by heating the plasma in an air-forced oven at 70 °C, because the label from ^3H -glucose is lost only to body water [18]. After re-hydration, the samples were dissolved in Triton-toluene scintillation liquid and counted for radioactivity in a β -counter (Beckman 7800).

Radioactive specific activity (RSA) was calculated for each plasma sample. Thirty-one individual curves of RSA were analysed using a computerized 'peeling off' procedure and served for calculations of glucose mass (GM, $\text{g}\cdot\text{kg}^{-1}\text{ BW}^{0.75}$), glucose entry rate (GER, $\text{g}\cdot\text{min}^{-1}\text{ kg}^{-1}\text{ BW}^{0.75}$) and glucose irreversible loss rate (GILR, $\text{g}\cdot\text{min}^{-1}\text{ kg}^{-1}\text{ BW}^{0.75}$) as described previously [8]. Expression of glucose metabolism on a $\text{BW}^{0.75}$ basis was in order to enable comparisons between large-framed Finn \times Awassi (approximately 65 kg BW) and small-framed F1 Booroola (approximately 45 kg BW) crossbred ewes. Ewes were sorted into three classes (1, 2 and 3) according to the number of fetuses corresponding to the number of lambs born.

Non-esterified fatty acids (NEFA) and insulin in pre-prandial plasma (sampled before administration of radioactive glucose) were analysed as described before [8].

2.3. Statistical analyses

The differences between treatment means were evaluated by using a repeated measures procedure with sheep (corn treatment \times number of fetuses) as the error term in the GLM procedure, using corn treatment and the number of fetuses as main effects, and their interaction, in variance analysis. The stage of pregnancy (no. of days pregnant) was used as a co-variant [13].

3. RESULTS

3.1. Glucose metabolism and stage of pregnancy

Stage of pregnancy (no. of days pregnant) within the range of this study did not affect glucose metabolism. Because the effect of the pregnancy stage throughout this study was not significant, no confounded effects of pregnancy stage and dietary levels could be expected.

3.2. Glucose metabolism and feeding level

The feeding level tended to affect GM positively ($P < 0.07$; *table 1*). A significant positive effect of feeding level on GER ($P < 0.0002$) was found: GER was lowest in ewes fed 1.0 M (3.42 $\text{mg}\cdot\text{min}^{-1}\text{ kg}^{-1}\text{ BW}^{0.75}$), higher for 1.25 M (6.18 $\text{mg}\cdot\text{min}^{-1}\text{ kg}^{-1}\text{ BW}^{0.75}$) and highest for 2.0 M (7.94 $\text{mg}\cdot\text{min}^{-1}\text{ kg}^{-1}\text{ BW}^{0.75}$). Values for GILR were also lowest ($P < 0.0006$) in ewes fed 1.0 M (2.57 $\text{mg}\cdot\text{min}^{-1}\text{ kg}^{-1}\text{ BW}^{0.75}$), while they did not differ between ewes fed 1.25 M and 2.0 M (4.99 and 5.79 $\text{mg}\cdot\text{min}^{-1}\text{ kg}^{-1}\text{ BW}^{0.75}$, respectively). The interaction between corn treatment and feeding level on GER tended to be significant ($P < 0.10$).

3.3. Glucose metabolism and corn treatment

Corn treatment had no effect on GM but affected GER and GILR (*table 1*). Ewes fed HEC had higher GER than HWC-fed coun-

Table 1. Effects of corn treatment (CT), feeding level (FL), number of fetuses (NF) and their interactions on the glucose mass (GM, g·kg⁻¹ BW^{0.75}), rates of glucose irreversible loss and entry rate (GILR and GER, mg·min⁻¹·BW^{0.75}) and the concentrations in plasma of insulin (mU·L⁻¹) and non-esterified fatty acids (NEFA, µM·L⁻¹) in ewes fed the experimental diets: least square means and the root of mean square errors (RMSE).

	Extruded corn grain (n = 14)			Whole corn grain (n = 17)			RMSE	Main effects (P <)			Inter-action
	Single (n = 3)	Twin (n = 6)	Triplet (n = 5)	Single (n = 7)	Twin (n = 5)	Triplet (n = 5)		Corn treatment	Feeding level	Number of fetuses	
GM											
1.0 M	158	-	219	62	-	194	72	NS	0.07	0.07	NS
1.25 M	250	159	212	251	84	252					
2.0 M	-	208	237	194	189	-					
GILR											
1.0 M	1.60	-	2.51	1.41	-	4.77	1.87	0.07	0.001	0.004	CT × FL 0.10
1.25 M	6.18	4.30	10.63	2.20	2.20	4.80					
2.0 M	-	5.52	9.66	5.49	4.47	-					
GER											
1.0 M	2.41	-	4.55	1.63	-	5.09	1.99	0.04	0.001	0.002	NF × FL 0.05
1.25 M	6.19	4.59	13.50	2.67	4.18	6.09					
2.0 M	-	7.48	11.33	8.10	6.65	-					
Insulin											
1.0 M	27.8	-	23.1	9.5	-	33.0	11.2	NS	NS	0.10	CT × NF 0.07
1.25 M	37.5	16.0	15.0	46.0	9.0	46.7					
2.0 M	-	26.4	-	27.1	20.8	-					
NEFA											
1.0 M	97	-	376	184	-	90	205	NS	NS	NS	NS
1.25 M	152	291	286	180	335	349					
2.0 M	-	266	403	120	281	-					

terparts (weighted means 7.01 and 5.74 mg·min·kg⁻¹ BW^{0.75}; $P < 0.04$; table 1). A similar trend was found for GILR (weighted means 5.45 and 4.18 mg·min·kg⁻¹ BW^{0.75}; $P < 0.07$). A tendency ($P < 0.10$) for the effect of the corn treatment × feeding level interaction was noted for GILR but not for GER. Corn treatment did not affect glucose metabolism at 1.0 M level. At 1.25 M level, GILR and GER were higher in the HEC than in the HWC group only in triplet-bearing ewes ($P < 0.02$ and $P < 0.005$, respectively). No differences were observed between HEC and HWC at the 2.0 M level (where comparisons could be made only for twin-bearing ewes).

3.4. Glucose metabolism and the number of fetuses

The overall effect of the number of fetuses on GM, GER and GILR was positive ($P < 0.07$, 0.002 and 0.004, respectively; table 1 and figure 1).

Ewes bearing single fetuses or twins exhibited similar GER and GILR, which were lower than in triplet-bearing ewes ($P < 0.05$). More detailed analysis shows that at the 1.0 M feeding level, GER and GILR were not affected by the number of fetuses in HEC-fed ewes, but tended ($P < 0.10$) to be greater in HWC-fed ewes bearing triplets than in those bearing singles.

However, at 1.25 and 2.0 M feeding levels, ewes bearing triplets and fed HEC had greater ($P < 0.05$) GER and GILR than twin-bearing counterparts fed the same diet, whereas no significant effect of the number of fetuses on glucose metabolism was noted within HWC-fed ewes.

3.5. Effects of corn treatment, feeding level and the number of fetuses on insulin and NEFA

No effect of corn treatment on insulin or NEFA was noted, but the corn treatment ×

number of fetuses interaction on insulin tended ($P < 0.07$) to be significant.

Insulin concentration tended to be highest in the plasma of triplet-bearing ewes, intermediate in single-bearing ewes, and lowest in those bearing twins (33.4, 26.6 and 21.8 mU·L⁻¹, respectively; $P < 0.10$). A trend was also found for NEFA concentration. It was highest in plasma from triplet-bearing ewes, intermediate in ewes bearing twins, and lowest in those bearing singles (332, 280 and 138 meq/L, respectively; $P < 0.10$, figure 2).

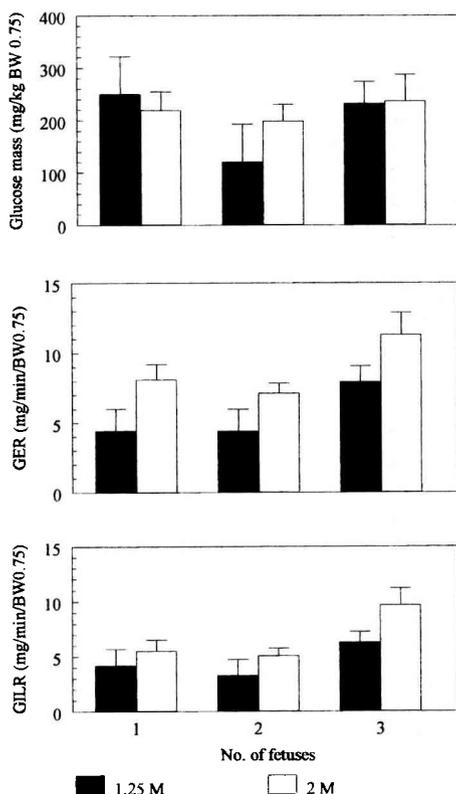


Figure 1. Rates of glucose mass (GM, mg·kg⁻¹ BW^{0.75}), irreversible loss (GILR, mg·min·kg⁻¹ BW^{0.75}) and entry rate (GER, mg·min·kg⁻¹ BW^{0.75}) in ewes bearing single, twin or triplets at mid-pregnancy and fed 1.25 or 2.0 × maintenance level (least square means ± standard error).

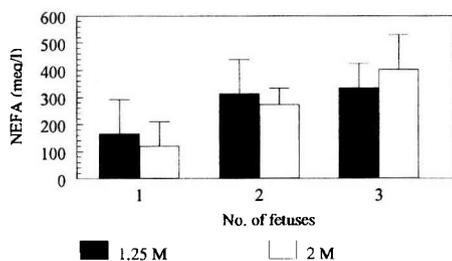


Figure 2. Concentration of non-esterified fatty acids (NEFA, $\mu\text{M}\cdot\text{L}^{-1}$) in ewes bearing single, twin or triplets at mid-pregnancy and fed 1.25 or 2.0 \times maintenance level (least square means \pm standard error).

4. DISCUSSION

4.1. Relationship between corn treatment and glucose metabolism

In a previous study in non-pregnant ewes, we demonstrated higher GER when the diet included whole corn grain, compared to ground or extruded grain [8]. In contrast, in the present study, GER was higher in pregnant ewes fed extruded corn than in those fed whole corn grain (*table 1*). This last finding is in agreement with our previous report that ewes fed diets containing extruded corn gave birth to heavier lambs than ewes fed diets with whole or ground corn [10]. Feeding HEC is associated with high levels of propionate in ruminal fluid [8]. Therefore, the discrepancy between pregnant and non-pregnant ewes may result from differences in gluconeogenesis from propionate between pregnant and non-pregnant ewes, possibly mediated by modifications of the hormonal environment in pregnancy.

Although propionate is an excellent substrate for gluconeogenesis in sheep [19], an excess supply of propionate to the liver may result in saturation of the gluconeogenetic process, as was shown in cows by Grohn et al. [4]. Therefore, it seems that the ability of pregnant ewes to synthesize more glucose from propionate could explain the contradicting results of feeding HEC versus HWC to pregnant or non-pregnant ewes.

Steel and Leng [16] reported higher rates of gluconeogenesis in 100 d pregnant ewes than in non-pregnant ewes. Wilson et al. [17] reported that a higher percentage of the propionate resource is diverted to glucose as pregnancy proceeds: approximately 37 % in ewes in mid-pregnancy and 55 % in late pregnancy. This is in agreement with the finding by Smith and Walsh [14] that the activity of key-enzymes for gluconeogenesis, such as pyruvate carboxylase, increases dramatically during pregnancy. It is suggested that pregnant ewes benefited more from the abundance of propionate supplied by feeding HEC than non-pregnant sheep, owing to more efficient gluconeogenesis.

In the present study, concentrations of circulating insulin were generally higher in sheep at mid-pregnancy than in non-pregnant sheep [8]. While feeding HEC to non-pregnant sheep was associated with a higher concentration of circulating insulin than feeding HWC [8, 9], no such effect was observed in pregnant sheep in the present experiment, probably because the effect of HEC on insulin was confounded with that of pregnancy. Insulin depresses gluconeogenesis and this action is six-fold more potent in pregnant than in non-pregnant ewes [5]. However, Brockman and Laarveld [2] showed that insulin is an inhibitor of gluconeogenesis from lactate but not from propionate [3]. In other words, suppression of gluconeogenesis by insulin is probably not higher in HEC- than in HWC-fed sheep during pregnancy.

4.2. Relationship between energy intake and glucose metabolism

The positive relationship between energy intake and glucose (*table 1*) was documented in previous studies in pregnant ewes [1, 15]. The present data show that the relationship between the level of intake and GILR is not merely linear in pregnant sheep, in accordance with the observation made by Hough et al. [7]. This relationship was different in HEC- and HWC-fed ewes: a large increase

in GER and GILR was noted from 1.0 to 1.25 M in HEC-fed ewes, whereas this increase was moderate in HWC counterparts (*table 1*).

4.3. Relationship between the number of embryos and glucose metabolism

Glucose metabolism was positively related to the number of fetuses (*figure 1* and *table 1*). Ewes bearing triplets exhibited higher GER and GILR than counterparts bearing one or two lambs. This finding is contradictory to Prior and Christenson [12], who found no differences in the glucose turnover rate measured in ewes bearing 1–3 fetuses at d 109 of pregnancy. Wilson et al. [17], reported that glucose production was similar in ewes bearing single and twin lambs in mid (d 76) and late (d 120) pregnancy, as was found in the present study. Our study indicates that the number of fetuses affects glucose metabolism only at a feeding level above maintenance, as was shown by Wilson et al. [17].

To summarize, our study shows that: i) the glucose metabolism in pregnant ewes can be increased by extruding the grain component of the diet, which may contribute to heavier birth weight in lambs; ii) the increase in glucose metabolism was particularly prominent in ewes with triplets; iii) the dietary level interacts with the number of fetuses and grain treatment on glucose metabolism.

REFERENCES

- [1] Barry T.N., Manley T.R., Glucose and protein metabolism during late pregnancy in triplet-bearing ewes given fresh forage ad lib, *Br. J. Nutr.* 54 (1985) 521–533.
- [2] Brockman R.P., Laarveld B., Effect of insulin on gluconeogenesis and the metabolism of lactate in sheep, *Can. J. Physiol. Pharmacol.* 64 (1986) 1055–1059.
- [3] Brockman R.P., Laarveld B., Effect of insulin on the utilization of propionate by sheep, *Br. J. Nutr.* 64 (1990) 95–101.
- [4] Grohn Y., Bruss M.L., Huffmann E.F., Nonlinear pharmacokinetics and conversion to glucose of intravenous sodium propionate in cattle, *J. Dairy Sci.* 70 (1987) 2272–2279.
- [5] Hay W.W., Chin-Chu L.J., Mezmarich H.K., Effects of high levels of insulin on glucose utilization and glucose production in pregnant and non-pregnant sheep, *Proc. Soc. Exp. Biol. Med.* 189 (1988) 275–284.
- [6] Hinch G.N., Crosbie S.F., Kelly R.W., Owens J.L., Davis G.H., Influence of birth weight and litter size on lamb survival in high fecundity Booroola-Merino crossbred flocks, *N.Z. Agric. Res.* 28 (1985) 31–38.
- [7] Hough G.M., McDowell G.H., Annison E.F., Williams A.J., Glucose metabolism in pregnant and lactating ewes, *Proc. Nutr. Soc. Aust.* 10 (1985) 153.
- [8] Landau S., Nitsan Z., Zoref Z., Madar Z., The influence of processing corn grain on glucose metabolism in ewes, *Reprod. Nutr. Dev.* 32 (1992) 231–240.
- [9] Landau S., Bor A., Leibovich H., Zoref Z., Nitsan Z., Madar Z., The effect of ruminal starch degradability in the diet of Booroola crossbred ewes on induced ovulation rate and prolificacy, *Anim. Rep. Sci.* 38 (1995) 97–108.
- [10] Landau S., Zoref Z., Nitsan Z., Madar Z., The influence of extruding corn grain in diets fed to Finn × Awassi crossbred ewes during late pregnancy on birth weight of lambs, *Can. J. Anim. Sci.* 77 (1997) 141–147.
- [11] National Research Council, Nutrient requirements for sheep, National Academy of Science, Washington, DC, 1985.
- [12] Prior R.L., Christenson R.K., Uterine blood flow and nutrient uptake during late gestation in ewes with different number of fetuses, *J. Anim. Sci.* 46 (1978) 201–210.
- [13] SAS Institute Inc., SAS/STAT User's guide, Release 6.03 Edition, Cary, NC, 1988.
- [14] Smith R.W., Walsh A., Effects of pregnancy and lactation on the activities in sheep liver of some enzymes of glucose metabolism, *J. Agric. Sci. Camb.* 98 (1982) 5623–565.
- [15] Steel J.W., Leng R.S., Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 2. Synthesis of glucose from ruminal propionate, *Br. J. Nutr.* 30 (1973) 475–489.
- [16] Steel J.W., Leng R.S., Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 1. The kinetics of glucose metabolism, *Br. J. Nutr.* 30 (1973) 451–474.
- [17] Wilson S., MacRae J.C., Buttery P.J., Glucose production and utilization in non-pregnant, pregnant and lactating ewes, *Br. J. Nutr.* 50 (1983) 303–316.
- [18] Wolfe R.R., Specific applications: Glucose metabolism, in: Tracers in Metabolic Research. Radioisotope and Stable Isotope/Mass Spectrometry Methods, Alan R. Liss Publications, New York, 1984, pp. 113–129.
- [19] Yacoub C., Remesy C., Demigne C., Particularités de l'utilisation des substrats glucoformateurs par les hépatocytes isolés de mouton, *Reprod. Nutr. Dev.* 28 (1988) 191–192.