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SUBSTRATE MEDIATED TESTS ON PREDISPOSITIONS TO MILK PRODUCTION AND DISEASES[1]

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Substrat yükleme uygulamalarının süt verimi ve hastalıklara predispozisyon üzerine etkisi

Özet: *Yükleme testlerinin süt verimi ve adaptasyon üzerine etkisi süt sığırlarında incelenmiştir. Yüksek süt verimi üzerine enerji metabolizmasının önemli etkisi vardır. Bu nedenle genotip tarafından belirlenen süt verimi ile endokrin sistem özellikleri arasında yakın bir ilişki vardır.*

Araştırmada uygulanan testler metabolik faaliyetleri zorlamaya yönelmiştir. Araştırma materyali olarak 10 *çift tek yumurta ikizi düve ve onların birinci laktasyon verimleri kullanılmıştır. Bir ay süre ile kontrollu beslenen hayvanlar* 18 *saat aç bırakıldıktan sonra intravenöz olarak propiyonat veya butirat infuzyonu yapılmıştır. Bir saat içinde tespit edilen metabolik reaksiyonlar yönünden ikiz eşler arasında benzerlikler tespit edilmiştir. Ayrıca metabolik reaksiyonlar ile süt verimi ve kompozisyonu arasında da önemli korelasyonlar bulunmuştur.*

Elde edilen sonuçlar, genç hayvanlarda yapılacak provokasyon testlerinden hayvanların ilerideki verim performanslarını tahmin etmenin mümkün olabileceğini göstermiştir.

Summary: *İndividual predispositions on milk performance and adaptability are studied with loading tests in dairy cattle. High milk performance has strong effects on the energy metabolism. Therefore, differences in the ability of milk production which is caused by the genotype seem to be based in a high degree on characteristics of the endocrine system. For the experiment,* 10 *pairs of monozygous twins have been used in the*

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age of heifers and during the onset of the first lactation. Defined consecutive stimuli were set by a controlled feeding for one month, a food deprivation for 18 hours, and infusions of propionate or butyrate. During a period of about one hour the reactions of individual animals could be measured with several metabolic parameters. These metabolic reactions, reveal a strong resemblance within twin pairs and therefore indicate a reasonable heritability. Furthermore, the reactions of heifers correlate significantly to the amount and composition of the milk which is produced later on in the lactation period. From the results follows that the developed substrate provocation tests reveal informations for young animals concerning the heritable performance during later age.

Introduction

By use of hyperphysiological doses loading tests of animals can challenge distinct metabolic reactions. Loading tests were applied to study the dispositions or consequences of high performance in animal breeding. For example, in dairy cattle high milk performance has strong effects on the energy metabolism. During the high yielding period at the onset of lactation, cows reach generally a negative energy balance (1). This situation has to be adjusted by feeding intake, mobilization of body stores and reduction of peripheric tissue requirements. Furthermore, the ability of metabolic reactions depends on the substrate demand of mammary gland. Therefore, gluconeogenesis and partitioning of substrates in the body are of central significance. Both are controlled hormonally so that genotypic differences in the ability of milk production may partyl be based on characteristics of the endocrine system (5).

The loading tests are developed by us in order to estimate the dispositions for different performances in dairy cattle, their physiological base and the involved genes or gene complexes. Moreover, the tests were used to describe the consequences of high production levels and to recognize the ability of performances beyond lactation. A propionate infusion test was developed to get information of gluconeogenetic pathways and a butyrate infusion test was used to measure the metabolism of ketones.

Material and Methods

Ten pairs of monozygous twins of the breed German Friesian were used as heifers between 12 and 18 months of age and as cows at the onset of their first lactations. Data of milk yield and appearance of clinical ketosis were registered. The loading arrangement consists of a controlled initial feeding and a food deprivation for 18 h followed by a i.v. infusion. For the propionate infusion test 0.04 g propionate / kg $^{0.75}$ BW / min for 20 min, and for the butyrate infusion test 0.02 g butyrate / kg $^{0.75}$ BW / nin for 20 min were applied. In some individuals both infusion were repeated after a minimal interval of 7 days. Several blood samples were drawn for measuring the reactions before, during, and after infusions. From the blood, the following parameters of energy metabolism were examined: insulin, glucose, volatile fatty acids, free fatty acids and ketones. Furthermore, GOT, Total Bilirubin and GIDH were determined to test the liver function. Besides, clinical observations, breath and heart frequencies as well as body temperature were recorded. The statistical evaluation of data includes the temporal development of the measured parameters during loading reactions, variances between and within twin pairs as well as correlations between loading reactions of heifers at one side and on the other side their milk performance later on and the incidence of clinical ketosis during early lactation.

Results

Some data of the *propionate infusion* test are summarized in *Figure* 1. After infusion of propionate the heifers reacted with a short termed, but large increase of the infused volatil fatty acid. The increases of blood glucose and insulin concentrations were higher in heifers than in cows. Thereby, for insulin two separate peakes could be distinguished in the heifers *(Figure 1)*. The free fatty acid values declined infusion and returned to maximum values after the loading period. This was valid for cows and heifers but with higher levels for the cows. As example of the clinical parameters, *Figure* 1 gives the respiratory frequencies. They rose for heifers and cows during the infusion period but returned shortly afterwards to the basis values before infusion. Altogether, the clinical parameters showed that the loading was terminated within a period less than 1 h.

Data of twin pairs regarding reactions to the propionate loading tests are given in *Figure* 2. After propionate infusion of heifers the blood glucose concentrations did achieve remarkable differences between twin pairs which correspond to the milk fat content during subsequent lactation. Consequently, in most cases the variation within twin pairs

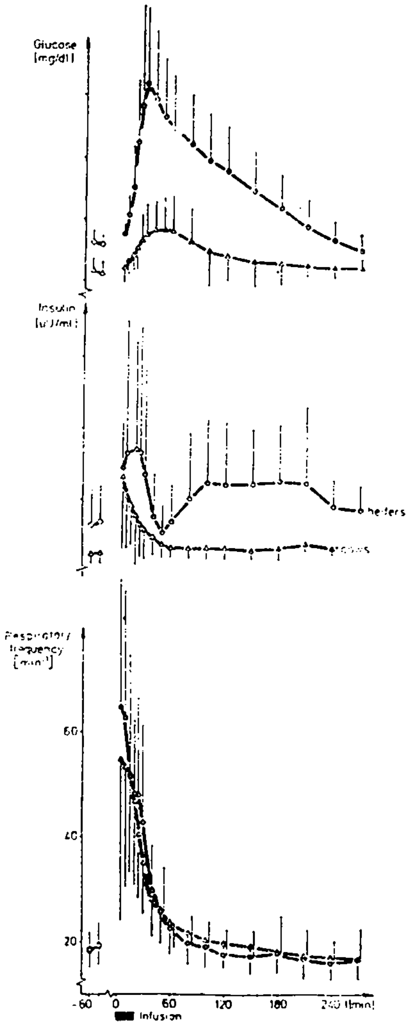

Figure 1 Reactions of glucose, insulin and respiratory frequency during anf after propionate infusion.

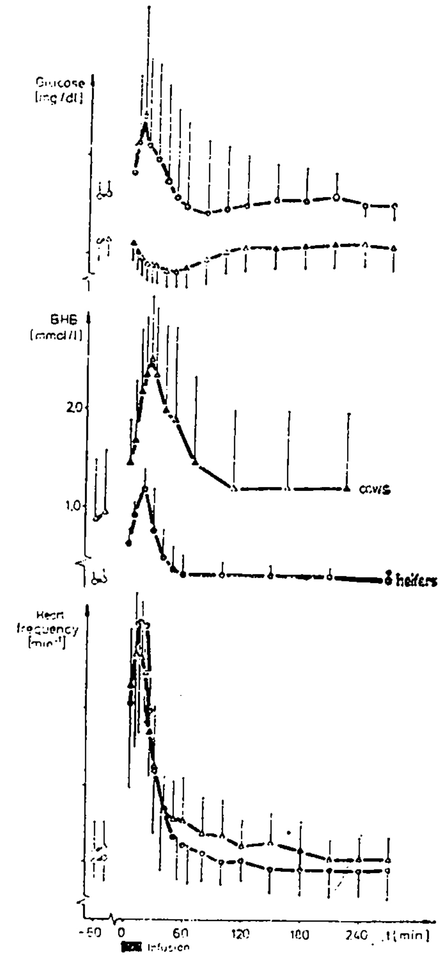

Figure 3 Reactions of glucose, BHB and heart frequency during and after butyrate infusion

was more similar than between them, as shown in *Table* 1 for insulin, glucose and respiratory frequency. *Table* 2 displays the correlations between reactions of heifers after propionate loading and their subsequent milk yields. Thereby, the concentrations of some volatile fatty

Table 1: Variances between and within twin pairs of heifers after propionate infusion.

Criteria of measurement (1)	MQ	df	MQ	df	F-test
Insulin (μU / ml)	1.037.89	8	151.91	9	$p \leq 0.01$
Glucose (mg / dl)	4.714.38	9	483.46	10	$p < 0.001$
Respiratory frequency (min^{-1})	72.10	9	9.49	10	$p \leq 0.01$

1- Arithmetic mean of differences between reaction and basic values.

Table 2: Correlations between loading reactions as heifers after propionate infusion and the later milk performance.

Criteria of mesurement (1)	Milk yield	Fat content	Protein content	Fat yield	Protein yield
Butyric acid (μg / ml)	-0.57*	0.05	0.07	-0.71**	-0.60*
Insulin (μU / ml)	-0.19	0.72**	0.45	0.32	-0.01
Glucose (mg / dl)	0.00	0.49	-0.30	0.37	0.11
Respiratory frequency (min^{-1})	0.38	0.00	0.20	0.49	0.48

1) Arithmetic mean of differences between reaction and basic values.
*$p \leq 0.05$; **$p \leq 0.01$

acids were found negatively correlated with the yield of milk, milk fat and milk protein. Further, it has to be mentioned the positive correlations between the blood concentrations of insulin and glucose as well as the heart frequencies on one side and the subsequent milk fat contents on the other side.

By *butyrate infusion* the concentrations of butyrate as well as insulin did rise in the venous blood. The concentrations of heifers and cows changed at the same manner but on different levels because of their different physiological stages (Figure 3). Regarding the ketone bodies, heifers as well as cows showed a rapid increase followed by a decline (Figure 3). Additionally, in cows the maximum values reached nearly ketotic levels. As an example of the clinical parameters, Figure 3 illustrates similarities between heifers and cows by using the heart frequencies after butyrate loading. All parameters examined recovered their physiological values within less than one hour.

Similarities within twin pairs concerning their reactions on butyrate infusion are given in *Figure* 4 for blood glucose concentrations of two twin pairs in an age of heifers. As seen on the example of two twin pairs, the differences of glucose concentrations as heifers did correspond to their subsequent milk yield. This observation was also valid

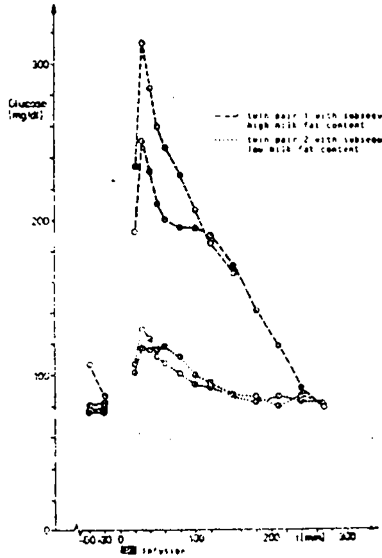

Fig 2 Glucose reactions of two twin oairs as heifers after propionate infusion.

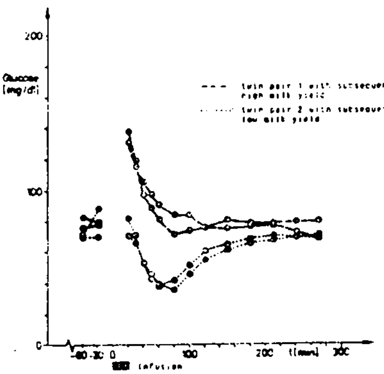

Fig 4 Glucose reactions of two twin pairs as heifers after butyrate infusion.

for the whole material, as seen in *Table* 3. Altogether, the concentrations of insulin, glucose and BHB as well as the heart frequencies varied less within twin pairs of heifers than between them. As documented in *Table* 4 for the butyrate loading test, glucose reactions of heifers correlate negatively with the subsequent yield of milk, milk fat and milk protein. Moreover, significant correlations were found between the blood concentrations of BHB and iso-butyric acid of heifers on one hand and the subsequent contents of milk fat and milk protein on the other hand. Furthermore, in heifers the values of clinical parameters, as body temperature and heart frequency, were positively correlated with milk content during the following first lactation.

Table 3: Variances between and within twin pairs of heifers after butyrate infusion

Criteria of measurement (1)	MQ	df	MQ	df	F-test
Insulin (μU / ml)	9.017.37	9	1.991.63	10	$p \leq 0.05$
Glucose (mg / dl)	1.897.41	9	127.52	10	$p \leq 0.001$
BHB (mmol / l)	701.62	9	55.46	10	$p \leq 0.001$
Heart frequency (min^{-1})	75.45	9	11.67	10	$p \leq 0.01$

1- Arithmetic mean of differences between reaction and basic values.

Table 4: Correlations between loading reactions as heifers after butyrate infusion and the later performance

Criteria of measurement	Parameters	Milk yield	Fat content	Protein content	Fat yield	Protein yield	Ketosis
Iso-Butyric acid (µg / ml)	mean value (1)	-0.19	0.54*	0.61*	0.15	0.03	0.51*
Insulin (mU / ml)	relatively mean value (2)	0.32	0.32	-0.31	0.18	0.24	0.21
Glucose (mg / dl)	relatively mean value (2)	0.07	0.01	0.18	0.08	0.00	-0.46
	T / 2	-0.73**	0.27	0.35	-0.76**	-0.69**	-0.47
BHB (mmol / l)	relatively mean value (2)	0.59*	-0.55*	-0.46	0.36	0.47	0.22
Heart frequency (min^{-1})	relatively mean value (2)	-0.03	0.16	0.63*	0.07	0.23	0.12

1- Arithmetic mean of reaction values.

2- Arithmetic mean of differences between reaction and basic values.

*p ≤ 0.05; **p ≤ 0.001

Discussion

The results of the loading tests are in accordance with measurements done under physiological aspects (2, 6, 7, 8). The physiological interpretation of the results is described by H. Fuhrmann et al (4). Whereas this discussion should answer to the following questions:

— What is characterized by the loading tests?

— Are there limitations of the data?

— What should be the further methodical developments?

— What types of applications are possible in cattle breeding?

The principal characteristic of the developed loading tests can be described as a defined provocation of the metabolism of cattle. For it, a temporal sequence of initial standardized feeding, food depriviation and infusion of substrates was used. The obtained metabolic reactions were repeatable, variable between individuals, heritable and causally correlated to the disposition of the performance traits regarded. The loading tests could be handled at an early age, were simple, economical and timesaving.

However, at the present stage, the interpretation of the results have to be limited. First of all, only 20 animals could be examined. Then, the animals were kept under the specific environment of one farm and were tested solely as heifers of a certain age. Furthermore, the results are only indirectly associated with the criteria relevant for milk performance. Thus, special methodical investigations and criteria have to be added in order to link the loading reactions directly with quantitative parameters of metabolism and its regulation. Moreover, data obtained from animals of different ages, both sexes and different breeds should be studied. Finally, for genetical application, appropriate family material (e.g. half sibs) and relevant informations (e.g. estimations of breeding values) have to be considered.

But already at this stage, the results of the loading tests can be interpreted as informative for an extended evaluation of milk performance. Animal production requires not only data recording and selection for performance traits, but also informations about the underlying heritable physiological dispositions (3). Moreover, the described loading tests can be applied in practical cattle breeding, to test individual bulls in order to choose sires for the A.I.. Additionally, the loading test can be implemented in the progeny tests of bull-sires. That may be done by testing random samples of daughters. Finally, with the help of loading tests bull mothers can be selected. The need of an extended data recording occur with the modern techniques of multiple ovulation and embryo transfer.

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