

## CHAPTER 4. DATA AND STATISTICAL ANALYSES

## 1. Production data

## 1.1 Body weight

The body weight in kilograms determined weekly on two consecutive days was used to calculate a weighted average of two observations. The results of the average body weight for each week of the experiment are presented in Table 14 and Figure 13 (n=10) as weekly, weighted average body weights. All results are given as the mean of all cows  $\pm$  standard deviation (SD), with the SD as a percentage of the mean, or coefficient of variation (CV).

Table 14. Weekly mean body weight

Week	Mean $\pm$ SD	CV
1	612.0 $\pm$ 59.3 kg	9.7%
2	602.8 $\pm$ 58.9 kg	9.8%
3	594.3 $\pm$ 59.4 kg	10.0%
4	589.3 $\pm$ 59.7 kg	10.1%
5	589.5 $\pm$ 62.8 kg	10.7%
6	582.8 $\pm$ 66.3 kg	11.4%
7	587.5 $\pm$ 65.4 kg	11.1%
8	580.8 $\pm$ 61.4 kg	10.6%
9	576.3 $\pm$ 57.3 kg	9.9%
10	577.8 $\pm$ 55.0 kg	9.5%
11	562.8 $\pm$ 53.4 kg	9.5%
12	558.6 $\pm$ 60.1 kg	10.8%

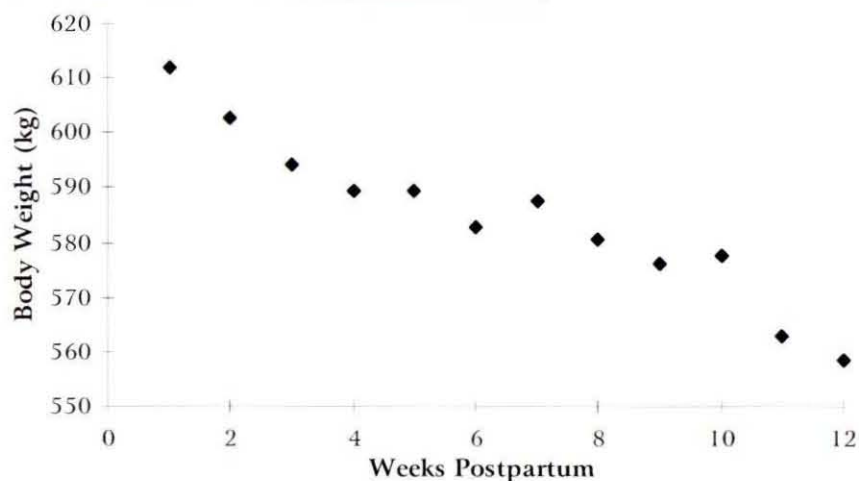


Figure 13. Weekly mean body weight

### 1.2 Body condition score

The average BCS of two observations, from two consecutive days was determined (1=emaciated, 5=obese) for each cow. The average BCS by week postpartum is given in Table 15 and Figure 14.

Table 15. Weekly mean BCS

Week	Mean $\pm$ SD	CV
1	3.2 $\pm$ 0.2	5.9%
2	3.2 $\pm$ 0.2	6.5%
3	3.1 $\pm$ 0.2	6.5%
4	3.1 $\pm$ 0.2	7.8%
5	3.1 $\pm$ 0.2	7.7%
6	3.0 $\pm$ 0.3	8.3%
7	3.0 $\pm$ 0.3	8.3%
8	3.0 $\pm$ 0.3	9.2%
9	2.9 $\pm$ 0.3	8.6%
10	2.9 $\pm$ 0.3	8.5%
11	2.9 $\pm$ 0.2	8.3%
12	2.9 $\pm$ 0.2	8.3%

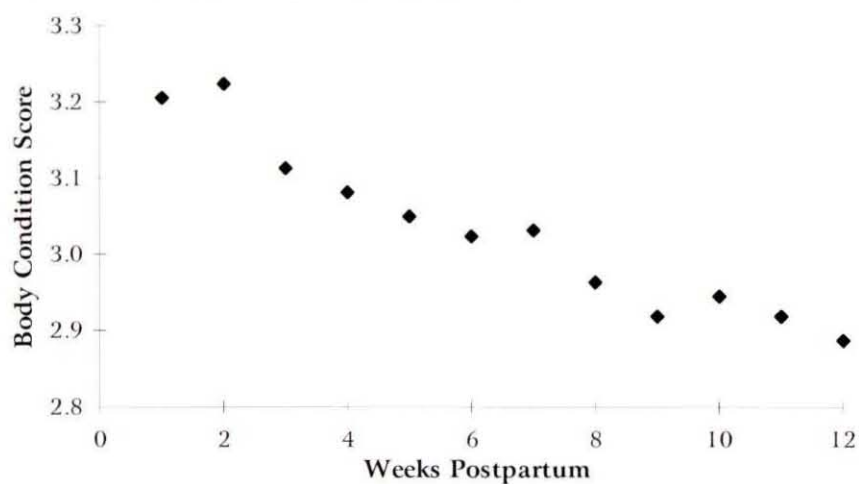


Figure 14. Weekly mean body condition score

### 1.3 Milk production

The milk production was determined for the morning and afternoon milkings. The weighted average for each week was determined from these daily milk production records (Table 16 and Figure 15).

Table 16. Weekly mean milk production

Week	Mean $\pm$ SD	CV
1	20.4 $\pm$ 4.0 kg/d	19.8%
2	28.2 $\pm$ 5.3 kg/d	18.9%
3	29.6 $\pm$ 5.4 kg/d	18.2%
4	29.5 $\pm$ 5.5 kg/d	18.5%
5	30.0 $\pm$ 5.6 kg/d	18.8%
6	30.1 $\pm$ 5.3 kg/d	17.6%
7	29.5 $\pm$ 4.5 kg/d	15.4%
8	28.6 $\pm$ 5.2 kg/d	18.3%
9	28.2 $\pm$ 5.2 kg/d	18.6%
10	27.9 $\pm$ 6.1 kg/d	21.7%
11	28.2 $\pm$ 6.6 kg/d	23.4%
12	26.2 $\pm$ 5.3 kg/d	20.4%

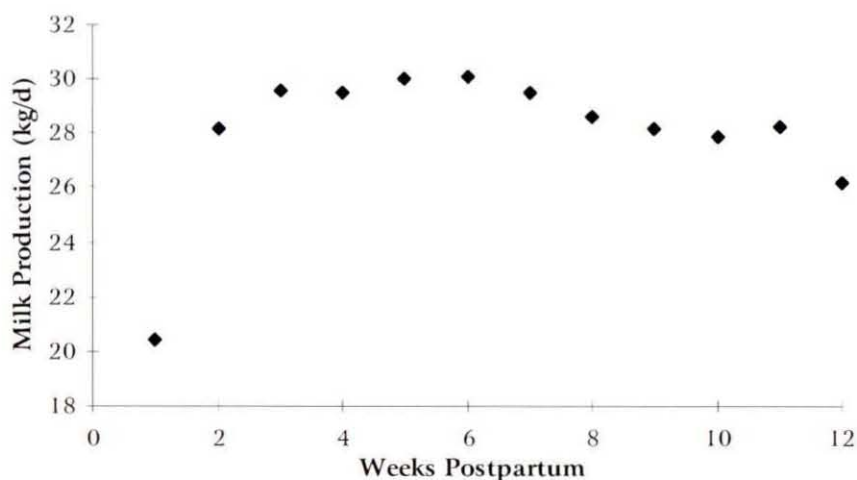


Figure 15. Weekly mean milk production

During the experimental period the mean milk production of the 3 days prior to metabolic test (Figure 16) days exhibited a decline, especially during the periods of energy restriction.

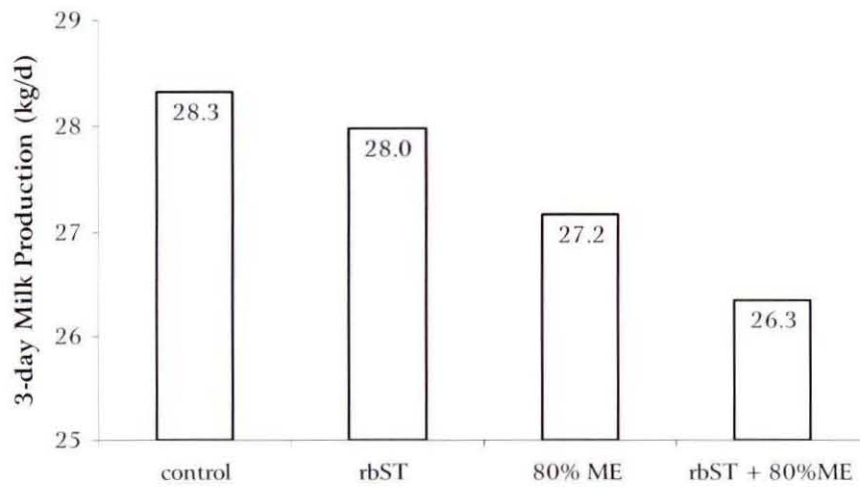


Figure 16. Mean milk production prior to metabolic tests

## 2. Whole-blood data

### 2.1 Data collected during the insulin challenge

#### 2.1.1 The control period

The glucose concentration in whole-blood was periodically determined from 30 minutes before insulin injection (t-30) up to 180 minutes after insulin injection (t180). These values are presented as a weighted average for all cows (n=10), at each time point  $\pm$  SD and CV (Table 17 and Figure 17).

Table 17. Insulin challenge whole-blood glucose concentration (control)

Time	Mean $\pm$ SD	CV
t -30	48.5 $\pm$ 2.8 mg/dL	5.7%
t -20	49.0 $\pm$ 3.1 mg/dL	6.3%
t -10	48.8 $\pm$ 3.3 mg/dL	6.7%
t 0	48.6 $\pm$ 3.2 mg/dL	6.7%
t 10	44.2 $\pm$ 4.0 mg/dL	9.1%
t 20	37.6 $\pm$ 3.0 mg/dL	8.0%
t 25	35.4 $\pm$ 3.5 mg/dL	9.9%
t 30	33.7 $\pm$ 3.6 mg/dL	10.6%
t 35	32.0 $\pm$ 3.7 mg/dL	11.5%
t 40	31.8 $\pm$ 2.7 mg/dL	8.6%
t 45	32.8 $\pm$ 2.5 mg/dL	7.5%
t 50	34.2 $\pm$ 2.5 mg/dL	7.4%
t 55	35.8 $\pm$ 3.2 mg/dL	9.1%
t 60	36.8 $\pm$ 3.5 mg/dL	9.5%
t 65	37.9 $\pm$ 3.9 mg/dL	10.3%
t 70	38.6 $\pm$ 4.3 mg/dL	11.1%
t 80	40.3 $\pm$ 3.8 mg/dL	9.3%
t 90	41.8 $\pm$ 3.4 mg/dL	8.2%
t 100	42.6 $\pm$ 4.1 mg/dL	9.5%
t 120	44.0 $\pm$ 3.9 mg/dL	8.8%
t 150	46.2 $\pm$ 3.8 mg/dL	8.2%
t 180	47.8 $\pm$ 4.4 mg/dL	9.2%

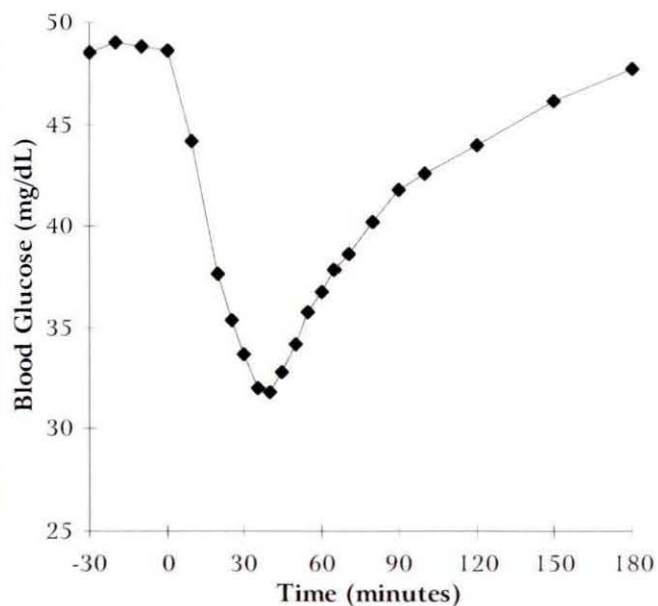


Figure 17. Insulin challenge whole-blood concentration (control)

### 2.1.2 The rbST period

Whole-blood glucose concentration data collected  $\pm 7$  days after recombinant bST treatment is presented in Table 18 and Figure 18. Individual values were used to calculate the weighted average for all cows.

Table 18. Insulin challenge whole-blood glucose concentration (rbST)

Time	Mean $\pm$ SD	CV
t -30	43.9 $\pm$ 3.4 mg/dL	7.8%
t -20	44.3 $\pm$ 3.8 mg/dL	8.5%
t -10	44.0 $\pm$ 4.2 mg/dL	9.6%
t 0	44.4 $\pm$ 4.2 mg/dL	9.6%
t 10	40.8 $\pm$ 3.1 mg/dL	7.5%
t 20	36.0 $\pm$ 2.8 mg/dL	7.9%
t 25	33.9 $\pm$ 2.3 mg/dL	6.8%
t 30	32.7 $\pm$ 2.5 mg/dL	7.6%
t 35	31.5 $\pm$ 2.7 mg/dL	8.7%
t 40	30.8 $\pm$ 2.3 mg/dL	7.4%
t 45	30.5 $\pm$ 2.6 mg/dL	8.5%
t 50	31.2 $\pm$ 3.6 mg/dL	11.4%
t 55	32.6 $\pm$ 4.3 mg/dL	13.2%
t 60	33.0 $\pm$ 4.8 mg/dL	14.5%
t 65	33.8 $\pm$ 4.8 mg/dL	14.3%
t 70	34.7 $\pm$ 4.9 mg/dL	14.2%
t 80	36.1 $\pm$ 4.8 mg/dL	13.3%
t 90	36.9 $\pm$ 4.8 mg/dL	13.1%
t 100	37.8 $\pm$ 4.5 mg/dL	11.8%
t 120	39.7 $\pm$ 4.8 mg/dL	12.0%
t 150	40.7 $\pm$ 4.5 mg/dL	11.2%
t 180	41.5 $\pm$ 4.8 mg/dL	11.6%

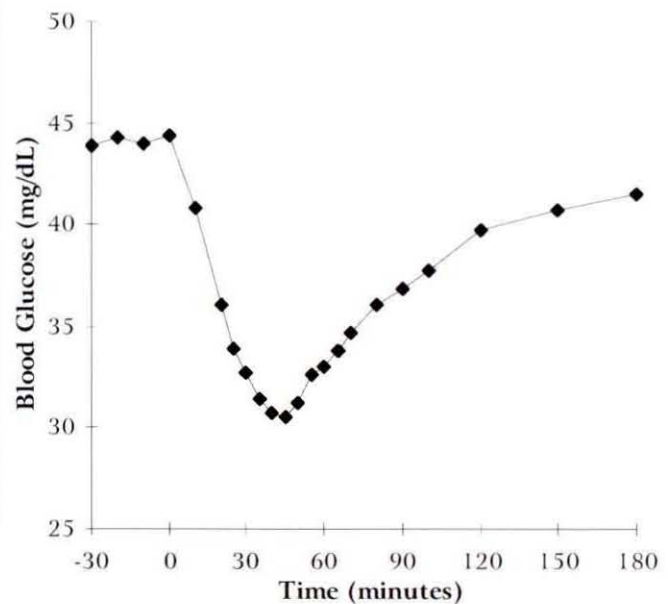


Figure 18. Insulin challenge whole-blood concentration (rbST)

### 2.1.3 The 80% ME period

The average whole-blood glucose concentration results of the insulin challenge, performed 1 week after energy restriction are presented in Table 19 and Figure 19.

Table 19. Insulin challenge whole-blood glucose concentration (80% ME)

Time	Mean $\pm$ SD	CV
t -30	45.8 $\pm$ 4.3 mg/dL	9.3%
t -20	45.8 $\pm$ 4.4 mg/dL	9.5%
t -10	45.9 $\pm$ 3.7 mg/dL	8.2%
t 0	45.5 $\pm$ 3.9 mg/dL	8.5%
t 10	42.1 $\pm$ 3.5 mg/dL	8.4%
t 20	35.2 $\pm$ 3.1 mg/dL	8.7%
t 25	32.9 $\pm$ 3.5 mg/dL	10.8%
t 30	30.9 $\pm$ 3.9 mg/dL	12.5%
t 35	29.3 $\pm$ 3.9 mg/dL	13.4%
t 40	29.2 $\pm$ 2.9 mg/dL	9.9%
t 45	30.1 $\pm$ 2.8 mg/dL	9.2%
t 50	30.9 $\pm$ 2.7 mg/dL	8.7%
t 55	32.7 $\pm$ 3.2 mg/dL	9.8%
t 60	33.5 $\pm$ 3.9 mg/dL	11.7%
t 65	34.9 $\pm$ 4.4 mg/dL	12.7%
t 70	35.8 $\pm$ 4.4 mg/dL	12.3%
t 80	38.2 $\pm$ 4.1 mg/dL	10.8%
t 90	39.7 $\pm$ 4.3 mg/dL	10.9%
t 100	40.9 $\pm$ 4.8 mg/dL	11.7%
t 120	42.9 $\pm$ 4.1 mg/dL	9.7%
t 150	43.1 $\pm$ 3.5 mg/dL	8.2%
t 180	44.9 $\pm$ 3.8 mg/dL	8.5%

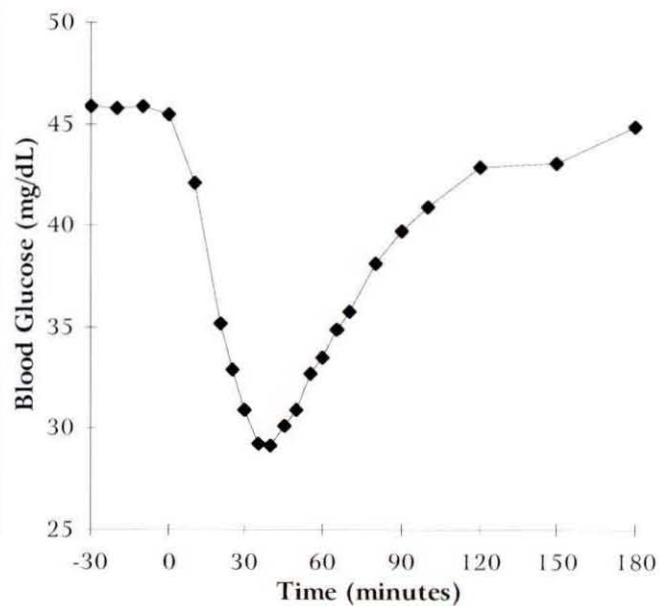


Figure 19. Insulin challenge whole-blood concentration (80% ME)

### 2.1.4 The 80% ME + rbST period

Whole-blood data collected periodically during the insulin challenge performed to test the combined effects of 80% of metabolizable energy and recombinant bST treatments, is presented in Table 20 and Figure 20.

Table 20. Insulin challenge whole-blood glucose concentration (80% ME + rbST)

Time	Mean $\pm$ SD	CV
t -30	46.7 $\pm$ 2.9 mg/dL	6.2%
t -20	46.6 $\pm$ 2.6 mg/dL	5.6%
t -10	46.6 $\pm$ 2.6 mg/dL	5.6%
t 0	46.4 $\pm$ 3.4 mg/dL	7.3%
t 10	43.5 $\pm$ 3.2 mg/dL	7.3%
t 20	37.5 $\pm$ 2.9 mg/dL	7.7%
t 25	35.2 $\pm$ 3.0 mg/dL	8.5%
t 30	33.3 $\pm$ 2.6 mg/dL	7.8%
t 35	31.5 $\pm$ 2.7 mg/dL	8.5%
t 40	30.7 $\pm$ 2.6 mg/dL	8.4%
t 45	31.1 $\pm$ 3.1 mg/dL	10.1%
t 50	32.1 $\pm$ 4.2 mg/dL	12.9%
t 55	33.1 $\pm$ 4.7 mg/dL	14.3%
t 60	33.8 $\pm$ 4.6 mg/dL	13.7%
t 65	34.8 $\pm$ 5.2 mg/dL	14.8%
t 70	36.7 $\pm$ 5.2 mg/dL	14.2%
t 80	38.1 $\pm$ 5.1 mg/dL	13.4%
t 90	39.3 $\pm$ 3.9 mg/dL	9.9%
t 100	40.8 $\pm$ 3.7 mg/dL	9.1%
t 120	42.3 $\pm$ 3.2 mg/dL	7.7%
t 150	43.5 $\pm$ 2.6 mg/dL	5.9%
t 180	44.6 $\pm$ 3.2 mg/dL	7.1%

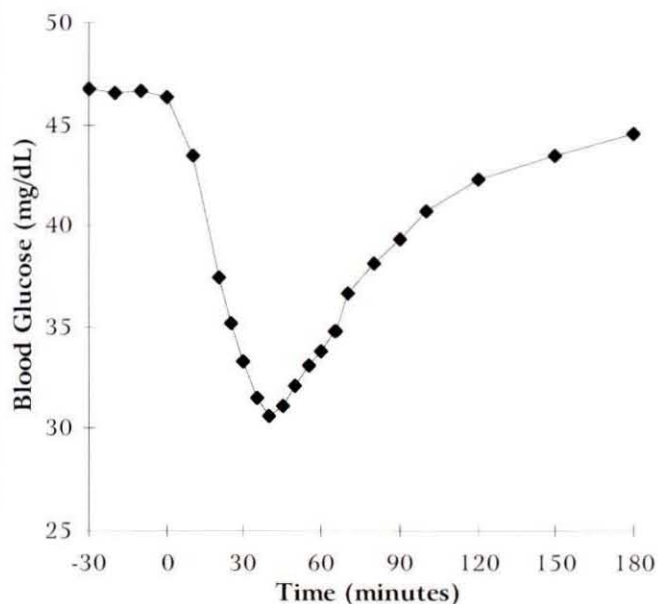


Figure 20. Insulin challenge whole-blood concentration (80% ME + rbST)

## 2.2 Effect of treatments in the insulin challenge

### 2.2.1 The baseline glucose concentration

The individual baseline glucose concentrations determined at 10-minute intervals from t-30 to t0 were analyzed using repeated measures between periods (SAS, 1994). There was no significant effect of time of sampling on glucose concentration ( $P < 0.7005$ ), but there was a significant effect ( $P < 0.0001$ ) of period on the individual baseline measurements (Table 21 and Figure 21).

Table 21. The insulin challenge baseline whole-blood concentration

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 48.7 $\pm$ 3.0 mg/dL	6.1%
2	rbST	<sup>b</sup> 44.1 $\pm$ 3.8 mg/dL	8.6%
3	80% ME	<sup>c</sup> 45.7 $\pm$ 3.9 mg/dL	8.6%
4	80% ME + rbST	<sup>d</sup> 46.6 $\pm$ 2.8 mg/dL	6.0%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

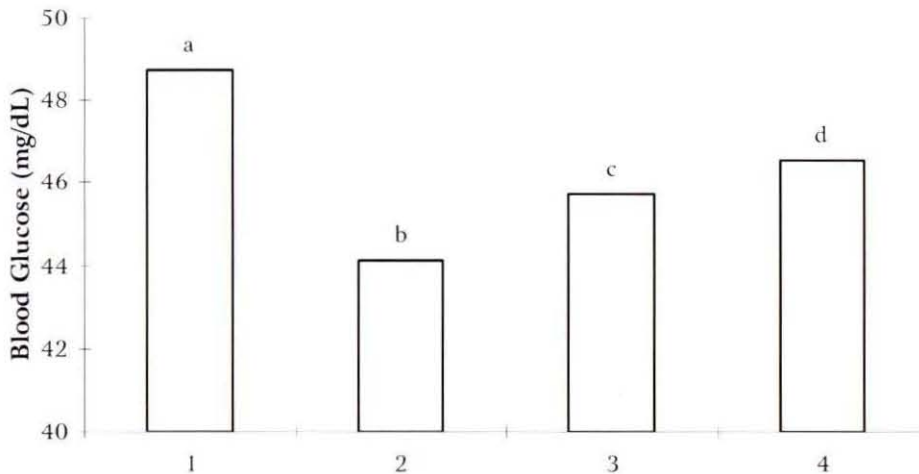


Figure 21. The insulin challenge baseline (whole-blood)

### 2.2.2 The baseline glucose AUC

The area under the successive glucose concentration values was calculated by summation of the areas of the trapezoids under the baseline curve (AUC), between  $t-30$  to  $t_0$  (paragraph 3.7.2, Materials and methods). Period significantly ( $P < 0.0006$ ) affected the insulin challenge baseline AUC and the values are presented in Table 22 and Figure 22 as the average for all cows ( $n=10$ ), during each period. The whole-blood glucose AUC of the 80% ME + rbST period tended to be different from the control ( $P < 0.0679$ ) and the rbST ( $P < 0.0571$ ) periods.

Table 22. The insulin challenge baseline whole-blood AUC

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 1464.2 $\pm$ 91.6 mg $\times$ min	6.3%
2	rbST	<sup>b</sup> 1324.4 $\pm$ 117.4 mg $\times$ min	8.9%
3	80% ME	<sup>b</sup> 1373.0 $\pm$ 120.9 mg $\times$ min	8.8%
4	80% ME + rbST	<sup>ab</sup> 1397.6 $\pm$ 81.9 mg $\times$ min	5.9%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

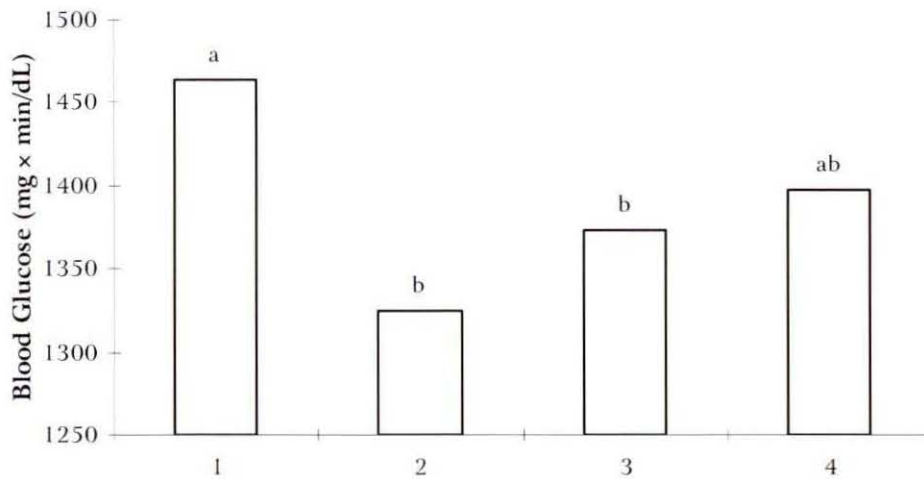


Figure 22. The insulin challenge baseline AUC (whole-blood)

### 2.2.3 The glucose AUC response to insulin injection (t0 - t30)

The response AUC from t0 to t30 was calculated by summation of the areas of the trapezoids under the response curve, corrected for the baseline AUC. The corrected AUC values are presented in Table 23 and Figure 23. Significant differences between periods were observed ( $P < 0.0228$ ) and differences tended to reach significance between the rbST and 80% ME period ( $P < 0.0794$ ) as well as the control and 80% ME + rbST period ( $P < 0.0600$ ).

Table 23. The insulin challenge whole-blood response AUC

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> -235.7 $\pm$ 54.8 mg $\times$ min /dL	23.2%
2	rbST	<sup>b</sup> -172.8 $\pm$ 45.8 mg $\times$ min /dL	26.5%
3	80% ME	<sup>ab</sup> -218.9 $\pm$ 62.4 mg $\times$ min /dL	28.5%
4	80% ME + rbST	<sup>ab</sup> -190.3 $\pm$ 28.1 mg $\times$ min /dL	14.8%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

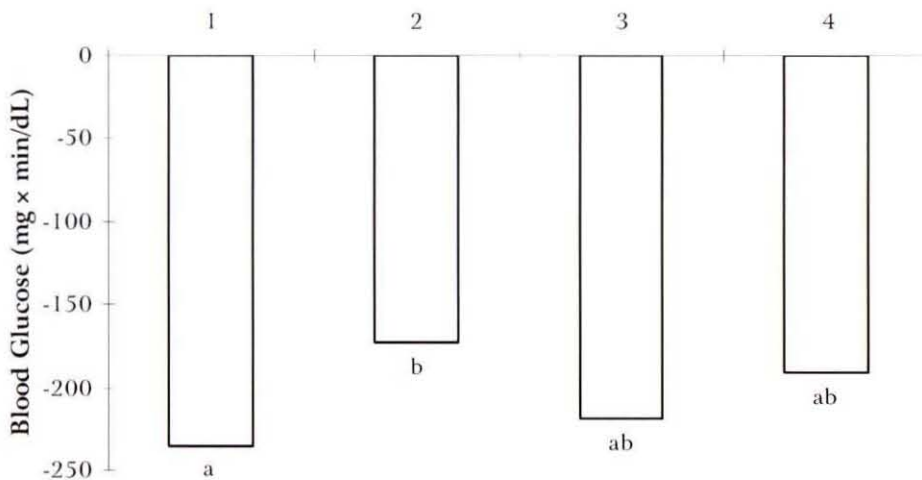


Figure 23. The insulin challenge response AUC (whole-blood)

### 2.2.4 The maximum glucose response

The maximum glucose response was determined by deducting the minimum glucose concentration recorded during each insulin challenge, from the average baseline glucose concentration for each challenge. There were statistically significant differences ( $P < 0.0148$ ) in the glucose response between periods (Table 24 and Figure 24). Additionally, there was a tendency ( $P < 0.0646$ ) for the 80% ME + rbST period to be different from the rbST period.

Table 24. The insulin challenge maximum response

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 18.3 $\pm$ 3.3 mg/dL	18.2%
2	rbST	<sup>b</sup> 14.3 $\pm$ 3.5 mg/dL	24.4%
3	80% ME	<sup>a</sup> 17.9 $\pm$ 4.5 mg/dL	25.4%
4	80% ME + rbST	<sup>ab</sup> 16.8 $\pm$ 2.3 mg/dL	13.5%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

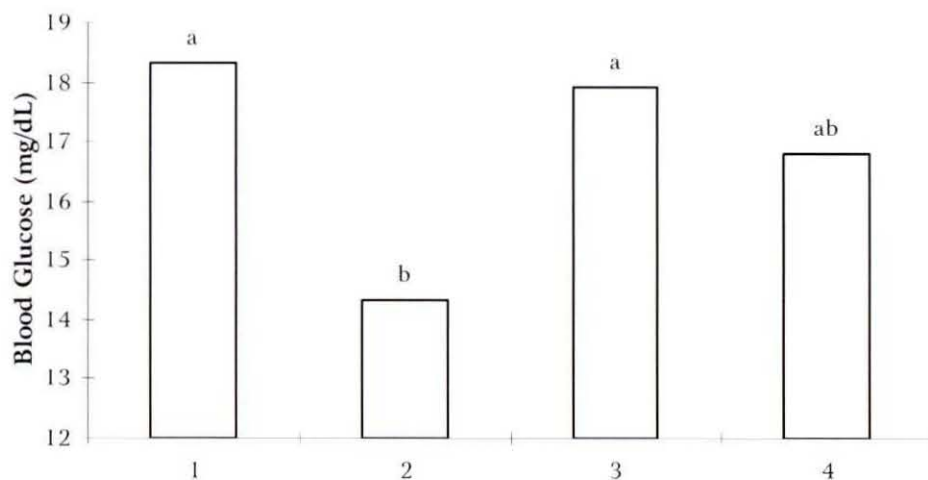


Figure 24. The insulin challenge maximum response

### 2.2.5 Time to reach the maximum glucose response

The time to minimum whole-blood glucose concentration was recorded individually for each insulin challenge and affected by treatments ( $P < 0.0112$ ). These differences are presented in Table 25 and Figure 25 although it should be kept in mind that the difference between periods 1 and 4 reached statistical significance ( $P < 0.05$ ), due to rounding of the probability, which was strictly speaking only a tendency toward significance ( $P < 0.0528$ ). The significant decrease in the timing of the rbST response also lead to the fact that the hyperglycaemic clamp was delayed during this period as the whole-blood glucose concentration had not yet stabilized and not returned to baseline concentration by t210. There was also a tendency ( $P < 0.0584$ ) for the difference between the rbST and 80% ME periods.

Table 25. The time to reach maximum glucose response

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 42.0 $\pm$ 6.7 minutes	16.1%
2	rbST	<sup>b</sup> 48.5 $\pm$ 7.5 minutes	15.4%
3	80% ME	<sup>ab</sup> 42.5 $\pm$ 6.8 minutes	15.9%
4	80% ME + rbST	<sup>b</sup> 46.0 $\pm$ 5.2 minutes	11.2%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

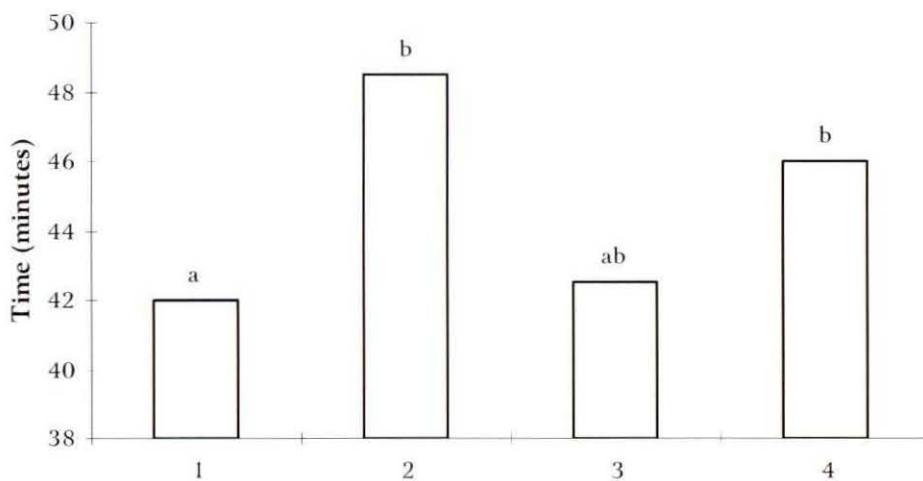


Figure 25. The time to reach maximum response

## 2.3 Data collected during the hyperglycaemic clamp

### 2.3.1 The control period

The glucose concentrations in whole-blood periodically determined from t-30 before glucose injection, up to up to t120 minutes after initiation of glucose infusion are represented as a weighted average (n=10) at each time point (Table 26 and Figure 26).

Table 26. Glucose clamp whole-blood glucose concentration (control)

Time	Mean $\pm$ SD	CV
t -30	46.9 $\pm$ 3.6 mg/dL	7.7%
t -20	47.1 $\pm$ 3.9 mg/dL	8.2%
t -10	47.8 $\pm$ 4.4 mg/dL	9.2%
t 0	48.0 $\pm$ 4.6 mg/dL	9.6%
t 5	60.9 $\pm$ 5.3 mg/dL	8.8%
t 10	70.1 $\pm$ 5.4 mg/dL	7.7%
t 15	77.7 $\pm$ 7.2 mg/dL	9.3%
t 20	84.7 $\pm$ 7.7 mg/dL	9.1%
t 25	88.6 $\pm$ 7.3 mg/dL	8.2%
t 30	92.9 $\pm$ 7.8 mg/dL	8.4%
t 40	95.9 $\pm$ 6.5 mg/dL	6.8%
t 50	96.6 $\pm$ 6.7 mg/dL	7.0%
t 60	97.7 $\pm$ 6.9 mg/dL	7.0%
t 70	97.4 $\pm$ 6.3 mg/dL	6.5%
t 80	97.2 $\pm$ 5.6 mg/dL	5.8%
t 90	97.2 $\pm$ 4.7 mg/dL	4.8%
t 100	96.1 $\pm$ 4.9 mg/dL	5.1%
t 110	95.1 $\pm$ 4.9 mg/dL	5.1%
t 120	94.5 $\pm$ 4.3 mg/dL	4.5%

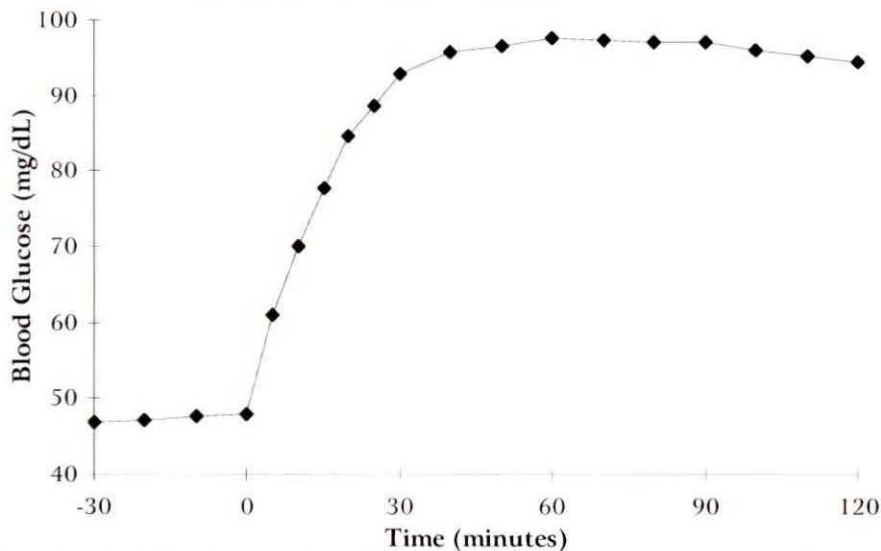


Figure 26. Glucose clamp whole-blood concentration (control)

### 2.3.2 The rbST period

The mean whole-blood glucose concentrations for each time point of the hyperglycaemic clamp, performed in the rbST period are presented in Table 27 and Figure 27.

Table 27. Glucose clamp whole-blood glucose concentration (rbST)

Time	Mean $\pm$ SD	CV
t -30	41.1 $\pm$ 4.3 mg/dL	10.5%
t -20	41.4 $\pm$ 4.5 mg/dL	10.8%
t -10	41.6 $\pm$ 4.8 mg/dL	11.5%
t 0	42.0 $\pm$ 4.9 mg/dL	11.7%
t 5	54.3 $\pm$ 4.6 mg/dL	8.5%
t 10	63.0 $\pm$ 5.8 mg/dL	9.1%
t 15	70.1 $\pm$ 6.7 mg/dL	9.6%
t 20	77.3 $\pm$ 6.6 mg/dL	8.6%
t 25	83.2 $\pm$ 6.2 mg/dL	7.5%
t 30	87.9 $\pm$ 7.1 mg/dL	8.1%
t 40	89.1 $\pm$ 7.8 mg/dL	8.8%
t 50	91.5 $\pm$ 7.3 mg/dL	8.0%
t 60	93.0 $\pm$ 7.7 mg/dL	8.3%
t 70	92.9 $\pm$ 6.4 mg/dL	6.9%
t 80	93.8 $\pm$ 7.0 mg/dL	7.4%
t 90	94.0 $\pm$ 7.3 mg/dL	7.7%
t 100	93.8 $\pm$ 6.1 mg/dL	6.5%
t 110	93.2 $\pm$ 6.7 mg/dL	7.2%
t 120	91.8 $\pm$ 5.5 mg/dL	6.0%

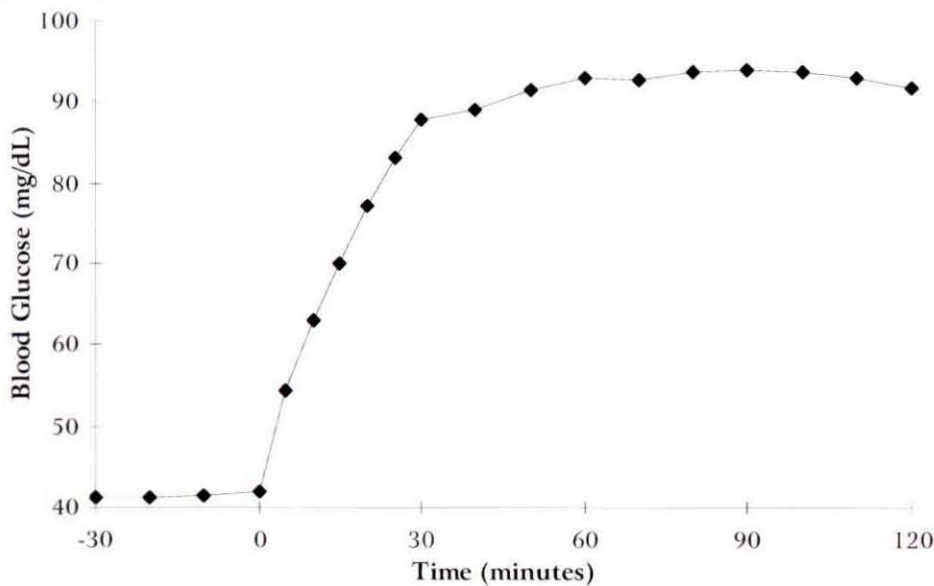


Figure 27. Glucose clamp whole-blood concentration (rbST)

### 2.3.3 The 80% ME period

The collection of whole-blood glucose concentration data followed 1 week after initiation of energy restriction to 80% of the estimated ME requirement. Data is presented in Table 28 and Figure 28.

Table 28. Glucose clamp whole-blood glucose concentration (80% ME)

Time	Mean $\pm$ SD	CV
t -30	45.0 $\pm$ 4.2 mg/dL	9.4%
t -20	45.6 $\pm$ 3.7 mg/dL	8.2%
t -10	45.3 $\pm$ 4.1 mg/dL	9.0%
t 0	46.0 $\pm$ 4.9 mg/dL	10.6%
t 5	59.1 $\pm$ 4.4 mg/dL	7.4%
t 10	67.2 $\pm$ 4.3 mg/dL	6.5%
t 15	75.5 $\pm$ 4.4 mg/dL	5.9%
t 20	81.5 $\pm$ 5.3 mg/dL	6.5%
t 25	89.1 $\pm$ 6.3 mg/dL	7.1%
t 30	93.3 $\pm$ 4.7 mg/dL	5.0%
t 40	94.0 $\pm$ 5.4 mg/dL	5.7%
t 50	95.2 $\pm$ 5.6 mg/dL	5.9%
t 60	96.2 $\pm$ 5.0 mg/dL	5.2%
t 70	95.6 $\pm$ 5.0 mg/dL	5.2%
t 80	95.3 $\pm$ 4.8 mg/dL	5.0%
t 90	96.6 $\pm$ 6.2 mg/dL	6.4%
t 100	95.6 $\pm$ 5.5 mg/dL	5.7%
t 110	94.4 $\pm$ 6.3 mg/dL	6.7%
t 120	92.9 $\pm$ 6.1 mg/dL	6.6%

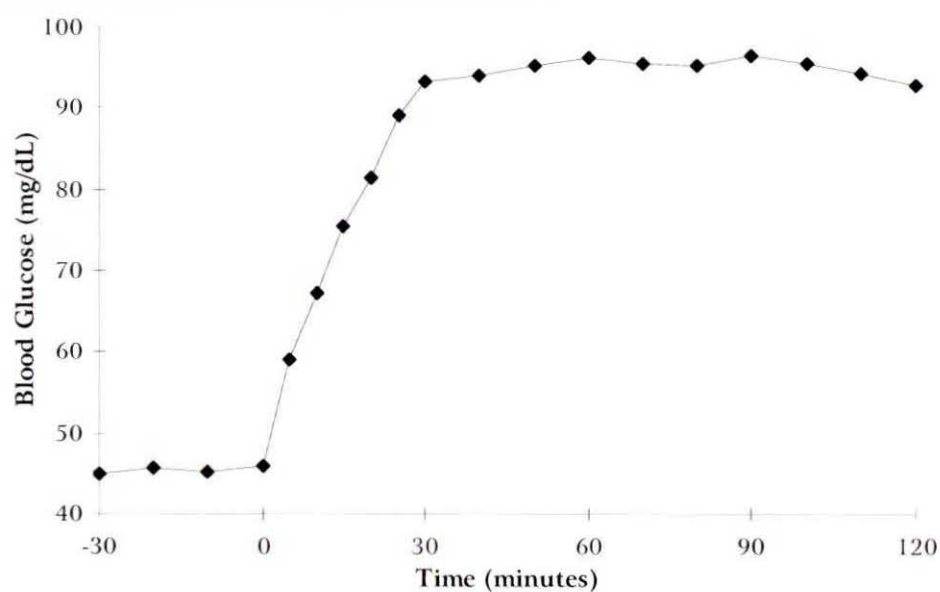


Figure 28. Glucose clamp whole-blood concentration (80% ME)

### 2.3.4 The 80% ME + rbST period

The mean whole-blood glucose concentrations determined from t-30 to t120 of hyperglycaemia in the 80% ME + rbST period are presented in Table 29 and Figure 29.

Table 29. Glucose clamp whole-blood glucose concentration (80% ME + rbST)

Time	Mean $\pm$ SD	CV
t -30	43.9 $\pm$ 2.9 mg/dL	6.7%
t -20	44.2 $\pm$ 2.5 mg/dL	5.6%
t -10	44.7 $\pm$ 3.3 mg/dL	7.3%
t 0	45.3 $\pm$ 3.2 mg/dL	7.0%
t 5	58.0 $\pm$ 3.7 mg/dL	6.4%
t 10	68.7 $\pm$ 3.3 mg/dL	4.8%
t 15	76.1 $\pm$ 4.2 mg/dL	5.5%
t 20	83.5 $\pm$ 4.7 mg/dL	5.6%
t 25	90.0 $\pm$ 4.5 mg/dL	5.0%
t 30	92.7 $\pm$ 4.5 mg/dL	4.9%
t 40	94.5 $\pm$ 4.9 mg/dL	5.2%
t 50	96.2 $\pm$ 6.4 mg/dL	6.7%
t 60	97.1 $\pm$ 5.9 mg/dL	6.1%
t 70	97.6 $\pm$ 4.3 mg/dL	4.4%
t 80	97.8 $\pm$ 4.9 mg/dL	5.0%
t 90	97.9 $\pm$ 5.5 mg/dL	5.6%
t 100	98.0 $\pm$ 5.9 mg/dL	6.0%
t 110	97.0 $\pm$ 5.7 mg/dL	5.8%
t 120	95.7 $\pm$ 4.7 mg/dL	5.0%

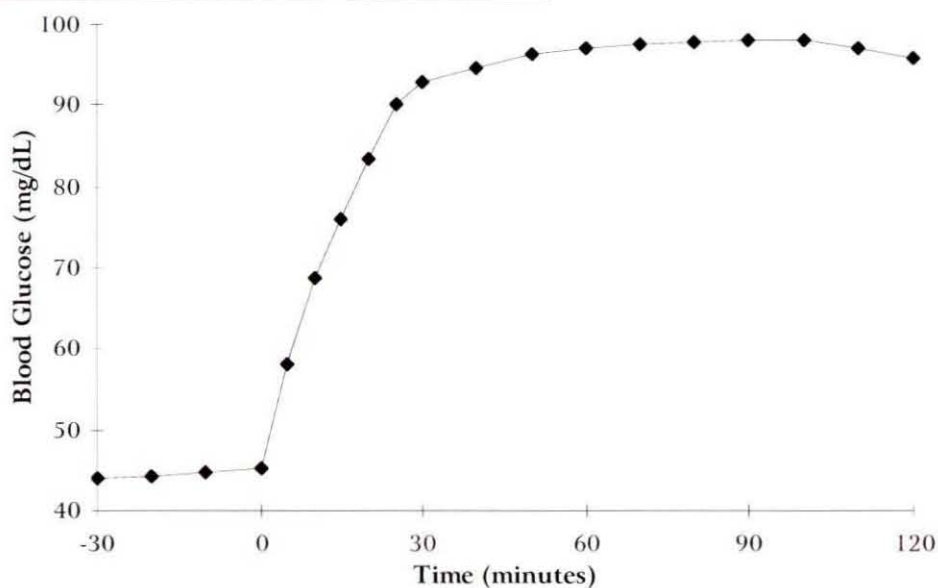


Figure 29. Glucose clamp whole-blood concentration (80% ME + rbST)

## 2.4 Effect of treatments in the hyperglycaemic clamp

### 2.4.1 The baseline glucose concentration

The individual whole-blood glucose concentrations determined at 10-minute intervals from  $t-30$  to  $t0$  were analyzed using repeated measures between periods (Table 30 and Figure 30). There was a small, but significant effect of time of sampling ( $t-30$ ,  $t-20$ ,  $t-10$  and  $t0$ ) on blood glucose concentrations ( $P < 0.0011$ ), which is described in more detail in the Materials and Methods (paragraph 5.3.1). Treatments affected blood glucose concentrations of the baseline preceding the glucose clamp ( $P < 0.0001$ ).

Table 30. The glucose clamp baseline whole-blood concentration

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 47.5 $\pm$ 4.0 mg /dL	8.4%
2	rbST	<sup>b</sup> 41.5 $\pm$ 4.4 mg /dL	10.7%
3	80% ME	<sup>c</sup> 45.5 $\pm$ 4.1 mg /dL	9.0%
4	80% ME + rbST	<sup>d</sup> 44.5 $\pm$ 2.9 mg /dL	6.6%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

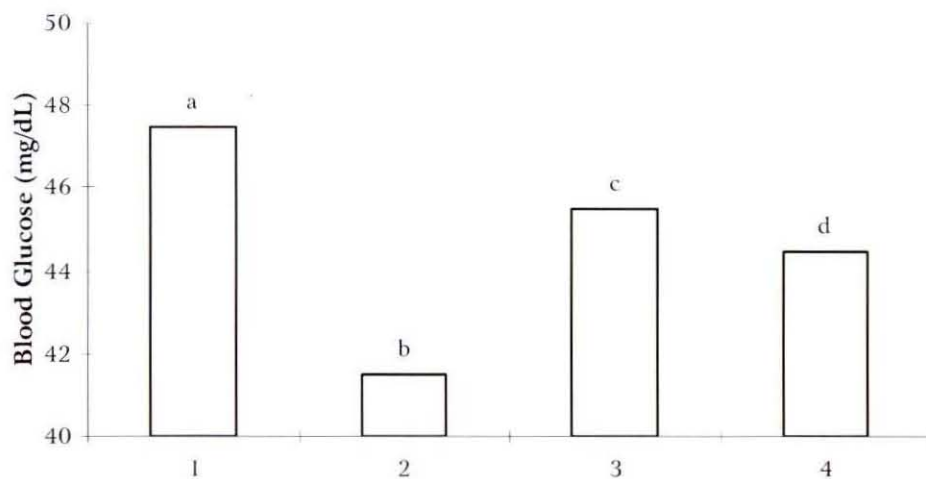


Figure 30. The glucose clamp baseline (whole-blood)

### 2.4.2 The total glucose infusion rate

The total amount of glucose infused during the entire clamp (120 minutes) was unaffected by period ( $P < 0.7114$ ), as it included the rise toward clamped hyperglycaemia, where experimental protocols were similar for all cows (Table 31 and Figure 31).

Table 31. The glucose clamp total GIR

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 2.8 $\pm$ 0.4 mg / kg $\times$ min	15.1%
2	rbST	<sup>a</sup> 2.8 $\pm$ 0.4 mg / kg $\times$ min	15.6%
3	80% ME	<sup>a</sup> 2.9 $\pm$ 0.4 mg / kg $\times$ min	14.7%
4	80% ME + rbST	<sup>a</sup> 2.9 $\pm$ 0.4 mg / kg $\times$ min	13.9%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

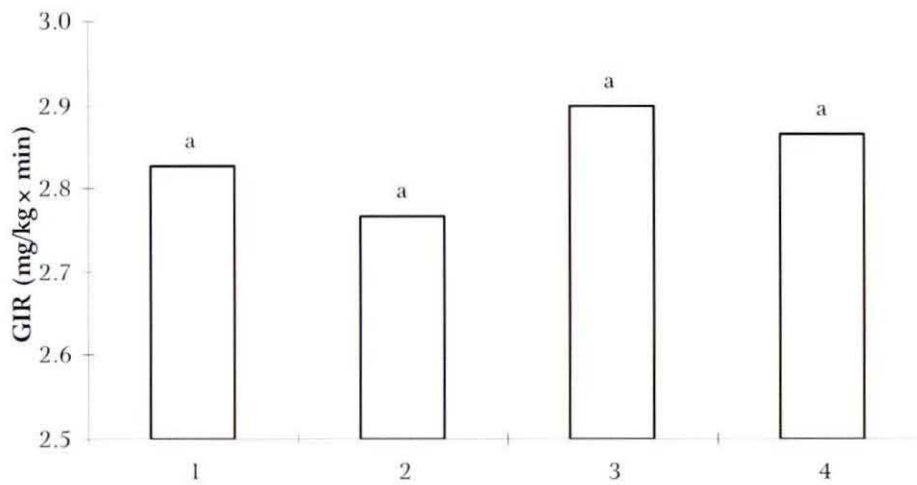


Figure 31. The glucose clamp total GIR

### 2.4.3 The steady-state glucose infusion rate

The amount (grams) of glucose infused during every 10-minute period of the final 40 minutes (t80 to t120) and the fasting body weight was used to calculate the SSGIR. The SSGIR was not affected by the individual time point measurements ( $P < 0.1574$ ), but was affected by period ( $P < 0.0001$ ) as presented in Table 32 and Figure 32. The difference between the control and 80% ME period tended to reach significance ( $P < 0.0774$ ).

Table 32. The glucose clamp SSGIR

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 2.3 $\pm$ 0.5 mg / kg $\times$ min	19.9%
2	rbST	<sup>b</sup> 2.1 $\pm$ 0.4 mg / kg $\times$ min	19.6%
3	80% ME	<sup>a</sup> 2.3 $\pm$ 0.5 mg / kg $\times$ min	21.5%
4	80% ME + rbST	<sup>c</sup> 2.2 $\pm$ 0.5 mg / kg $\times$ min	23.3%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

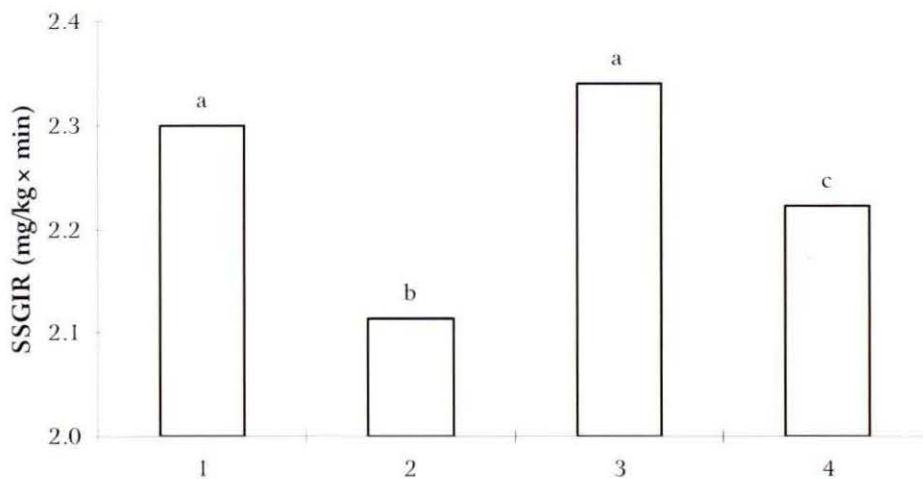


Figure 32. The glucose clamp SSGIR

### 3. Plasma data

#### 3.1 Data collected during the basal period

##### 3.1.1 Individual glucose concentrations

Plasma samples harvested after jugular venipuncture up to week 7 were analyzed for plasma glucose concentration. Plasma was collected fortnightly, on two consecutive days and data presented as the weighted average for all cows ( $n=10$ ) for each sampling day (Table 33 and Figure 33).

Table 33. Individual basal plasma glucose concentrations

Day	Mean $\pm$ SD	CV
7	58.2 $\pm$ 8.3 mg/dL	14.3%
8	57.1 $\pm$ 6.0 mg/dL	10.6%
21	59.7 $\pm$ 5.0 mg/dL	8.4%
22	61.1 $\pm$ 4.9 mg/dL	8.0%
35	62.5 $\pm$ 4.7 mg/dL	7.4%
36	62.9 $\pm$ 2.4 mg/dL	3.9%
49	62.9 $\pm$ 2.9 mg/dL	4.5%
50	59.5 $\pm$ 2.6 mg/dL	4.3%

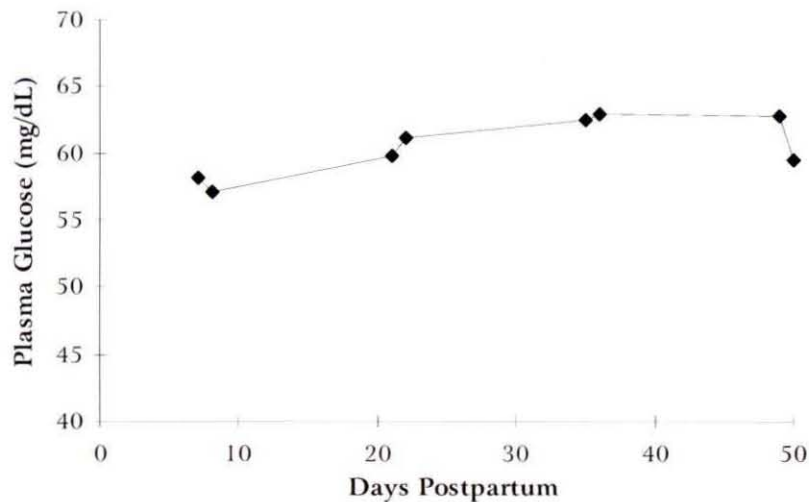


Figure 33. Individual basal plasma glucose concentration

### 3.1.2 Average glucose concentration

The weighted average of each week was calculated from the data presented in Table 33. The weekly average basal plasma glucose concentrations were calculated as a weighted average of the two consecutive samples and are presented in Table 34 and Figure 34.

Table 34. Mean basal plasma glucose concentrations

Week	Mean $\pm$ SD	CV
1	57.6 $\pm$ 6.4 mg/dL	11.1%
3	60.2 $\pm$ 4.8 mg/dL	8.0%
5	62.7 $\pm$ 3.0 mg/dL	4.7%
7	61.2 $\pm$ 2.5 mg/dL	4.0%

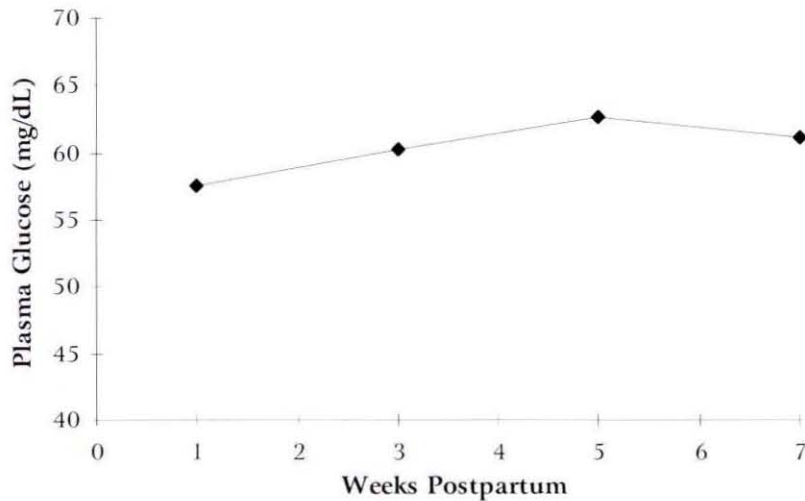


Figure 34. Mean basal plasma glucose concentration

### 3.2 Data collected during the insulin challenge

#### 3.2.1 The control period

The glucose concentration in plasma was periodically determined from t-30 up to t150 minutes, relative to insulin injection. The data for the control period are presented in Table 35 and Figure 35. Values represent a weighted mean for all cows (n=10) for each plasma sample.

Table 35. Insulin challenge plasma glucose concentration (control)

Time	Mean $\pm$ SD	CV
t -30	66.4 $\pm$ 3.7 mg/dL	5.6%
t -20	67.3 $\pm$ 4.9 mg/dL	7.3%
t -10	67.7 $\pm$ 4.3 mg/dL	6.4%
t 0	66.9 $\pm$ 4.3 mg/dL	6.4%
t 30	45.3 $\pm$ 4.6 mg/dL	10.1%
t 60	50.1 $\pm$ 5.6 mg/dL	11.2%
t 90	57.0 $\pm$ 5.6 mg/dL	9.7%
t 120	59.9 $\pm$ 6.1 mg/dL	10.2%
t 150	62.9 $\pm$ 5.7 mg/dL	9.1%

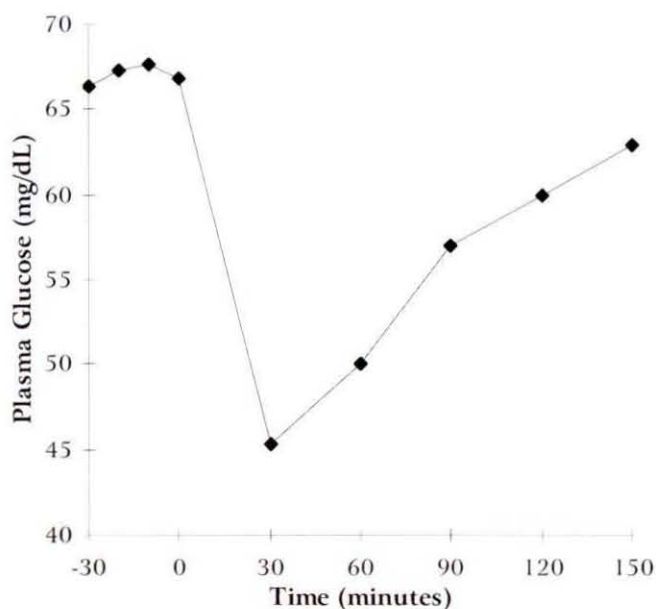


Figure 35. Insulin challenge plasma concentration (control)

### 3.2.2 The rbST period

The plasma glucose concentration data periodically collected after insulin injection, for the rbST treatment period are presented in Table 36 and Figure 36.

Table 36. Insulin challenge plasma glucose concentration (rbST)

Time	Mean $\pm$ SD	CV
t -30	60.0 $\pm$ 5.0 mg/dL	8.3%
t -20	60.3 $\pm$ 4.9 mg/dL	8.2%
t -10	60.3 $\pm$ 5.0 mg/dL	8.3%
t 0	60.8 $\pm$ 5.3 mg/dL	8.8%
t 30	43.8 $\pm$ 4.4 mg/dL	10.0%
t 60	44.8 $\pm$ 6.7 mg/dL	15.0%
t 90	49.6 $\pm$ 6.6 mg/dL	13.2%
t 120	53.8 $\pm$ 6.6 mg/dL	12.2%
t 150	55.9 $\pm$ 6.4 mg/dL	11.5%

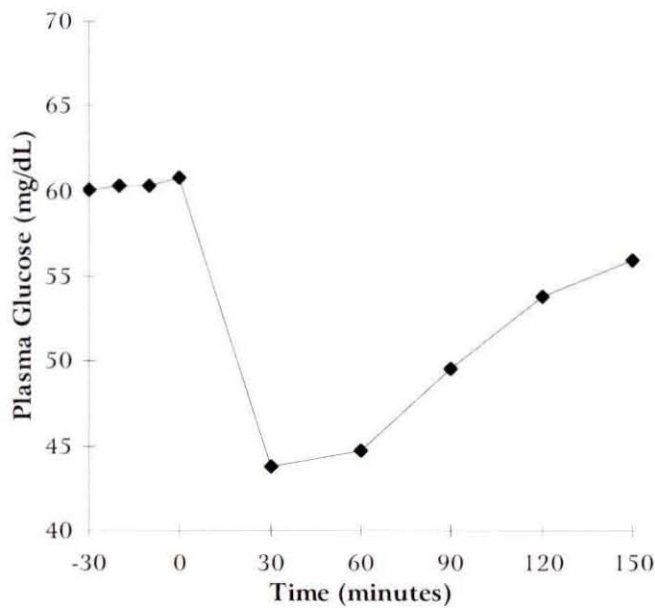


Figure 36. Insulin challenge plasma concentration (rbST)

### 3.2.3 The 80% ME period

Data of the 80% ME period was collected  $\pm 7$  days after energy restriction was commenced (Table 37 and Figure 37) and represents the average for all cows of plasma harvested in the insulin challenge.

Table 37. Insulin challenge plasma glucose concentration (80% ME)

Time	Mean $\pm$ SD	CV
t -30	63.9 $\pm$ 4.8 mg/dL	7.5%
t -20	64.2 $\pm$ 5.4 mg/dL	8.3%
t -10	63.9 $\pm$ 5.2 mg/dL	8.1%
t 0	63.1 $\pm$ 5.5 mg/dL	8.8%
t 30	42.5 $\pm$ 5.2 mg/dL	12.4%
t 60	46.1 $\pm$ 6.0 mg/dL	13.0%
t 90	54.5 $\pm$ 5.9 mg/dL	10.8%
t 120	57.5 $\pm$ 5.6 mg/dL	9.7%
t 150	58.6 $\pm$ 5.0 mg/dL	8.5%

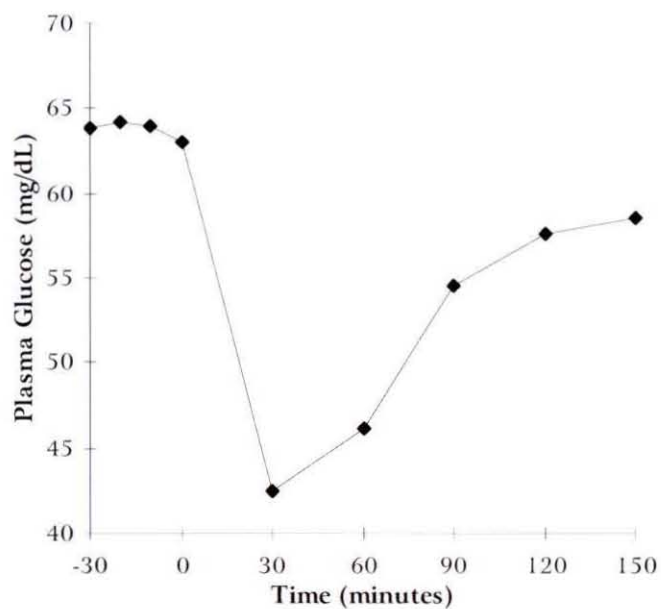


Figure 37. Insulin challenge plasma concentration (80% ME)

### 3.2.4 The 80% ME + rbST period

The glucose concentration in plasma was determined approximately 1 week after recombinant bST administration, during energy restriction (Table 38 and Figure 38).

Table 38. Insulin challenge plasma glucose concentration (80% ME + rbST)

Time	Mea	± SD	CV
t -30	63.7	± 4.1 mg/dL	6.4%
t -20	63.7	± 3.1 mg/dL	4.9%
t -10	65.1	± 4.1 mg/dL	6.4%
t 0	64.6	± 4.5 mg/dL	7.0%
t 30	45.7	± 3.4 mg/dL	7.4%
t 60	46.7	± 6.1 mg/dL	13.1%
t 90	53.8	± 5.9 mg/dL	10.9%
t 120	57.5	± 4.4 mg/dL	7.7%
t 150	59.2	± 3.2 mg/dL	5.5%

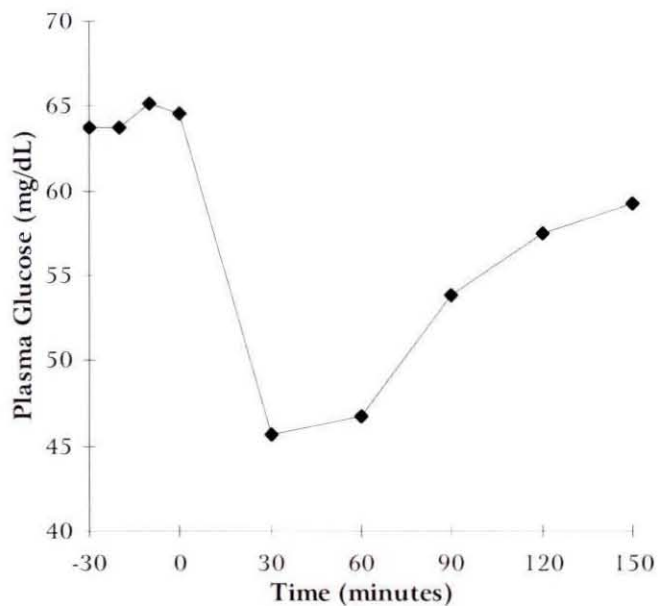


Figure 38. Insulin challenge plasma concentration (80% ME + rbST)

### 3.3 Effect of treatments in the insulin challenge

#### 3.3.1 The baseline glucose concentration

The individual glucose concentrations determined at 10-minute intervals from  $t-30$  to  $t0$  were analyzed using repeated measures between periods. There was no significant effect of time of sampling ( $P < 0.1409$ ) on glucose concentration, while period significantly affected the baseline plasma glucose concentrations ( $P < 0.0001$ ). The small difference between the 80% ME and the 80% ME + rbST period (Table 39 and Figure 39) tended to reach significance ( $P < 0.0755$ ).

Table 39. The insulin challenge baseline plasma concentration

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 67.1 $\pm$ 4.2 mg /dL	6.3%
2	rbST	<sup>b</sup> 60.4 $\pm$ 4.9 mg /dL	8.1%
3	80% ME	<sup>c</sup> 63.8 $\pm$ 5.0 mg /dL	7.9%
4	80% ME + rbST	<sup>c</sup> 64.3 $\pm$ 3.9 mg /dL	6.1%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

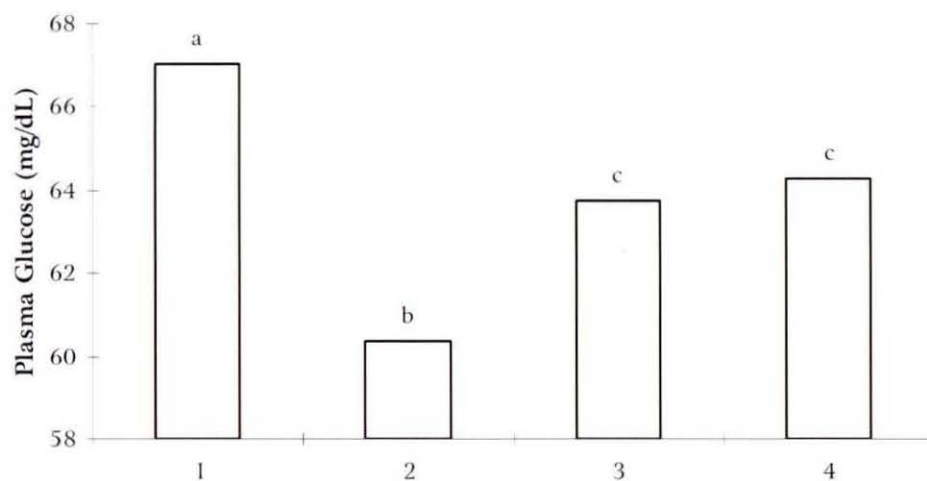


Figure 39. The insulin challenge baseline (plasma)

### 3.3.2 The baseline glucose AUC

The average baseline AUC calculated by summation of the areas of the trapezoids under the insulin challenge baseline (t-30 through t0) was affected by treatment ( $P < 0.0004$ ) and results summarized in Table 40 and Figure 40.

Table 40. The insulin challenge baseline plasma AUC

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 2016.2 $\pm$ 128.8 mg $\times$ min /dL	6.4%
2	rbST	<sup>b</sup> 1810.5 $\pm$ 148.4 mg $\times$ min /dL	8.2%
3	80% ME	<sup>c</sup> 1915.7 $\pm$ 153.0 mg $\times$ min /dL	8.0%
4	80% ME + rbST	<sup>ac</sup> 1929.9 $\pm$ 111.9 mg $\times$ min /dL	5.8%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

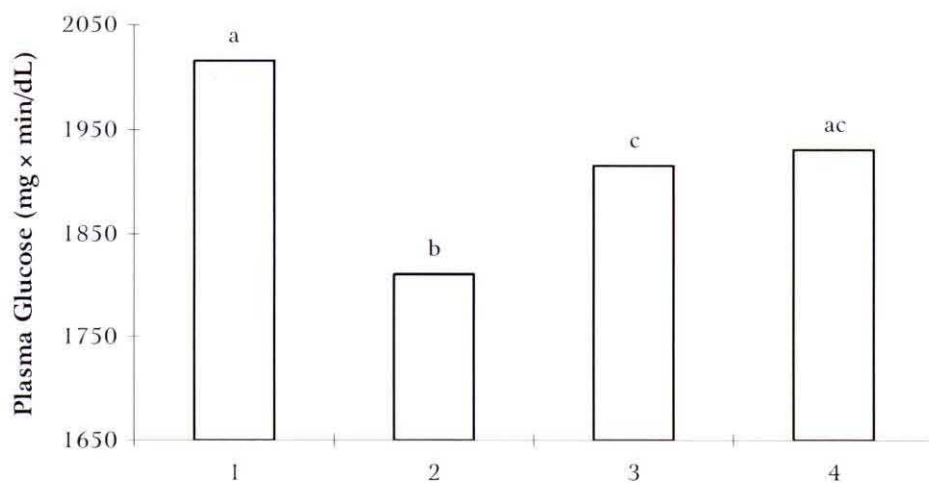


Figure 40. The insulin challenge baseline AUC (plasma)

### 3.3.3 The glucose AUC response to insulin injection (t0 - t30)

The AUC of the plasma glucose response phase was calculated by summation of the areas of the trapezoids between t0 to t30. These values were corrected for the baseline AUC to yield the corrected AUC above the curve. The differences between periods ( $P < 0.0034$ ) are summarized in Table 41 and Figure 41. The difference between period 1 and period 4 reached statistical significance ( $P < 0.05$ ), but it must be noted that this was due to rounding ( $P < 0.0514$ ), while the difference between period 3 and period 4 tended to reach significance ( $P < 0.0734$ ).

Table 41. The insulin challenge plasma response AUC

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> -333.8 $\pm$ 62.8 mg $\times$ min /dL	18.8%
2	rbST	<sup>b</sup> -241.3 $\pm$ 60.3 mg $\times$ min /dL	25.0%
3	80% ME	<sup>ac</sup> -333.1 $\pm$ 84.5 mg $\times$ min /dL	25.4%
4	80% ME + rbST	<sup>bc</sup> -275.2 $\pm$ 37.9 mg $\times$ min /dL	13.8%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

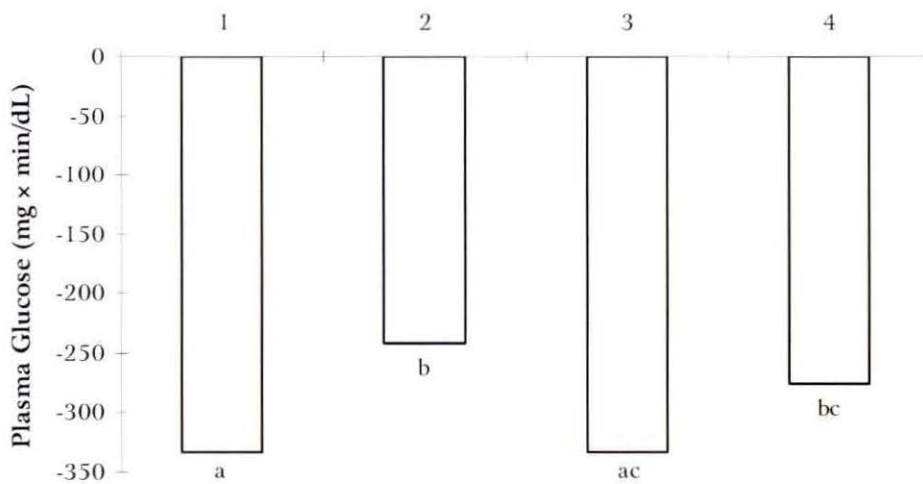


Figure 41. The insulin challenge response AUC (plasma)

### 3.4 Data collected during the hyperglycaemic clamp

#### 3.4.1 The control period

The glucose concentration in plasma was periodically determined from t-30 to t120 minutes after initiation of glucose infusion, in the control period (Table 42 and Figure 42). Values are presented as the weighted average for all cows of each plasma sample  $\pm$  SD and CV.

Table 42. Glucose clamp plasma glucose concentration (control)

Time	Mean $\pm$ SD	CV
t -30	64.6 $\pm$ 6.1 mg/dL	9.4%
t -20	64.5 $\pm$ 6.1 mg/dL	9.4%
t -10	65.4 $\pm$ 7.0 mg/dL	10.7%
t 0	65.7 $\pm$ 6.7 mg/dL	10.2%
t 20	115.4 $\pm$ 11.4 mg/dL	9.9%
t 40	130.3 $\pm$ 9.1 mg/dL	7.0%
t 60	132.7 $\pm$ 10.4 mg/dL	7.9%
t 80	133.8 $\pm$ 8.0 mg/dL	6.0%
t 90	133.9 $\pm$ 5.4 mg/dL	4.0%
t 100	132.0 $\pm$ 5.9 mg/dL	4.5%
t 110	130.3 $\pm$ 5.9 mg/dL	4.5%
t 120	129.5 $\pm$ 5.6 mg/dL	4.3%

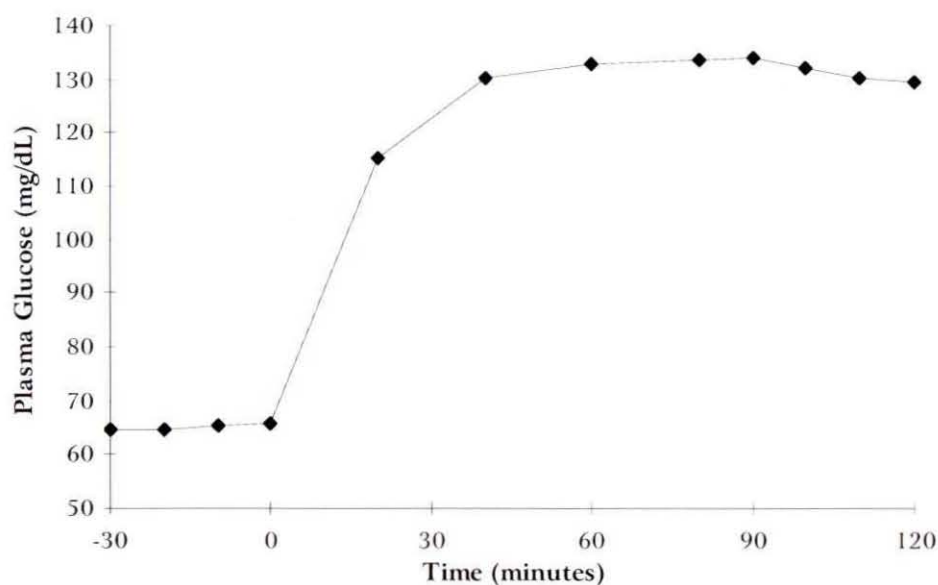


Figure 42. Glucose clamp plasma concentration (control)

### 3.4.2 The rbST period

Plasma glucose data collected for the rbST period is presented as the weighted average for each time point in Table 43 and Figure 43.

Table 43. Glucose clamp plasma glucose concentration (rbST)

Time	Mean $\pm$ SD	CV
t -30	55.4 $\pm$ 5.4 mg/dL	9.8%
t -20	55.8 $\pm$ 7.0 mg/dL	12.6%
t -10	55.3 $\pm$ 6.0 mg/dL	10.8%
t 0	54.9 $\pm$ 6.8 mg/dL	12.5%
t 20	105.1 $\pm$ 9.9 mg/dL	9.4%
t 40	121.1 $\pm$ 9.0 mg/dL	7.4%
t 60	125.3 $\pm$ 9.2 mg/dL	7.3%
t 80	125.3 $\pm$ 8.9 mg/dL	7.1%
t 90	126.6 $\pm$ 9.2 mg/dL	7.3%
t 100	127.2 $\pm$ 6.9 mg/dL	5.4%
t 110	128.0 $\pm$ 7.5 mg/dL	5.8%
t 120	125.4 $\pm$ 6.0 mg/dL	4.8%

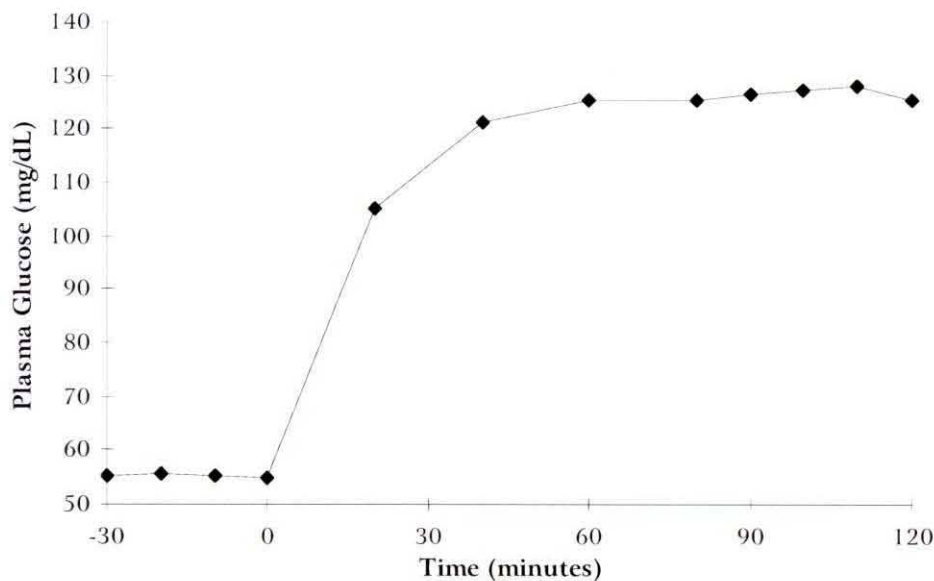


Figure 43. Glucose clamp plasma concentration (rbST)

### 3.4.3 The 80% ME period

The plasma glucose concentration of periodically collected samples of the 80% ME period is presented in Table 44 and Figure 44.

Table 44. Glucose clamp plasma glucose concentration (80% ME)

Time	Mean $\pm$ SD	CV
t -30	61.8 $\pm$ 6.4 mg/dL	10.3%
t -20	62.1 $\pm$ 4.6 mg/dL	7.4%
t -10	61.4 $\pm$ 5.5 mg/dL	8.9%
t 0	62.4 $\pm$ 6.5 mg/dL	10.4%
t 20	110.8 $\pm$ 6.4 mg/dL	5.7%
t 40	128.3 $\pm$ 6.5 mg/dL	5.1%
t 60	130.7 $\pm$ 6.1 mg/dL	4.7%
t 80	129.7 $\pm$ 7.0 mg/dL	5.4%
t 90	132.0 $\pm$ 8.0 mg/dL	6.0%
t 100	129.5 $\pm$ 7.8 mg/dL	6.1%
t 110	127.4 $\pm$ 11.0 mg/dL	8.6%
t 120	126.2 $\pm$ 10.7 mg/dL	8.4%

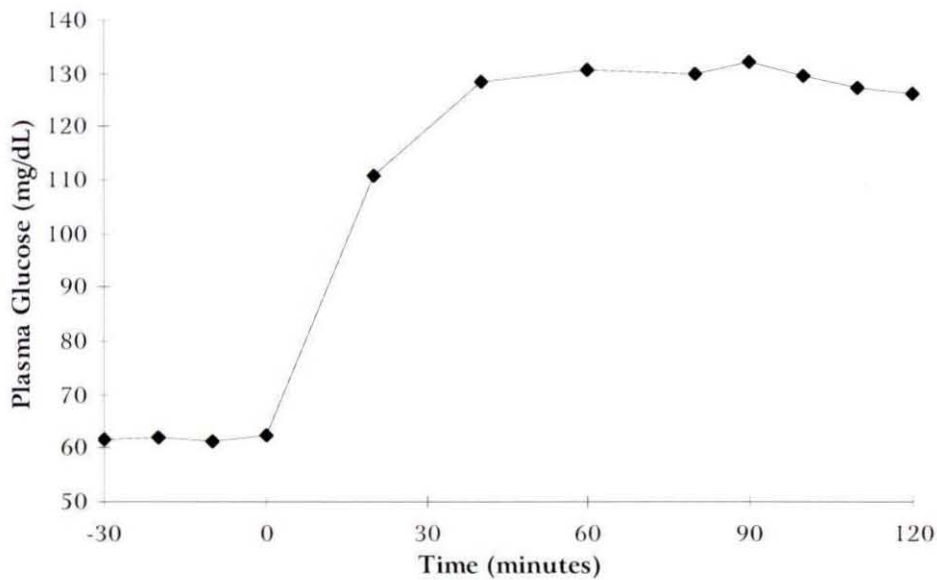


Figure 44. Glucose clamp plasma concentration (80% ME)

### 3.4.4 The 80% ME + rbST period

Approximately 1 week following recombinant bST injection during the energy restriction phase, plasma glucose samples were collected during 2 hours of hyperglycaemia and the mean plasma glucose concentration results are presented in Table 45 and Figure 45.

Table 45. Glucose clamp plasma glucose concentration (80% ME + rbST)

Time	Mean $\pm$ SD	CV
t -30	61.2 $\pm$ 3.3 mg/dL	5.4%
t -20	60.5 $\pm$ 3.5 mg/dL	5.7%
t -10	61.7 $\pm$ 4.7 mg/dL	7.6%
t 0	62.0 $\pm$ 4.6 mg/dL	7.4%
t 20	114.3 $\pm$ 6.2 mg/dL	5.4%
t 40	129.6 $\pm$ 7.3 mg/dL	5.6%
t 60	127.3 $\pm$ 7.7 mg/dL	6.1%
t 80	129.6 $\pm$ 6.8 mg/dL	5.2%
t 90	131.2 $\pm$ 8.4 mg/dL	6.4%
t 100	133.4 $\pm$ 6.7 mg/dL	5.0%
t 110	132.2 $\pm$ 6.9 mg/dL	5.2%
t 120	129.7 $\pm$ 7.2 mg/dL	5.5%

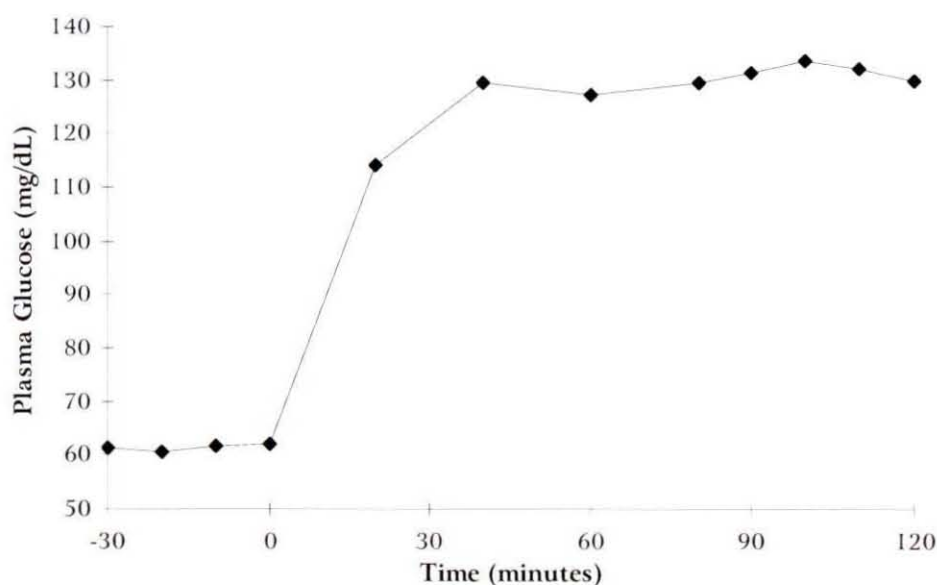


Figure 45. Glucose clamp plasma concentration (80% ME + rbST)

### 3.5 Effect of treatments in the hyperglycaemic clamp

#### 3.5.1 The baseline glucose concentration

The individual plasma glucose concentrations determined at 10-minute intervals from  $t-30$  up to  $t_0$  were analyzed using repeated measures between periods. These values were unaffected by the time of sampling ( $P < 0.5195$ ), but significantly affected by treatment periods ( $P < 0.0001$ ). This data is summarized in Table 46 and Figure 46.

Table 46. The glucose clamp baseline plasma concentration

Period	Description	Mean $\pm$ SD	CV
1	Control	<sup>a</sup> 65.0 $\pm$ 6.2 mg/dL	9.6%
2	rbST	<sup>b</sup> 55.4 $\pm$ 6.1 mg/dL	11.0%
3	80% ME	<sup>c</sup> 61.9 $\pm$ 5.6 mg/dL	9.0%
4	80% ME + rbST	<sup>c</sup> 61.4 $\pm$ 3.9 mg/dL	6.4%

Different superscripts (a,b,c,d) indicate statistically significant differences ( $P < 0.05$ ) between periods.

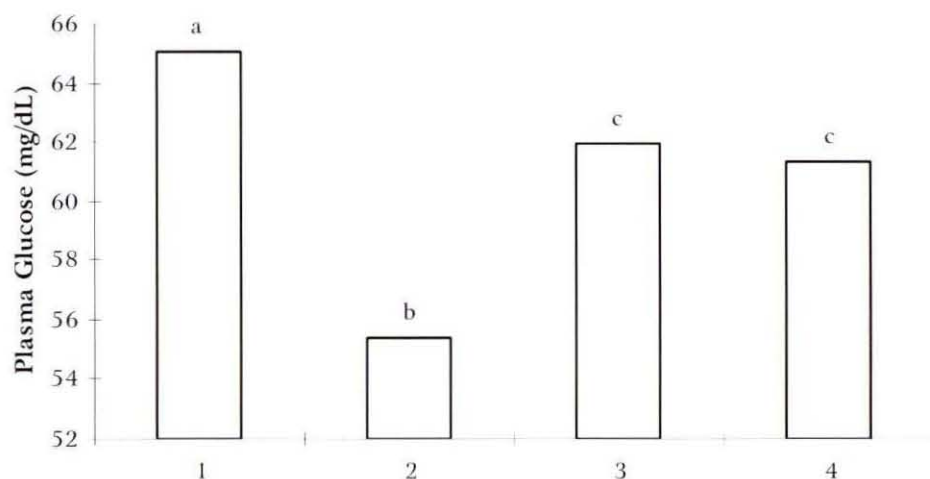


Figure 46. The glucose clamp baseline (plasma)