

Nutrition and colostrum production in sheep. 1. Metabolic and hormonal responses to a high-energy supplement in the final stages of pregnancy

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Abstract. We tested the hypothesis that supplementation with cracked maize during the last week of pregnancy would provide ewes with a substrate for glucose and enhance the synthesis of lactose and, consequently, their production of colostrum. Thirty single- and 30 twin-bearing ewes were fed lucerne hay and half of each group was supplemented daily with 0.75 kg per head cracked maize during the last week of pregnancy. Colostrum production and the endocrine patterns in the animals were investigated. Supplementation with maize more than doubled the mass of colostrum available at birth in unsupplemented ewes: 339 v. 145 g in single-bearing ewes and 536 v. 197 g in twin-bearing ewes ($P < 0.001$). The total colostrum produced in the 10 h after birth was also significantly increased by supplementation: 730 v. 475 g in single-bearing ewes and 1259 v. 631 g in twin-bearing ewes ($P < 0.01$). The colostrum in the supplemented ewes was also more liquid with a viscosity score of 5.8 compared with 5.7 and 4.5 in unsupplemented single- and twin-bearing ewes ($P < 0.01$). Supplemented ewes had higher concentrations of lactose in their colostrum at parturition (2.6% v. 1.8% in single-bearing ewes and 2.5% v. 1.4% in twin-bearing ewes; $P < 0.01$). The plasma concentrations of progesterone and growth hormone in supplemented ewes were lower, whereas those of IGF-I and insulin were higher, all consistent with a higher capacity to produce colostrum. It is concluded that a high-energy supplement, like maize, fed to ewes in the last week of gestation increases their capacity to produce colostrum for their lambs, particularly for ewes bearing twins.

Extra keywords: glucose, growth hormone, insulin, insulin-like growth factor-I, lactogenesis, lactose, maize, progesterone.

Introduction

An adequate supply of colostrum in the first few hours after birth has a major influence on the survival of newborn mammals because it is the most important source of energy and the only source of immunoglobulins and water. In the sheep (Pattinson *et al.* 1995), the presence of colostrum in the stomach has also been shown to facilitate the ability of a lamb to recognise its mother (Goursaud and Nowak 1999). This is essential for a successful interaction between mother and young soon after birth.

Twin born lambs are particularly at risk because, compared with single-bearing ewes, multiple-bearing ewes produce less colostrum, particularly in the early post-partum period (Alexander and Davies 1959; Banchemo *et al.* 2003). This may be even more important in grazing Merino and Merino-derived ewes because they cannot meet their metabolisable energy (ME) requirements for late pregnancy. Grazing ewes often do not have enough glucose as a proportion of their total ME intake. The role of glucose in the synthesis of colostrum

was demonstrated by Barry and Manley (1985), who infused 175 g day⁻¹ glucose into the abomasum of triplet-bearing Romney × Booroola Merino ewes for the last 6 weeks of gestation and increased colostrum production threefold compared with control ewes that had a higher ME intake but from forage alone. Exogenous glucose can be used directly for gut metabolism and this should make more glucose available to the mammary gland for lactose synthesis by sparing endogenously synthesised glucose (Nocek and Tamminga 1991).

In addition, there is a positive relationship between energy intake and hepatic blood flow and the mechanism appears to be triggered by an increase in the amount of volatile fatty acids, mainly propionate, crossing the wall of the rumen and reaching the liver (Wieghart *et al.* 1986). The increase in hepatic blood flow may, in turn, increase the rate of withdrawal of progesterone from the blood (Parr 1992; Parr *et al.* 1993), which will hasten the onset of lactogenesis (Hartmann *et al.* 1973).

Ruminants fed a forage diet have little glucose available for absorption (Leng 1970; Lindsay 1970; Bergman *et al.* 1974). However, with diets containing a high concentration of starch, such as corn, large amounts of starch may pass into the small intestine and contribute a significant amount of glucose (Armstrong and Smithard 1979; Nocek and Tamminga 1991). In addition, the amount of starch reaching the small intestine should increase during late pregnancy because the fetus/es compress the rumen, thereby increasing the rate of passage of digesta from the rumen (Weston 1988). If the same applies for a cereal grain fed to ewes in late pregnancy, the amount of starch reaching the small intestine should be high. Therefore, ewes fed pasture or a roughage diet should benefit from being supplemented with a cereal grain in late pregnancy, especially if the grain is milled to a particle size that facilitates unrestricted passage from the rumen (Poppi *et al.* 1980).

On this basis, we hypothesised that supplementation with a large amount of cracked maize during the last week of pregnancy would provide the ewes with glucose from post-ruminal digestion of the starch and that this would enhance the synthesis of lactose (Linzell 1967; Knowlton *et al.* 1998a; Rigout *et al.* 2002) and, consequently, the production of colostrum. Therefore, in the present study, we measured the responses in colostrum production and endocrine patterns in single- and twin-bearing ewes fed lucerne hay and supplemented with cracked maize during the last week of pregnancy. A preliminary report of some of this work has been presented previously (Banchero *et al.* 2002).

Materials and methods

The present experiment was conducted in accordance with the directives of the National Institute of Agricultural Research (INIA) concerning the use of animals for experimentation.

Experimental treatments

The experiment was conducted at the Experimental Unit 'Palo a Pique' of INIA Treinta y Tres, Uruguay (35°S), in August 2001 using adult Corriedale ewes selected from a flock of 400 that had been synchronised using a single dose of 160 µg delprostenate (Glandinex™; Gramon Laboratories, Uruguay) and mated during the second oestrus after synchronisation with 9% Corriedale rams. The ewes were scanned for pregnancy on Day 85 after the introduction of the rams when 30 ewes bearing single fetuses (48.2 ± 1.23 kg; body condition score 1.52 ± 0.02) and 30 ewes bearing twin fetuses (52.3 ± 1.28 kg; body condition score 1.54 ± 0.03) were selected. The ewes grazed natural pastures (9% crude protein (CP) and 60% dry matter digestibility (DMD)) from mating until Day 105 of gestation, when they started grazing improved pastures (19% CP and 70% DMD) to maintain a body condition score of approximately 1.5 (scale 1–5).

The experimental ewes were brought indoors on Day 130 after mating (Day –17 before expected parturition; Day 0 = parturition), placed in individual pens and fed at an estimated 100% of ME requirements according to the Ministry of Agriculture, Fisheries and Food (MAFF 1975). Each twin-bearing ewe was offered a daily basal diet of 1.4 kg lucerne hay dry matter (DM; 18% CP and 8.6 MJ ME per kg DM). Single-bearing ewes were offered a basal diet of 1.0 kg DM lucerne hay daily. All ewes were offered a mineral lick and water *ad libitum*. Fourteen

Table 1. Mean metabolisable energy offered (MJ per ewe daily) to single- and twin-bearing Corriedale ewes during the experimental period

Control values are 100% of requirements according to MAFF (1975)

Treatment (days)	Birth type			
	Single-bearing ewes		Twin-bearing ewes	
	Control	Supplemented	Control	Supplemented
134–135	8.6	11.5	12	14.9
136–137	8.6	13.9	12	17.3
138–139	8.6	16.3	12	19.7
140–147 (parturition)	8.6	17.5	12	20.9

days before the expected time of parturition, half the twin-bearing ewes and half the single-bearing ewes were offered a supplement of cracked maize. The ewes were offered the cracked maize (88% DM, 9.6% CP and 13.6 MJ ME per kg DM) gradually: 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 kg day⁻¹ from Day 134 (Day –13) to Day 139 (Day –8) of gestation and the full amount of 0.75 kg day⁻¹ from Day –7 until parturition (Day 0; Table 1).

Feeding and feed intake

The lucerne hay was offered each morning at 0800 hours. The cracked maize was offered to the supplemented ewes 30 min later. The amount of each type of feed refused by each ewe was weighed and recorded every day before feeding.

Live weight and body condition

Bodyweight and condition were recorded once a week from Day 90 of gestation until lambing. In the animal house, animals were weighed and scored for body condition at 0730 hours, before feeding.

Udder measures, colostrum production, viscosity and composition

Udder volume was calculated using the formula of Bencini and Purvis (1990) on Days –10, –5, –3 and –1 before parturition and immediately after birth. The full volume of the udder at birth was calculated from its linear dimensions and the empty volume was obtained by subtracting the amount of colostrum that could be expressed from it at parturition.

The ewes were observed 24 h day⁻¹ and, immediately after parturition, they received an intramuscular injection of 5 IU oxytocin (Hipofamina™; Dispert Laboratories, Montevideo, Uruguay) and one teat was completely hand milked and covered with tape to prevent sucking. The other teat was left uncovered for the lamb. The colostrum was weighed and classified on appearance and consistency according to the eight-point scale of McCance and Alexander (1959), where 0 is no expressible secretion, 1 is a clear serous straw-coloured liquid grading to 7, which is an opaque white liquid similar to normal milk. A sample of 20 mL colostrum was kept with a preservative (50 µL of 10% potassium dichromate) for analysis of colostrum constituents. The milking was repeated 1, 3, 6 and 10 h after lambing. The colostrum was weighed and a sample was kept each time for analysis of components. At lambing, the colostrum collected represented prenatal accumulation and subsequent yields represented the quantities secreted since the previous milking. Colostrum samples were analysed for fat, lactose and protein using a Milkoscan 133 (Foss Electric, Hillerød, Denmark).

Blood sampling

Blood was sampled from the jugular vein of the ewes into 10-mL heparinised tubes immediately before feeding at 0700 hours: once a week from Day 115 to Day 142 of gestation; twice a day (0700 and 1900 hours) until lambing; immediately after birth and 1, 3, 6 and 10 h later. Jugular blood was taken from each lamb immediately after birth. Blood

samples were chilled to 4°C and centrifuged within 1 h at 3000g for 10 min. The plasma was divided equally into two plastic tubes and stored at -20°C until assay for hormones and metabolites. Blood glucose was measured soon after bleeding using a single drop of whole blood and a Medisense²® blood glucose metre and sensor electrodes (Medisense, Bedford, MA, USA).

Assays of hormones and metabolites

β -Hydroxybutyrate and urea

β -Hydroxybutyrate (β -OHB) was measured using a spectrophotometric assay (McMurray *et al.* 1984) and urea was measured using an enzymatic ultraviolet test based on the urease/GLDH method (Reference?) on a Roche Cobas Mira-S autoanalyser (Roche, City?, State?, Country?). All samples for each metabolite were run in a single assay.

Insulin

Insulin was measured in duplicate by the double-antibody radioimmunoassay (RIA) method of Hales and Randle (1963) as modified by Basset and Wallace (1966) and described by Tindal *et al.* (1978). Mean concentrations in low-, medium- and high-quality controls were 5.4, 12.4 and 33 μ U mL⁻¹, respectively. The intra- and interassay coefficients of variation were 6.6% and 13.1%, respectively, and the minimum detection limit was 1.09 μ U mL⁻¹.

Growth hormone

Growth hormone (GH) was measured in duplicate using the RIA described by Downing *et al.* (1995). Mean concentrations in low-, medium- and high-quality controls were 17.4, 25.1 and 40 ng mL⁻¹, respectively. The intra- and interassay coefficients of variation were 6.4% and 2.4%, respectively, and the minimum detection limit was 0.49 ng mL⁻¹.

Insulin-like growth factor-I

Insulin-like growth factor (IGF)-I was measured in duplicate with the chloramine-T RIA method described by Gluckman *et al.* (1983). Mean concentrations in low- and high-quality controls were 1.0 and 1.3 ng mL⁻¹, respectively. The intra- and interassay coefficients of variation were 7.1% and 5.7%, respectively, and the minimum detection limit was 0.153 ng mL⁻¹.

Progesterone

Progesterone was measured using a double-antibody RIA after extraction with hexane, as described previously by Gales *et al.* (1997). Mean concentrations in low-, medium- and high-quality controls were 1.4, 2.1 and 3.6 ng mL⁻¹, respectively. The intra- and interassay coefficients of variation were 9.7% and 4.5%, respectively, and the minimum detection limit was 0.03 ng mL⁻¹.

Lamb birthweight and identification

Lambs were weighed and identified soon after birth. Each lamb was allowed to suck the uncovered teat and, if colostrum production was judged to be insufficient for their requirements based on Mellor and Murray (1986), lambs were given a supplement of warm colostrum.

Statistical analysis

Least-squares analysis of variance was used to analyse the effect of body condition, birth type and/or supplementation on udder volume, weight and constituents of colostrum, ewe live weight, body condition, lamb birthweight and lamb plasma glucose, using the statistical programme SuperANOVA (Abacus Concepts 1989). Interactions between birth type and treatments were tested for and are shown only where they

are significant. Regression analysis was used to establish correlations between colostrum production and udder volume. Changes in hormone concentration in plasma were subjected to repeated-measures analysis of variance using the statistical programme GENSTAT (GENSTAT 1993). Effects were considered to be significant when the level of probability was 5% or less. All results are presented as the mean \pm s.e.m.

Results

Feed intake, live weight and body condition

The ewes consumed all the lucerne hay and cracked maize offered during the first 7 days of housing. However, supplemented single- and twin-bearing ewes reduced their hay intake by 4% and 8%, respectively, in the last week of pregnancy (from Day -8 to parturition). Twin-bearing ewes were heavier ($P < 0.05$) but had similar body condition to single-bearing ewes from Day 85 of gestation until parturition (Fig. 1). There were no differences in either live weight or body condition between supplemented and unsupplemented ewes after the period of supplementation.

Udder development

Twin-bearing ewes had bigger ($P < 0.05$) udders than single-bearing ewes at Days -10 and -3 (Fig. 2). At parturition, both the full and empty udders were bigger ($P < 0.05$) in twin-bearing compared with single-bearing ewes. Udders (measured empty) were 43% bigger ($P < 0.05$) in twin-bearing compared with single-bearing ewes. Supplementation further improved udder development and also total udder volume during the last days of pregnancy. The effect was significant on Day -3 ($P < 0.05$) and, at birth, udders were, on average, 49% bigger ($P < 0.05$) in supplemented ewes compared with non-supplemented ewes.

Production of colostrum

At lambing, the supplemented ewes had accumulated more than double the weight of colostrum observed in unsupplemented ewes (Table 2) and the response was greater, albeit not significantly, in twin-bearing ewes. After parturition, supplemented ewes consistently produced more colostrum than unsupplemented ewes and the weights differed significantly in the first hour and from 6–10 h after lambing. The total colostrum produced (that accumulated at parturition plus the subsequent production from birth to 10 h) was higher in twin-bearing compared with single-bearing ewes and in supplemented compared with unsupplemented ewes. The colostrum accumulated at parturition was positively correlated ($P < 0.05$) with the volume of the udder at Days -10 ($n = 53$; $r = 0.27$), -5 ($n = 37$; $r = 0.60$), -3 ($n = 46$; $r = 0.68$) and -1 ($n = 38$; $r = 0.50$) prepartum and at parturition ($n = 56$; $r = 0.38$).

Viscosity of the colostrum

The viscosity score of the colostrum decreased for all groups from parturition up to 10 h after birth. There was an

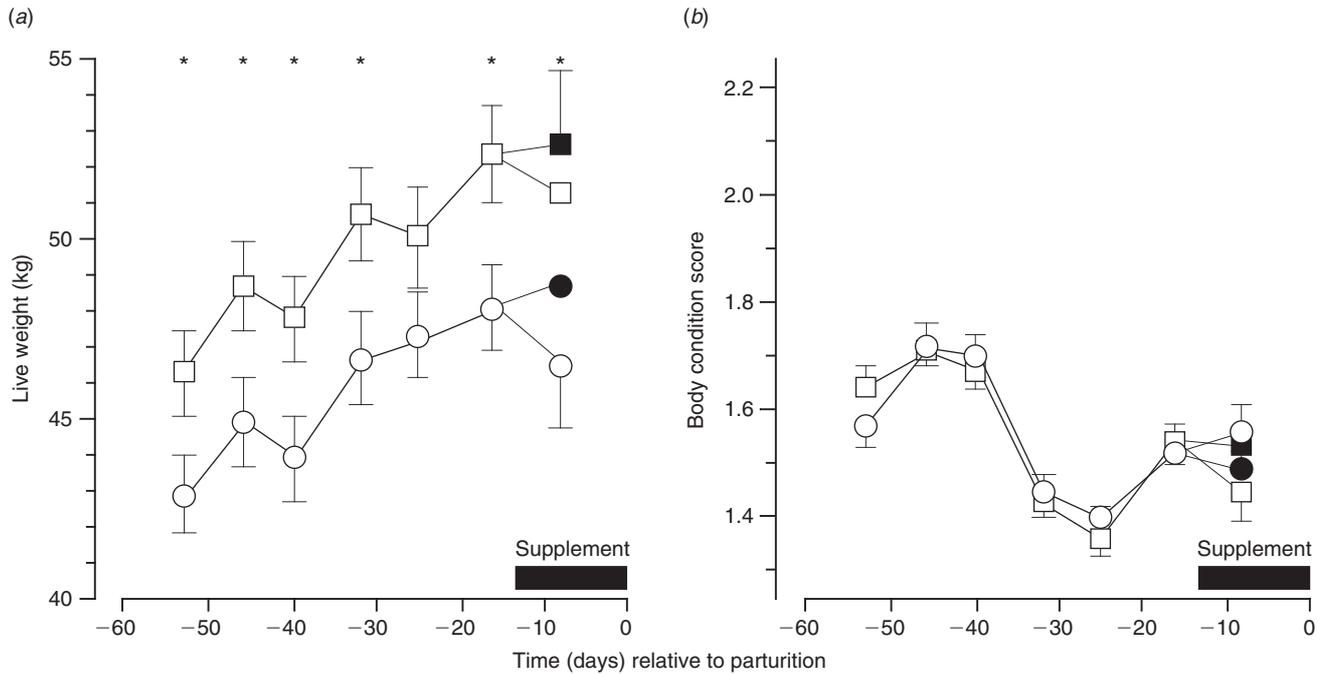


Fig. 1. Live weight (a) and body condition score (b) of Corriedale ewes bearing single (○) or twin fetuses (□) during the last 53 days of gestation and fed a supplement of cracked maize (closed symbols) or no supplement (open symbols) in the last week before lambing. * $P < 0.05$ for birth type.

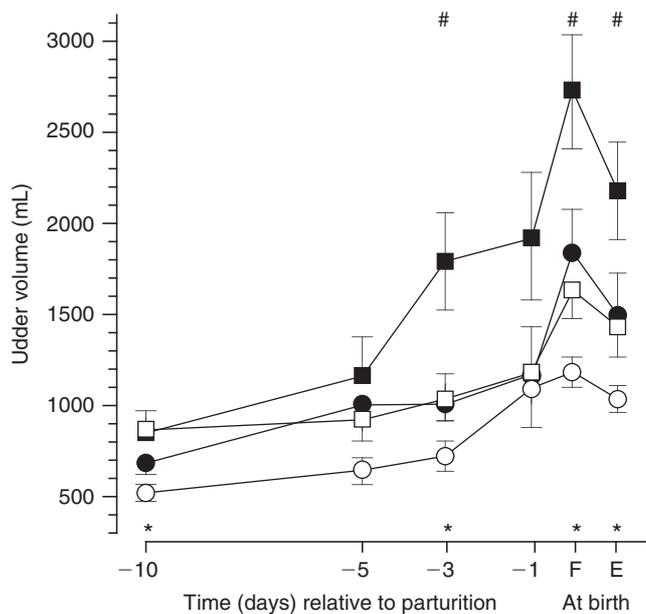


Fig. 2. Udder volume during the last 10 days of gestation in Corriedale ewes bearing single fetuses fed a supplement of cracked maize (●) or no supplement (○) and in ewes bearing twin fetuses supplemented (■) or unsupplemented (□). * $P < 0.05$ for birth type; # $P < 0.05$ for treatment; F = full udder volume; E = empty udder volume.

interaction between birth type and supplementation on the viscosity at parturition (Table 3; $P < 0.05$): twin-bearing ewes that had not been supplemented had more viscous colostrum ($P < 0.05$) than ewes in the other groups. Immediately after

parturition, unsupplemented ewes continued to produce more viscous colostrum than supplemented ewes but, by 10 h after birth, there was no difference between groups. Colostrum viscosity was positively correlated ($P < 0.05$) with colostrum weight at parturition ($n = 56$; $r = 0.34$) and with the yield of colostrum from parturition up to 1 h after birth ($n = 56$; $r = 0.43$). The viscosity score was not significantly correlated with colostrum yield 3 h after birth.

Production of colostrum constituents

The percentage composition of colostrum did not differ in single- and twin-bearing ewes at parturition (Table 4). Thereafter, the percentage of fat and protein remained similar for both groups of ewes, but single-bearing ewes had a higher ($P < 0.05$) percentage of lactose than twin-bearing ewes at 3 and 6 h after birth.

Supplementation affected all the main colostrum constituents at parturition. The percentages of fat and protein were lower in supplemented ewes, but lactose percentage was higher. After birth, the percentage of fat was the same regardless of supplementation. In contrast, protein percentage continued to be lower in supplemented ewes and this effect remained up to 6 h after parturition ($P < 0.05$). Lactose percentage remained higher ($P < 0.05$) in supplemented compared with unsupplemented ewes up to 3 h after birth.

For twin and single lambs, similar total amounts of protein and ME were available in colostrum at birth and during the following 10 h (Table 5), mainly because twin-bearing ewes produced nearly double the amount of total solids of

Table 2. Mean (\pm s.e.m.) colostrum accumulated (g) at parturition and secreted 0–1, 1–3, 3–6 and 6–10 h after parturition and total production of colostrum in single- and twin-bearing Corriedale ewes supplemented with maize or unsupplemented in the week before lambing

	Single		Twin		Significance	
	Control	Supplemented	Control	Supplemented	BT	Treat
Colostrum accumulated (g)						
At parturition	145 \pm 26	339 \pm 53	197 \pm 40	536 \pm 126	NS	***
Parturition to 1 h after birth	77 \pm 14	120 \pm 23	102 \pm 19	203 \pm 35	*	**
1–3 h after birth	66 \pm 9	79 \pm 22	90 \pm 14	163 \pm 40	*	NS
3–6 h after birth	69 \pm 14	80 \pm 18	96 \pm 16	147 \pm 42	NS	NS
6–10 h after birth	117 \pm 20	103 \pm 13	145 \pm 27	201 \pm 38	NS	*
Total colostrum produced (g)	475 \pm 70	730 \pm 89	631 \pm 95	1259 \pm 167	**	**

BT, birth type (single or twin); Treat, treatment (control or supplemented).

Significant differences for values within rows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. NS, values within rows are not significantly different.

Table 3. Mean (\pm s.e.m.) viscosity scores (scale 0–7) for colostrum present at parturition and secreted 0–1, 1–3, 3–6 and 6–10 h after parturition in single- and twin-bearing Corriedale ewes supplemented with maize or unsupplemented in the week before lambing

	Single		Twin		Significance		
	Control	Supplemented	Control	Supplemented	BT	Treat	BT \times Treat
Viscosity scores							
At parturition	5.7 \pm 0.2	5.8 \pm 0.3	4.5 \pm 0.4	5.8 \pm 0.1	*	**	*
Parturition to 1 h after birth	5.4 \pm 0.4	5.7 \pm 0.4	5.2 \pm 0.3	6.2 \pm 0.2	NS	*	NS
1–3 h after birth	6.1 \pm 0.4	6.5 \pm 0.1	5.6 \pm 0.4	6.4 \pm 0.2	NS	*	NS
3–6 h after birth	6.6 \pm 0.3	6.8 \pm 0.1	6.1 \pm 0.3	6.8 \pm 0.1	NS	NS	NS
6–10 h after birth	6.9 \pm 0.1	7.0 \pm 0.0	6.5 \pm 0.3	7.0 \pm 0.0	NS	*	NS

BT, birth type (single or twin); Treat, treatment (control or supplemented).

Significant differences for values within rows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. NS, values within rows are not significantly different.

Table 4. Components of colostrum at different milking times in single- and twin-bearing Corriedale ewes supplemented with maize or unsupplemented in the week before lambing

	Single		Twin		Significance	
	Control	Supplemented	Control	Supplemented	BT	Treat
Fat (%)						
At parturition	8.5 \pm 0.5	7.6 \pm 0.8	9.6 \pm 0.5	7.2 \pm 1.0	NS	*
Parturition to 1 h after birth	10.6 \pm 0.6	10.9 \pm 0.9	12.5 \pm 0.6	11.2 \pm 1.1	NS	NS
1–3 h after birth	10.2 \pm 0.7	11.5 \pm 0.6	11.6 \pm 0.6	11.6 \pm 1.0	NS	NS
3–6 h after birth	9.5 \pm 0.7	10.2 \pm 0.8	9.3 \pm 0.5	9.7 \pm 0.5	NS	NS
6–10 h after birth	8.2 \pm 0.5	9.2 \pm 0.4	9.5 \pm 0.6	9.0 \pm 0.7	NS	NS
Protein (%)						
At parturition	17.2 \pm 0.7	14.7 \pm 0.9	18.9 \pm 0.9	15.7 \pm 0.9	NS	***
Parturition to 1 h after birth	15.7 \pm 0.6	13.2 \pm 0.9	17.9 \pm 1.1	14.0 \pm 0.9	NS	**
1–3 h after birth	12.8 \pm 1.0	10.7 \pm 1.0	14.1 \pm 1.2	11.8 \pm 0.9	NS	*
3–6 h after birth	10.8 \pm 1.1	8.0 \pm 0.8	11.9 \pm 1.5	9.2 \pm 0.9	NS	*
6–10 h after birth	7.7 \pm 1.0	6.1 \pm 0.6	8.7 \pm 1.1	7.9 \pm 0.7	NS	NS
Lactose (%)						
At parturition	1.8 \pm 0.2	2.6 \pm 0.3	1.4 \pm 0.3	2.5 \pm 0.2	NS	***
Parturition to 1 h after birth	1.7 \pm 0.2	2.4 \pm 0.2	1.2 \pm 0.2	2.2 \pm 0.2	NS	***
1–3 h after birth	2.3 \pm 0.2	2.8 \pm 0.2	1.9 \pm 0.2	2.4 \pm 0.1	*	*
3–6 h after birth	3.0 \pm 0.2	3.4 \pm 0.2	2.6 \pm 0.3	2.9 \pm 0.1	*	NS
6–10 h after birth	3.6 \pm 0.2	3.9 \pm 0.1	3.5 \pm 0.2	3.6 \pm 0.1	NS	NS

Data are the mean \pm s.e.m.

BT, birth type (single or twin); Treat, treatment (control or supplemented).

Significant differences for values within rows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. NS, values within rows are not significantly different.

Table 5. Amount of metabolisable energy (from fat, lactose and protein) and protein secreted in the colostrum accumulated at parturition and produced from parturition up to 10 h after birth in single- and twin-bearing Corriedale ewes supplemented with maize or unsupplemented in the week before lambing

	Single		Twin		Significance	
	Control	Supplemented	Control	Supplemented	BT	Treat
ME secreted (MJ)						
At parturition	0.5 ± 0.1	1.1 ± 0.2	0.8 ± 0.2	2.1 ± 0.9	NS	*
Parturition to 10 h	1.4 ± 0.2	1.8 ± 0.3	2.0 ± 0.3	3.3 ± 0.5	**	*
Total ME	1.9 ± 0.3	2.9 ± 0.4	2.8 ± 0.5	5.4 ± 1.0	**	**
Protein secreted (g)						
At parturition	24.8 ± 4.5	47.3 ± 6.5	37.8 ± 7.8	74.9 ± 14.2	*	**
Parturition to 10 h	35.2 ± 4.7	38.4 ± 6.9	53.5 ± 8.4	73.8 ± 10.5	***	NS
Total protein	60 ± 8.5	85.7 ± 11.4	91.3 ± 14.5	148.7 ± 17.0	***	***

Data are the mean ± s.e.m.

BT, birth type (single or twin); Treat, treatment (control or supplemented); ME, metabolisable energy.

Significant differences for values within rows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. NS, values within rows are not significantly different.

single-bearing ewes. Moreover, lambs mothered by ewes supplemented with maize had nearly double the amount of available protein and ME at lambing and during the first 10 h of life compared with lambs mothered by unsupplemented ewes (Table 5; $P < 0.05$).

Hormones and metabolites related to nutritional state, udder development and lactogenesis

The data presented in this section are the mean of at least nine animals per treatment. The plasma concentration of glucose increased from Day -1 until 1 h after parturition and then decreased rapidly to 10 h post partum (Fig. 3). From Day -6 up to 10 h after birth, glucose concentrations were higher in supplemented compared with unsupplemented ewes ($P < 0.05$). There was no difference in plasma glucose between birth types. Supplemented ewes had higher levels of insulin than unsupplemented ewes, although differences were only significant ($P < 0.05$) on Days -7, -6, -3, -1 and 1 h after birth (Fig. 3). Insulin concentrations also differed between birth types, with single-bearing ewes having higher values on Days -4 and -3 and 1 h after birth compared with twin-bearing ewes.

In all groups, plasma GH concentrations increased over the last few days before parturition, then fell from 1 h after birth (Fig. 3). However, the timing of the onset of the rise differed between groups, with GH levels beginning to rise as early as Day -7 in unsupplemented twin-bearing ewes, but not starting until 12 h before parturition in the remaining groups. Unsupplemented ewes had higher levels of GH than supplemented ewes from Day -7 up to 10 h after birth. Twin-bearing ewes also had higher levels of GH than single-bearing ewes before parturition and up to 1 h after birth. The difference was significant on Days -11, -4 to -1, at parturition and 1 h after birth.

Plasma IGF-I concentrations increased gradually from Day -18 until 10 h after birth for both supplemented and

unsupplemented ewes (Fig. 3). However, supplemented ewes had higher ($P < 0.05$) values from Day -3 until 10 h after birth. Twin- and single-bearing ewes had similar levels of IGF-I with the exception of Day -1, when single-bearing ewes had higher ($P < 0.05$) levels of IGF-I than twin-bearing ewes.

Plasma β -OHB concentrations were higher ($P < 0.05$) in unsupplemented ewes than in supplemented ewes from Day -5 up to parturition (Fig. 4). There was no difference in β -OHB concentrations between single-bearing ewes and twin-bearing ewes. Plasma urea concentrations were higher ($P < 0.05$) in unsupplemented compared with supplemented ewes during the period evaluated (Fig. 4). The only difference in urea concentrations between single- and twin-bearing ewes was detected 12 h before parturition ($P < 0.05$).

The plasma concentrations of progesterone decreased ($P < 0.05$) before birth in all groups (Fig. 5). However, supplemented ewes began with a lower plasma concentration of progesterone than unsupplemented ewes from Day -7 up to 1 h after birth and the difference was significant from Days -6 to -1 and at parturition ($P < 0.05$). Twin-bearing ewes had higher concentrations of plasma progesterone than single-bearing ewes at parturition ($P < 0.05$).

Bodyweight and plasma glucose in lambs at birth

Twin lambs were lighter ($P < 0.05$) than single lambs (Table 6), but supplementation of the dam before parturition did not affect birthweights. The difference between single and twin lambs in terms of the plasma concentrations of glucose at birth were not significant (Table 6; $P < 0.10$), but lambs born to ewes supplemented with maize had higher concentrations than lambs born to unsupplemented ewes ($P < 0.05$).

Discussion

Supplementing twin-bearing ewes with cracked maize before lambing increased the colostrum accumulated at birth

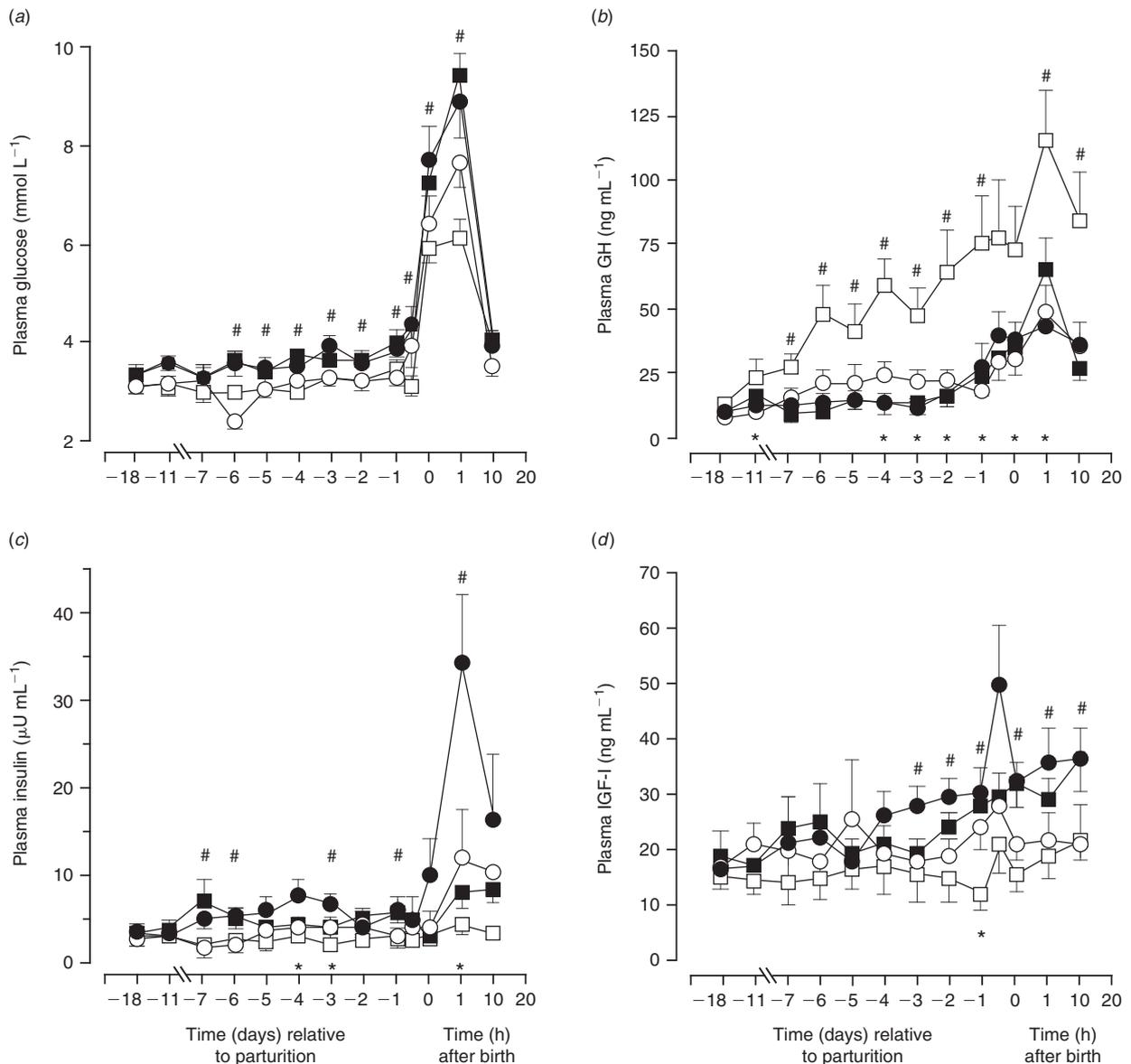


Fig. 3. Plasma concentrations of glucose (a), growth hormone (GH) (b), insulin (c) and insulin-like growth factor-I (IGF-I) (d) during the last 18 days of gestation and first 10 h after birth in Corriedale ewes bearing single fetuses fed a supplement of cracked maize (●) or no supplement (○) and ewes bearing twin fetuses supplemented with maize (■) or unsupplemented (□). * $P < 0.05$ for birth type; # $P < 0.05$ for treatment.

and its synthesis during the following 10 h, despite the unsupplemented ewes having been fed to meet their estimated requirements for energy (MAFF 1975). The reason seems to be that, without the maize supplement, the ewes had insufficient glucose. Indeed, the increased plasma glucose concentrations in supplemented ewes supports the hypothesis that cracked maize increases the entry rate of glucose (Knowlton *et al.* 1998a; Landau *et al.* 1999) and, subsequently, the mammary uptake of glucose and the synthesis of lactose by the mammary gland (Linzell 1974). The glucose supplied by the cracked maize may be used, in part, to provide the energy needed for gut metabolism (Nocek and Tamminga 1991;

Knowlton *et al.* 1998b), sparing endogenously synthesised glucose for mammary uptake and lactose synthesis.

The greater response in the yield of colostrum of twin-bearing ewes over single-bearing ewes after supplementation is consistent with the work of Hall *et al.* (1992). Normally, twin-bearing ewes develop larger udders than single-bearing ewes (Mellor 1988) with the capacity to synthesise more colostrum. However, for this to happen, facilitatory conditions for colostrum synthesis have to be met. In the present study, twin-bearing ewes had bigger udders than single-bearing ewes and the plasma concentrations of progesterone, the main regulator of the synthesis of colostrum,

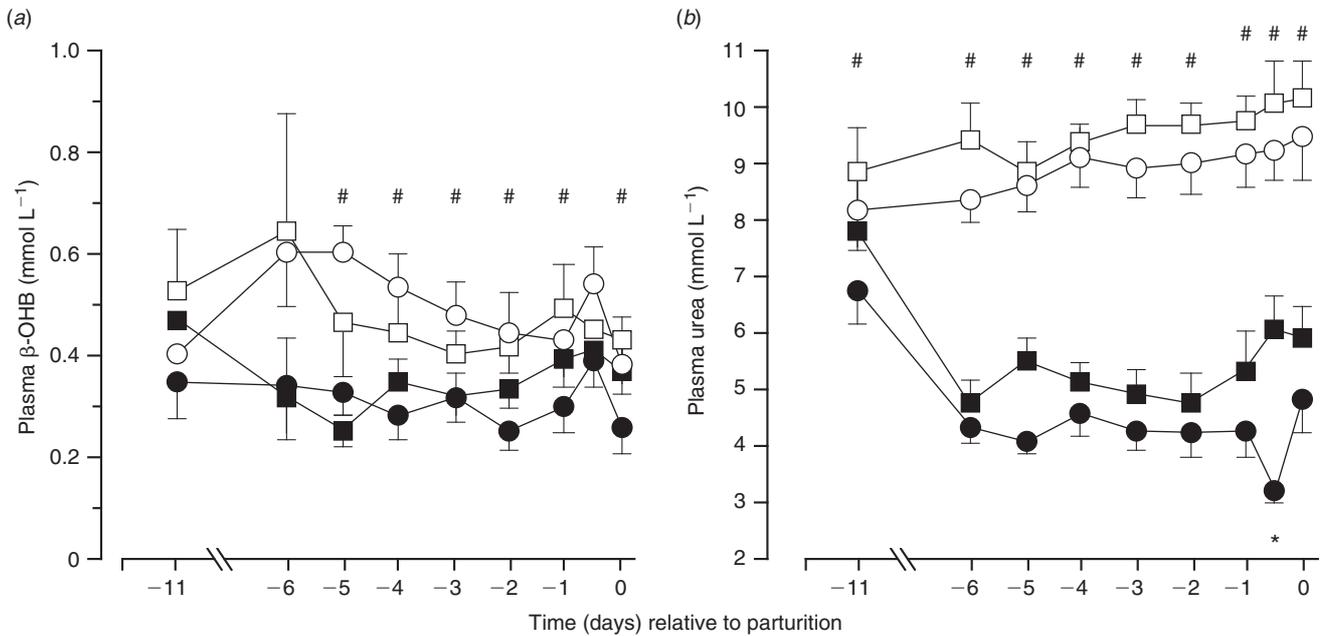


Fig. 4. Changes in plasma concentrations of β -hydroxybutyrate (β -OHB) (a) and urea (b) during the last 11 days of gestation in Corriedale ewes bearing single fetuses supplemented with maize (●) or unsupplemented (○) and ewes bearing twin fetuses supplemented with maize (■) or unsupplemented (□). * $P < 0.05$ for birth type; # $P < 0.05$ for treatment.

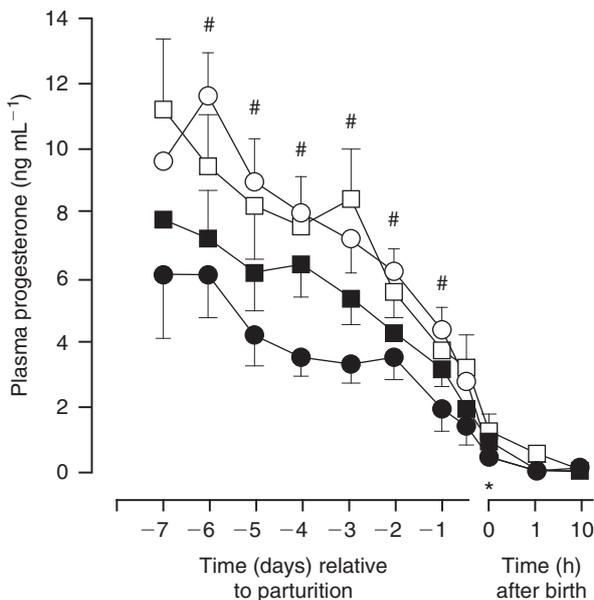


Fig. 5. Changes in plasma concentrations of progesterone during the last 7 days of gestation and first 10 h after birth in Corriedale ewes bearing single fetuses supplemented with maize (●) or unsupplemented (○) and ewes bearing twin fetuses supplemented with maize (■) or unsupplemented (□). * $P < 0.05$ for treatment; # $P < 0.05$ for birth type.

and glucose, the main substrate for synthesis of lactose, were adequate for the onset of lactogenesis in both twin- and single-bearing ewes supplemented with maize.

In addition to producing more colostrum than unsupplemented ewes, the supplemented ewes produced colostrum

that was less viscous. The low viscosity was associated with higher concentrations of lactose. Lactose is osmotically active (Leong *et al.* 1990) and draws water from the blood, which, in turn, decreases colostrum viscosity. The colostrum with the highest viscosity and the lowest lactose concentrations was observed in twin-bearing unsupplemented ewes. It seems that, without a supplement, even on an apparently complete diet, ewes bearing twins are less able than single-bearing ewes to meet their glucose requirements at the end of pregnancy.

The responses of the ewes to supplementation was reflected in differences in plasma concentrations of hormones and metabolites between supplemented and unsupplemented ewes. The concentration of progesterone during the prepartum period decreased in all ewes, but it decreased more rapidly in supplemented animals. Hartmann *et al.* (1973) suggested that the threshold level for plasma progesterone for the onset of lactogenesis is 1 ng mL⁻¹ and, in the present study, this notional threshold was reached between 12 h prepartum and parturition for twin- and single-bearing ewes supplemented with maize, as well as for unsupplemented single-bearing ewes, whereas it was reached between 12 h prepartum and 1 h post partum in twin-bearing unsupplemented ewes. Most of the colostrum present at birth was accumulated during these intervals. The extra colostrum produced by supplemented ewes was probably due to the higher availability of precursors for colostrum synthesis.

Insulin influences the partitioning of nutrients between the mammary gland and other tissues (Vernon 1989)

Table 6. Bodyweight and plasma glucose concentration in lambs at birth

	Single		Twin		Significance	
	Control	Supplemented	Control	Supplemented	BT	Treat
<i>n</i>	7	13	27	23		
Lamb birthweight (kg)	4.0 ± 0.2	4.1 ± 0.2	3.2 ± 0.1	3.3 ± 0.1	***	NS
Plasma glucose (mmol L ⁻¹)	1.7 ± 0.2	2.3 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	NS	*

Data are the mean ± s.e.m.

BT, birth type (single or twin); Treat, treatment (control or supplemented).

Significant differences for values within rows: **P* ≤ 0.05; ***P* ≤ 0.01; ****P* ≤ 0.001. NS, values within rows are not significantly different.

and circulating insulin concentrations were increased by supplementation. Even though insulin has no effect on the uptake of nutrients by the mammary gland in lactating cows (Lemosquet *et al.* 1997), high levels of insulin may decrease gluconeogenesis (Freychet 1990) and, in doing so, may spare more amino acids for colostrum synthesis (Knowlton *et al.* 1998b). The supplemented ewes had a lower concentration of protein in their colostrum than the control ewes, but this was due to a dilution effect (Kim *et al.* 2001) and, overall, they produced nearly twice the mass of protein seen in control ewes at lambing. In addition, high circulating concentrations of insulin can increase the plasma concentration of IGF-I (McGuire *et al.* 1995; Bequette *et al.* 2001) and this stimulates mammary growth and blood flow (Prosser *et al.* 1990; Delouis and Richard 1993). Increased mammary blood flow, in turn, may increase the uptake of glucose by the mammary gland (Bequette *et al.* 2001). Therefore, changes in insulin release that are induced by dietary treatments may affect the availability of milk precursors and, possibly, milk composition (Lemosquet *et al.* 1997).

For all treatments, the concentration of β -OHB was within the normal healthy range for sheep (<0.7 mmol L⁻¹; Aiello 1998), but the lower values observed in supplemented ewes suggest that these ewes had been mobilising less adipose tissue than unsupplemented ewes in order to meet their requirements in late pregnancy. Further evidence for this is seen in the lower fat percentage in the colostrum of supplemented ewes compared with that of unsupplemented ewes. An inhibition of adipose lypolysis or an increase in adipose lipogenesis (Lemosquet *et al.* 1997; Kennelly *et al.* 1999) can decrease the yield of C₁₈ fatty acids (Lemosquet *et al.* 1997; Hurtard *et al.* 2000) and, consequently, decrease the yield of milk fat. Despite the lower fat percentage in supplemented ewes, they managed to produce more total fat than unsupplemented ewes because they produced much more colostrum.

Finally, the protein in the diet of unsupplemented ewes appeared not to be utilised as efficiently as in supplemented ewes. The higher concentrations of urea in the plasma of unsupplemented ewes may be due to degradation of the lucerne protein to ammonia in the rumen and this would limit protein synthesis by the ruminal microbes. In contrast,

supplemented ewes receiving the same basal diet of lucerne may have used some of the starch from the corn as fermentable carbohydrate. This could have led to increased capture of the ammonia released from lucerne protein and, thus, more effective microbial protein synthesis.

In the presence of prolactin, insulin also becomes a growth factor for mammary tissue (Freychet 1990) and IGF-I plays a major role in mammary differentiation. The circulating concentrations of all three of these mammogenic hormones were increased by supplementation with maize and this may account for the better development of the udder in these ewes. However, GH concentrations were reduced by supplementation, an observation that is consistent with that of Mellor *et al.* (1987). In well-fed animals, GH acts through IGF-I to promote udder development and galactopoiesis but, when nutritional status is severely compromised, basal concentrations of IGF-I are lower and the ability of GH to increase IGF-I is blocked (Bauman and Vernon 1993), preventing stimulation of the mammary gland by GH through this pathway. This explains why unsupplemented twin-bearing ewes had poor udder development despite their high plasma GH levels. The lack of GH in supplemented ewes appears to be compensated for by the insulin–prolactin interaction and by glucose-driven increases in IGF-I secretion.

It has long been acknowledged that overfeeding ewes in the final weeks of pregnancy may cause excessive fetal growth, leading to dystocia and an increase in lamb mortality. However, the short duration of feeding needed to elicit the colostrum response had little or no effect on birthweight, as also reported by Murphy *et al.* (1996). This obviates concern about increasing the incidence of dystocia.

In conclusion, supplementation with a high-energy supplement during the last week of pregnancy greatly enhances the amount and viscosity of colostrum produced by ewes, particularly in those bearing twin lambs. From observations of the circulating concentrations of hormones and metabolites, it appears that these responses are due to the provision of extra glucose and the enhancement of lactose synthesis. This has been supported by another study in which alternative supplements have been tested (Banchero *et al.* 2004) and there is now a need to assess the implications of these outcomes for the survival of newborn lambs.

Acknowledgments

We thank Margaret Blackberry and John Beesley from the University of Western Australia for help with chemical analyses, the staff of the National Institute of Agricultural Research (INIA) in Uruguay for technical help and the Division de Laboratorios Veterinarios (DILAVE) for the preparation of samples for entry into Australia.

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Manuscript received 7 October 2003; revised and accepted 3 June 2004.