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Insulin Sensitivity of Heifers on Different Diets

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Sternbauer K, Luthman J: Insulin sensitivity of heifers on different diets. Acta vet. scand. 2002, 43, 107-114. – The hyperinsulinemic euglycemic clamp technique was used to investigate the effect on insulin sensitivity of 2 different diets used in practical cattle feeding in calves. Ten 4 to 5-month-old heifer calves were allocated to 2 feeding groups, LO or HI, to obtain growth rates of 400 g/day or 900 g/day. The heifers were fed and housed individually for 5 weeks. Growth rates close to calculated rates were obtained with the diets used. Weekly blood samples were collected from the jugular vein for analysis of glucose, insulin, cortisol, total serum protein, urea, cholesterol and non-esterified fatty acids. During week 5, insulin sensitivity was estimated using the hyperinsulinemic euglycemic clamp technique. Insulin sensitivity did not differ between the groups, but the plasma glucose levels were higher during weeks 3 and 4 for the HI group compared to the LO group. It may be concluded that the amount of concentrate in the diet was too low to induce changes in either the basal plasma insulin levels or the insulin sensitivity in the HI group.

hyperinsulinemic euglycemic clamp; insulin; glucose; feeding; cattle; ruminants.

Introduction

Ruminants are generally considered to be less sensitive to insulin than non-ruminants and it has been shown that the insulin mediated glucose disposal is significantly lower in hay-fed sheep than is earlier reported in monogastric animals (*Janes et al.* 1985). However, insulin is of major importance for glucose homeostasis and for the partitioning of nutrients to the different tissues also in ruminants. The tissue sensitivity to insulin is of special interest in lactating animals. The glucose uptake by the udder is insulin-independent (*Hove* 1978, *Debras et al.* 1989) and lactating dairy cows show reduced insulin responsiveness to glucose (*Sano et al.* 1993), which means that more glucose becomes available for milk synthesis. Contradictory results were obtained in lactating beef cattle (*Sano et al.* 1991). Both the responsiveness of insulin to glucose and the tissue responsiveness to insulin were enhanced during lactation in beef cows, indicating that nutrients were deposited to peripheral tissues also during lactation. This metabolic difference between beef and dairy cows may partly explain why dairy cows are more prone to loose weight during lactation. *Metcalf & Weekes* (1990) found that lactating ewes fed a restricted diet showed weight loss and decreased tissue sensitivity to insulin. The reduced tissue sensitivity to insulin may be seen as a mechanism for maintaining lactation during a period of energy deficiency.

It is well known that the diet can influence tissue sensitivity to insulin and contribute to the development of insulin resistance in man (*Proietto et al.* 1999). Insulin resistance is defined as a condition where normal concentrations of the hormone produce a less than normal biological response (*Kahn* 1978). The mechanisms behind insulin resistance are not completely understood and seems to be a multifactorial result of a variety of genetic, cellular and environmental causes (*Ferrannini* 1998).

Feeding induced insulin resistance is also described in the bovine species. Veal calves can develop insulin resistance and show hyperglycemia and glucosuria as responses to feeding (*Hostettler-Allen et al.* 1994). A study of the effects of milk replacers with different contents of lactose, total sugar, protein and fat showed that calves fed the highest amount of total sugar showed a similar plasma glucose level at the end of the 15 weeks' feeding period as at the beginning. On the other hand, the insulin level was 3 times higher at the end. The calves thus showed the classical blood chemical picture indicating insulin resistance (*Hugi et al.* 1997). The effect of different diets has also been studied in sheep. Animals fed a concentrate based diet showed significantly higher insulin responsiveness than animals fed a roughage based diet. The energy content of the diets was equal (*Sano et al.* 1992). In a later study, restrictively fed sheep were found to be more sensitive to insulin than *ad libitum* fed animals (*Sano et al.* 1999).

The hyperinsulinemic, euglycemic clamp (HEC) test was originally developed for use in man (*DeFronzo et al.* 1979) and has been used with slight modification also in calves (*Sternbauer et al.* 1998ab). The HEC test has been considered to be the most exact method to study insulin sensitivity (*Hermans et al.* 1999). The endogenous insulin production is inhibited by an exogenous insulin infusion, using the principle of the glucose-insulin feedback mechanism, which makes it possible to quantify the insulin mediated disposal.

The aim of the present investigation was to study the effects of diets containing different amounts of concentrate on insulin sensitivity in young growing heifers. The intention was to use diets that do not deviate from diets used in practical cattle feeding. The HEC test was used for the study of the effect of concentrate feeding on insulin sensitivity.

Materials and methods

Experimental design

Ten female calves of the Swedish Red and White Breed were used. The age of the calves was 4-5 months and all originated from the same dairy herd. The calves were housed in individual pens and were allowed an acclimatization period of 9 days. The calves were fed 1 kg of concentrate/day during this period and had free access to hay.

The calves were allocated into 2 groups according to age and body weight. The average body weight was 100 kg in both groups, range 85-116 kg and 89-118 kg, respectively. The aim was to achieve growth rates of 400 g/day (LO group) and 900g/day (HI group). The amounts of concentrate necessary to obtain these levels were calculated using national feeding tables (*Spörndly* 1995).

The pelleted concentrate had the following composition: 32% barley, 36% oats, 11% soy bean meal, 10% molassed beat pulp, 7% rape seed meal and 2% molasses. The amounts of concentrate fed were 0.5 kg/day/calf in the LO group and 2.2 kg/day/calf in the HI group. The daily amount of concentrate was divided into 2 meals fed at 7 am and 3 pm.

Both groups were fed hay *ad libitum*. Unfortunately 2 batches of hay had to be used. The first batch (A) was used from arrival till week 2 and the second (B) from week 3 till the end of experiment. The amount of hay offered and the amount refused were recorded daily for each calf. Both groups had access to mineral stones with selenium (Saltslick®, Hansson & Møhring, Halmstad, Sweden).

The dry matter, energy and protein content of hay and concentrate are shown in Table 1.

During week 5, a HEC test was performed with one calf from each group per day until all calves had been tested. The first test was performed at 9 a.m. and food was withdrawn at 5 p.m. the day before. For all afternoon HEC tests at 2 PM, the

Table 1. Composition of feeds.

Feed	% DM	CP, g/kg DM	Digestable CP, g/kg DM	Metabolizable Energy, MJ/kg DM
Concentrate	88	184	142	11.5
Hay A	84	104	*64	9.6
Hay B	84	87	*58	9.1

*Calculated values according to *Pálson,* 1973.

feed was withdrawn at 7 a.m. the same day. The order between groups was shifted every other day. The individual body weights prior to the tests are shown in Table 4.

Hyperinsulinemic, euglycemic clamp test

The test was performed as previously described (*Sternbauer et al.* 1998a). The infusion was given through a jugular vein catheter. A priming dose of insulin, 3mU/kg b.w, (Actrapid® 100 IU/ml, Novo Nordisk, Pharma AB Malmö, Sweden) was given during the first min. Thereafter, the dose was lowered each min. A constant infusion (1 mU/kg b.w.) was given from the 10th min.

The mean fasting glucose level, obtained at the initiation of the trial, 4.7 mmol/l, was used as the euglycemic level. The glucose infusion (Glucose 100 mg/ml, Kabi Pharmacia, Uppsala, Sweden) started 5 min after initiation of insulin infusion. Blood was sampled from a catheter in the contra lateral jugular vein every 5th min. for analysis of plasma glucose (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton, CA,USA), and the glucose infusion rate was adjusted to keep the plasma level constant at the pre determined level. The infused volume was recorded every 20th min.

The infusions were given by means of a 3-channel infusion pump (IVAC Medsystem 2860, IVAC Scandinavia AB, Täby, Sweden).

The insulin-mediated glucose disposal (M) expressed as μmol/kg b.w./min, during stable plasma glucose concentrations (60-120 min) was calculated for each calf. The amount of the glucose infused and the body weight of the calf were used for the calculation.

The M/I index, defined as the amount of glucose metabolized per unit of plasma insulin, was used to express insulin sensitivity. The index was calculated by dividing the M-value with the mean insulin concentration during the last hour of the test and multiplying with 100 (*Pollare et al.* 1990).

Sampling for blood chemistry

The effects of the diets on glucose, insulin, cortisol, total serum protein, urea, cholesterol and NEFA (Non-Esterified Fatty Acids) were monitored weekly. Blood was collected by jugular puncture into evacuated glass tubes (Vacutainer® Becton Dickinson). Sampling was performed at 7 a.m. immediately prior to the morning feeding. Serum and plasma samples were stored at –18 °C until analyzed at the end of the study.

Analytical methods

Plasma glucose was analyzed in duplicate immediately after sampling and centrifugation, using the enzymatic glucose oxidase test (Beckman Glucose Analyzer II). A difference between duplicates exceeding 0.2 mmol/l was not accepted. Prior to each test performance, the instrument was controlled using bovine serum of a known concentration.

Insulin and cortisol were measured by radioimmuno assays (Pharmacia RIA 100, Pharmacia

Table 2. Energy consumption, MJ/day, and metabolizable crude protein, g, during four consecutive weeks in 5 heifers on a low energy diet (LO) and 5 heifers on a high energy diet (HI). Mean and SD within bars.

	WEEK							
	1		2		3		4	
	LO	HI	LO	HI	LO	HI	LO	HI
Mean energy consumption	28.3[a] (2.9)	41.3 (4.3)	28.3[a] (2.5)	37.4 (4.8)	30.6[a] (2.7)	37.3 (4.8)	29.2[a] (2.1)	36.8 (2.8)
Mean metabolizable crude protein	231 (21)	422 (31)	231 (17)	394 (35)	195 (14)	360 (24)	187 (11)	357 (15)

[a] Significant difference within a week between LO and HI groups ($p < 0.05$).

Diagnostics, Uppsala, Sweden and Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA, respectively) according to the manufacturers' instructions.

Cholesterol, urea, total serum protein and NEFA, were analysed using the Cobas FARA multichannel analyser (Roche, Basel, Schwitzerland) with reagents from the instrument manufacturer and Wako Chemicals GmbH, Neuss, Germany, respectively.

Statistics

Student's *t* test for unpaired values was used to compare M-values and M/I-indexes between the groups. Blood chemical data, energy intake and average daily weight gain were analyzed using repeated measures analysis of variance (*Littell et al.* 1991). The general linear models, GLM, procedures of SAS package (*SAS Inst. Inc.,* 1989), were used. P levels less than 0.05 were considered significant.

Results

All calves remained healthy and none showed signs of heat during the trial period. The average growth rate was 15% higher than the calculated value in the LO group and 9% lower in the HI group (Spørndly 1995). The daily weight gain was calculated for the first 4 weeks of the trial, as the HEC tests were performed during the 5th week and the animals needed to be fasted before the tests. As shown in Table 2, the intake of energy and protein was significantly higher in the HI group than in the LO group. The percentage intake of roughage of the total feed consumption varied from 83% to 88% in the LO group and from 45% to 63% in the HI group during the trial.

The average total weight gain during the first 4 weeks of the study, was significantly higher in the HI group (-23 ± 3 kg) than the LO group (13 ± 3 kg). The average daily weight gain, ADW, was 820 g and 460 g/day, respectively.

The mean body weights at week 4 in the HI group was 128 kg (range 120-144 kg) and in the LO group 118 kg (range 108-136 kg).

The means of the studied blood parameters are shown in Table 3. There were no significant differences in the mean values before treatment between the groups except for insulin, which was significantly higher in the LO group. However, the means of insulin did not differ at any other time points. Glucose was significantly higher in the HI group at weeks 4 and 5 and urea was significantly higher in the same group at weeks 2 and 3. The NEFA levels were numerically low in both groups, but the HI group was significantly higher at the end of the study.

Table 3. Values for the blood chemical parameters analyzed once a week during 4 consecutive weeks in 5 heifers on a low energy diet (LO) and 5 heifers on a high energy diet (HI). Mean and SD within bars.

	WEEK									
	0		1		2		3		4	
	LO	HI	LO	HI	LO	HI	LO	HI	LO	HI
Insulin, μU/ml	5.6[a] (1.1)	3.8 (1.2)	5.4 (0.2)	4.7 (0.7)	5.2 (0.2)	5.0 (1.8)	3.5 (0.9)	5.6 (1.8)	5.3 (1.1)	4.9 (1.9)
Glucose, mmol/l	4.9 (0.5)	4.9 (0.6)	5.1 (0.3)	5.4 (0.4)	5.2 (0.5)	5.4 (0.4)	4.7[a] (0.1)	5.6 (0.2)	4.8[a] (0.3)	5.5 (0.5)
Protein, g/l	70.4 (3.0)	66.0 (3.5)	71.2 (5.2)	65.8 (1.8)	68.0 (5.5)	62.4 (4.4)	66.8 (4.5)	64.8 (3.6)	68.6 (4.5)	68.4 (0.6)
Urea, mmol/l	2.7 (0.6)	2.9 (0.3)	1.6 (0.4)	2.0 (0.2)	1.5[a] (0.2)	2.0 (0.2)	1.5[a] (0.3)	2.5 (0.6)	2.5 (0.7)	3.1 (0.6)
NEFA, mmol/l	0.20 (0.12)	0.12 (0.04)	0.11 (0.04)	0.09 (0.03)	0.09 (0.08)	0.12 (0.04)	0.13 (0.03)	0.10 (0.03)	0.09[a] (0.01)	0.14 (0.04)
Cholesterol, mmol/l	1.7 (0.3)	2.1 (0.3)	2.1 (0.2)	2.1 (0.3)	1.9 (0.2)	2.2 (0.4)	1.6 (0.3)	1.8 (0.7)	1.5 (0.3)	1.7 (0.4)

[a] Significant difference within a week between LO and HI groups ($p < 0.05$).

The serum cortisol levels were low. Of all samples analyzed (n=50) 94% were less than 12 nmol/l and 70% of these were below the detection limits (<6 nmol/l).

Mean plasma glucose was significantly higher in the HI group than in the LO group at the time of the HEC test, 5.2 (± 0.4) mmol/l vs. 4.6 (± 0.2) mmol/l. Plasma insulin was 4.5 ± 2.6 μU/ml and 3.2 ± 0.5 μU/ml, this difference was not statistically significant.

The average plasma levels of glucose and insulin for each calf during the last hour of the HEC test are shown in Table 4. The average glucose level was calculated from 13 samples in each calf and the insulin levels from 3 samples. The average levels were used for the calculation of the insulin mediated glucose disposal, M and M/I index. Plasma glucose was remarkably high in one calf (No. 858). This calf was not excluded from the calculations.

The mean M-value and the M/I-index did not differ between groups. Both M and M/I-index showed large individual variation in both groups (Table 4).

Discussion

As a reflection of the dietary intake, plasma glucose was significantly higher in the HI group at weeks 3 and 4 of the trial (Table 3). Similar results were obtained by *Abeni et al.* (2000) using heifers of similar age and body weight and with the similar growth rates. Animals showing a gain of 900 g/day had significantly higher glucose levels than animals gaining 700 g/day. Insulin was not analyzed in this study.

The prefeeding insulin level was significantly higher in the LO group than in the HI group at the beginning of the study (Table 3). The available data give no explanation to this difference.

Table 4. Body weight on the day before euglycemic hyperinsulinemic clamp test. Mean p-glucose (13 samples) and p-insulin (3 samples) during the last hour of clamp test, M-value (glucose consumption) and M/I-index (M-value correlated to the average insulin level) in 5 heifers fed low energy and protein (LO) and in 5 heifers fed high energy and protein (HI). Mean and SD within bars.

	LO					HI				
	Calf					Calf				
	857	859	861	863	865	858	860	862	864	866
Body weight, kg	145	128	117	126	137	150	130	141	137	130
P-Glucose 60'- 120',	4.4	4.7	4.7	4.5	4.8	6.0[a]	4.7	4.6	4.5	4.7
mmol/l	(0.2)	(0.1)	(0.3)	(0.2)	(0.2)	(0.2)	(0.2)	(0.3)	(0.2)	(0.2)
P-Insulin 60'-120',	87	64	51	81	70	73	63	57	72	68
μU/ml	(7)	(4)	(3)	(2)	(3)	(4)	(1)	(4)	(1)	(2)
M-value, μmol glucose/kg b.w. and min^{-1}	19	22	14	15	16	25	19	30	14	23
M/I-index	22	34	28	18	23	34	31	53	19	34

[a] Stable glucose level was higher than chosen, but not excluded because glucose consumption is considered to be the same as for lower level.

The diets were identical in both groups prior to the study and the time of sampling was the same. However, the mean basal insulin levels were calculated from a single sampling of each animal and not from series. No further differences in insulin between the groups occurred during the course of the trial.

In contrast to the results of the present study, *Röpke et al.* (1994) reported a significant insulin response to the diets in heifers. Animals of similar body weights as in the present study and with a daily gain of about 1 200 g showed significantly higher insulin levels than animals gaining about 800 g/day. The difference in insulin became evident after about 2 weeks on the diets and the response was more pronounced in heifers than in bulls. Blood glucose was not analyzed in this study.

The role of the composition of the diet was studied by *Achmadi et al.* (1993). Non-pregnant sheep were fed 2 iso-caloric diets with equal nitrogen content, differing only in roughage/concentrate ratio, 100/1 and 30/70, respectively. After 3 weeks on the diets, insulin was significantly higher in the group fed most concentrate. However, insulin was analyzed in arterial blood in this study, while in the present study insulin was analyzed in venous blood.

It seems probable that the highest amount of concentrate used in the present study was insufficient to induce an increase of the basal insulin level.

NEFA was significantly higher in the HI group week 4. The higher levels of NEFA and glucose may be interpreted as indicators of decreased sensitivity insulin. However, the physiological relevance of these small differences between the groups may be questioned. Further, as shown in Table 4, M and M/I-index did not differ between the groups.

The insulin mediated glucose disposal (M) indicates the whole body glucose disposal. The main target tissues for glucose uptake is considered to be skeletal muscle and adipose tissue. The mean M-values and M/I-indexes reported in Table 4 are in agreement with results

obtained in calves of similar age (*Sternbauer et al.* 1998a and b).

Both the secretory rate of insulin and the tissue sensitivity to insulin can be seen as mechanisms necessary for maintaining glucose homeostasis and for the partition of nutrients to the tissues. Diets containing high amounts of glucogenic precursors can therefore be expected to increase the secretory rate of insulin and/or the tissue sensitivity to insulin. The results reported in the literature on the effects of diets on insulin secretion and tissue sensitivity to insulin are not entirely unanimous. The partly contradictory results (e.g. *Metcalf & Weekes* 1990, *Sano et al.* 1999) may be explained by the fact there are obviously a number of factors that influence insulin sensitivity e.g. physiological state of the animals and for how long time the experimental diets have been feed. Further, differences in methodology can not be excluded.

The influence of the diets on some blood chemical parameters is shown in Table 3. The only parameter that changed significantly was urea. The higher urea level in the HI group weeks 2 and 3 was probably a reflection of the higher protein intake in this group (*Preston et al.* 1965, *Prewitt et al.* 1971, *Coggins & Field* 1976).

The diabetogenic effect of glucocorticoids is well known (*Tappy et al.* 1994, *Sternbauer et al.* 1998b) and stressfull handling of the animals could therefore be expected to influence carbohydrate metabolism. However, the cortisol levels were low during the study period and therefore not interfering with the results.

In conclusion, the amount of concentrate used in the present study resulted in growth rates close to the calculated rates, 400 g/day and 900 g/day. Plasma glucose was significantly higher in the HI group, while neither the basal insulin nor insulin sensitivity changed. The amount of concentrate in the HI group was probably too low and the time on the diets too short to induce such changes.

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Sammanfattning

Insulinkänslighet hos kvigkalvar på 2 olika foderstater.

Effekten av två olika foderstater på insulinkänsligheten studerades hos kalv. Tio 4 till 5 månader gamla kvigkalvar fördelades i två olika grupper, LO eller HI med avsikten att uppnå 400g tillväxt per dag eller 900g tillväxt per dag. Kvigkalvarna utfodrades och uppstallades individuellt under fem veckor. Tillväxthastigheter nära de beräknade uppnåddes med de foderstater som användes i försöket. En gång per vecka under försökets första fyra veckor togs blodprov från jugularvenen. Efter avslutat försök analyserades proverna med avseende på glukos, kortisol, protein, urea, kolesterol och fria fettsyror (NEFA). Under vecka 5, bestämdes insulinkänsligheten med hjälp av hyperinsulinemisk euglykemisk clamp teknik.

Det var ingen skillnad i insulin känslighet mellan grupperna. Glukosnivåerna var högre under vecka 3 och 4 i HI gruppen jämfört med LO gruppen. Med undantag av glukos så fanns inga skillnader i insulin, urea, protein, NEFA, eller kortisol. Konklusion: foderstaten med den högsta tillväxten var tydligen inte tillräckligt hög för att åstadkomma förändringar vare sig i insulin nivåer eller insulinkänsligheten.

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