Relationship between certain parametres included in the glucose tolerance test in young heifers and their milk production traits in forthcoming lactation I

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The aim of the study was to establish the relationship between parametres included in the glucose tolerance test (GTT) of heifers aged 9-10 months and their subsequent milk yield and composition in lactation I. Used were 42 Holstein-Friesian heifers selected from the herd with a mean individual yield of 9600 kg milk/year with 4.32% fat and 3.44% protein. Over 24 hrs preceding the GTT the animals were offered only water and small amount of hay. After that time a "0" blood sample was withdrawn from each heifer and animals were infused intravenously with 40% water solution of glucose (the volume applied was equivalent to 1g glucose/kg $W^{0.75}$). Then nine blood samples were withdrawn at 6 min intervals, and glucose and insulin contents of serum were determined.
After calving, the heifers were maintained under uniform conditions and fed according to TMR system (INRA feeding standards) till the end of lactation I. Simple correlation coefficients were calculated between the area under the glucose and under insulin concentration curves (AGLUCC and AINSCC), ratio AGLUCC/AINSCC (GLIN combined index), maximum glucose concentration (GLUMAX) and maximum insulin concentration (INSMAX). Moreover, coefficients of regression were calculated of the GTT individual indicators on milk production traits and respective regression equations were derived. The GLIN index appeared to be the most suitable GTT parametre to predict future milk traits in dairy heifers. It can be anticipated that GTT may be employed in practical breeding work as an additional criterion of preliminary selection of heifers assigned for dairy herd replacement.

KEY WORDS: dairy cows / glucose tolerance test / heifers / insulin / milk / production traits

From the economical point of view the breeders are interested in predicting the production level of young dairy heifers before their first insemination. For this reason, the glucose tolerance test (GTT) was suggested by Staufenbiel *et al.* [2000]. Basing on GTT it was found that the concentration of insulin might play role as a metabolic marker suitable to predict the future milk yield and composition [Panicke *et al.* 2000, 2002, Xing *et. al.* 1993]. Moreover, a relation was identified between parametres of GTT in young bulls and milk yield of their daughters [Reinicke 1993, Panicke *et al.* 2000, Behn *et al.* 2003, Panicke and Staufenbiel 2003].

Information on predicting dairy traits in young heifers based on GTT is limited [Staufenbiel *et al.* 2000, Sasaki *et al.* 2003]

Facing the above the present study aimed at establishing the relationship between the elements included in GTT in young heifers and their future milk yield and composition in lactation I, and to suggest the GTT parametre(s) most suitable for breeders for early selection of replacement heifers prior to their insemination, as an additive selection criterion.

Material and methods

The study was conducted on 42 Holstein-Friesian heifers, maintained in a herd with a mean yield of 9600 kg milk/cow/year, containing 4.32% fat and 3.44% protein. The randomly selected heifers were kept in a loose barn and fed TMR diets composed of maize silage, grass silage and concentrate mixture with premix containing minerals and vitamins, The INRA feeding standards [Jarrige 1988] were followed to obtain a daily live weight gain of 750-800 g/heifer.

The glucose tolerance test (GTT) was conducted on heifers at the age of 9-10 months (mean body weight 309±42 kg) according to Reinicke [1993] and Staufenbiel *et al.* [2000]. Over 24 h before starting the test the animals were offered only water and small amount of meadow hay. After that time the 16G VASOFIX 1.7x50 cannulas were fixed into jugular veins and first samples of blood (samples "0") were withdrawn. Then, each heifer was given a single intravenous infusion (lasting about 3 min) with 1g glucose/kg $W^{0.75}$ as a 40% water solution (Glukose 40 Braun, B. Braun Melsungen AG, Melsungen, Germany). Next, nine blood samples were withdrawn at 6 min intervals to determine the glucose and insulin content of serum.

 The heifers were inseminated at the age of about 15 months. During pregnancy and lactation the complete TMR diet of corn silage, wilted grass silage and concentrates supplemented with mineral and vitamin mixture were applied according to INRA feeding standards [Jarrige 1988]. Water was available *ad libitum*. The heifers were milked twice a day, milk volume was individually measured and milk samples were taken from each milking of each cow once a month following the routine milk recording procedure. Fat, protein and lactose contents of milk were determined using MilkoScan 104/A/B.

Yield of energy-corrected milk (ECM) was calculated according to formula:

$$\text{ECM (kg/day)} = \text{milk (kg/day)} \times [38.3 \times \text{fat (g/kg)} + 24.2 \times \text{protein (g/kg)} + 16.54 \times \text{lactose (g/kg)} + 20.7]/3140 \text{ [Sjaunja } et\ al.\ 1990].}$$

The milk yield and composition during both the whole lactation and 305-days period were recorded. The mean milk yield for 305-days lactation I was 7718 kg (SD = 1563) containing 3.87% of fat and 3.46% of total protein (SD = 0.44 and 0.21, respectively).

Moreover, estimated were**:**
  − **the area under glucose concentration curve (AGLUCC);**
  − **the area under insulin concentration curve (AINSCC);**
  − **the ratio AGLUCC/AINSCC (GLIN combined index);**
  − **maximum concentration of glucose (GLUMAX);**
  − **maximum concentration of insulin (INSMAX).**

The concentration of insulin was determined with radioimmunoassay method using Insulin RIA Kits (Linco Research Inc., USA, nr cat. WP1 12K) while the concentration of glucose with the Vitros apparatus (GLU DT slides, Johnson & Johnson Clinical Diagnosis)

The Pearson's correlations were calculated between the GTT indicators and milk production traits. The AGLUCC and AINSCC were calculated by multiplying the time that passed between sampling (6 min) by glucose or insulin concentrations. Moreover, regression coefficients of GTT indicators on milk production traits were computed and respective regression equations were derived. In both cases the CORR procedure of the SAS package [SAS, 1999/2000] was used.

Results and discussion

Out of the GTT parametres investigated, only three (AGLUCC, AINSCC, and GLIN) correlated significantly with almost all milk traits considered (Tab. 1). Correlations of GMAX and IMAX with milk traits were not found significant, and as such are ignored in further discussion.

All correlation coefficients estimated between the AGLUCC and milk traits (except the protein content of milk for the whole lactation) were negative. It is concluded, therefore, that prediction would be possible of milk yield (actual and energy-corrected) and protein and fat yield during 305-days.

The correlation coefficients of AINSCC with individual milk traits were all positive, but of different strength. Significant correlation was identified between AINSCC and ECM for whole lactation ($r = 0.37$) and between the AINSCC and protein content during the whole lactation ($r = 0.46$). These insulin-based parametres have turned more useful for prediction of milk traits than those based on glucose. However, the most suitable for predicting the milk productive traits during lactation

Table 1. Simple correlation coefficients between the three selected GTT indicators and milk production traits in heifers

Milk production trait	Area under the curve		Combined GLIN index (ratio AGLUCC/AINSCC
	AGLUCC	AINSCC	
Milk field, 305-days (kg)	-0.34*	0.16	-0.29
Milk yield, whole lactation (kg)	-0.30	0.28	-0.38**
Energy-corrected milk (ECM) yield, 305-days (kg)	-0.38*	0.29	-0.39**
Energy-corrected milk (ECM) yield, whole lactation (kg)	-0.29	0.37**	-0.44**
Milk fat yield, 305-days (kg)	-0.35*	0.34**	-0.44**
Milk fat yield, whole lactation (kg)	-0.27	0.40**	-0.47**
Milk protein yield, 305-days (kg)	-0.32*	0.26	-0.39**
Milk protein yield, whole lactation (kg)	-0.25	0.35*	-0.46**
Milk fat + protein yield, 305-days (kg)	-0.35*	0.31	-0.43**
Milk fat + protein yield, whole lactation (kg)	-0.27	0.38**	-0.46**
Milk protein content, whole lactation (%)	0.15	0.46**	-0.33*

*$P \leq 0.05$;   **$P \leq 0.01$.

I seems to be the GLIN combination index. With except of milk yield during 305 days, the correlation coefficients between GLIN index and remaining milk traits were negative and significant (r = from -0.33 to -0.47).

Strict relationship exists between insulin content and glucose content of blood. Glucose is required for the synthesis of lactose, the ATP-genesis in the provision of NADPH, ribose synthesis in the pentosephosphate pathway and also for the genesis of glycerol in the synthesis of fat. Glucose is also essential for the normal functioning of the nerve system [Gabel and Voigt 2000]. The glucose supply of the udder from endogenous and exogenous sources may be regarded as set points in the system of regulation the secretion of milk.

In literature available there is only a limited information about the use of GTT in comparable study. Staufenbiel *et al*. [1999] evaluated the relationship between milk traits and selected indicators of GTT estimated on milking cows. Correlations of milk yield and milk components with AGLUCC, AINSCC and GLIN index reported by the authors cited are surprisingly similar to results obtained in the present study. It seems to be expedient to use the GTT on heifers at young age as the supplementary criterion in the selection of replacement animals.

The results of the recent investigations conducted on 620 bulls at the age of 340-450 days by Freyer *et al*. [2006] indicated that heritability coefficients for GTT indicators appeared promising for use of these animals in breeding.

 For three GTT indicators – AGLUCC**,** AINSCC and GLIN index – which were shown to be significantly correlated with milk traits (Tab. 1) the regression equations were derived for predicting the future production level of heifers (Tab. 2, 3, 4).

Table 2. Equations of linear regression of the area under curve of insulin concentration "x" on milk traits: $Y = ax + b$

Milk production trait	Regression equation ($Y = ax + b$)	Significance level	
		a	b
Milk field, 305-days (kg)	$Y=0.03375x + 7227$	ns	***
Milk yield, whole lactation (kg)	$Y=0.10096x + 7642$	ns	***
Energy-corrected milk (ECM) yield, 305-days (kg)	$Y=0.04775x +6857$	ns	***
Energy-corrected milk (ECM) yield, whole lactation (kg)	$Y=0.12187x +7246$	*	***
Milk fat yield, 305-days (kg)	$Y=0.00213x + 262$	*	***
Milk fat yield, whole lactation (kg)	$Y=0.00538x + 279$	**	***
Milk protein yield, 305-days (kg)	$Y=0.00166x + 239$	ns	***
Milk protein yield, whole lactation (kg)	$Y=0.00434x + 259$	*	***
Milk fat + protein yield, 305-days (kg)	$Y=0.00380x + 501$	ns	***
Milk fat + protein yield, whole lactation (kg)	$Y=0.00989x + 532$	**	***
Milk protein content, whole lactation (%)	$Y=0.00001325x + 3.33$	**	***

*P≤0.05;   **P≤0.01;   ***P≤0.001,   ns − not significant.

Table 3. Equations of linear regression of the area under curve the concentration of glucose "x" on milk traits: $Y = ax + b$

Milk production trait	Regression equation ($Y = ax + b$)	Significance level	
		a	b
Milk field, 305-days (kg)	$Y = -0.50755x + 9866$	*	***
Milk yield, whole lactation (kg)	$Y = -0.77784x + 12403$	ns	***
Energy-corrected milk (ECM) yield, 305-days (kg)	$Y = -0.45806x + 9490$	**	***
Energy-corrected milk (ECM) yield, whole lactation (kg)	$Y = -0.72180x + 12168$	ns	***
Milk fat yield, 305-days (kg)	$Y = -0.01624x + 361$	*	***
Milk fat yield, whole lactation (kg)	$Y = -0.02610x + 467$	ns	***
Milk protein yield, 305-days (kg)	$Y = -0.01508x + 327$	*	***
Milk protein yield, whole lactation (kg)	$Y = -0.02276x + 419$	ns	***
Milk fat + protein yield, 305-days (kg)	$Y = -0.03132x + 689$	*	***
Milk fat + protein yield, whole lactation (kg)	$Y = -0.05015x + 888$	ns	***
Milk protein content, whole lactation (%)	$Y = .00003078x + 3.40$	ns	***

*P≤0.05;   ***P≤0.001,   ns − not significant.

Only the coefficients for linear regression appeared significant. On the basis of Tables 2, 3 and 4 the authors anticipate that it would be probably possible to predict the fat yield and fat+protein yield of milk during the whole lactation on the basis of

AINSCC established in young heifers (Tab. 2). The AGLUCC seems to be less useful for prediction of milk traits (Tab. 3). The only possible trait to be estimated on the basis of AGLUCC was the 305-days ECM; prediction of the 305-days yield of milk and remaining milk traits appeared less accurate.

Equations of regression derived in this study and based upon the combined GLIN index (Tab. 4) could be useful in prediction of the level of all investigated milk traits with high accuracy. However, further studies are needed on more numerous group of animals to improve the accuracy of estimating the of regression coefficients.

Table 4. Equations of linear regression the ratio of area under curve the concentration of glucose "x" to the area under the curve concentration of insulin on milk traits: $Y = ax + b$

Milk production trait	Regression equation ($Y = ax + b$)	Significance level	
		a	b
Milk field, 305-days (kg)	$Y = -878.9x + 8348$	ns	***
Milk yield, whole lactation (kg)	$Y = -2009.4x + 10552$	**	***
Energy-corrected milk (ECM) yield, 305-days (kg)	$Y = -943.9x + 8229$	**	***
Energy-corrected milk (ECM) yield, whole lactation (kg)	$Y = -2215.6x + 10702$	**	***
Milk fat yield, 305-days (kg)	$Y = -40.9x + 322$	**	***
Milk fat yield, whole lactation (kg)	$Y = -93.0x + 424$	**	***
Milk protein yield, 305-days (kg)	$Y = -37.3x + 290$	**	***
Milk protein yield, whole lactation (kg)	$Y = -83.7x + 383$	**	***
Milk fat + protein yield, 305-days (kg)	$Y = -78.2x + 612$	**	***
Milk fat + protein yield, whole lactation (kg)	$Y = -175.6x + 802$	**	***
Milk protein content, whole lactation (%)	$Y = -0.13983x + 3.63$	*	***

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$, ns − not significant.

Obtaining fast genetic progress in dairy cattle is difficult as the figures characterizing their milk productivity are available from females usually older than 3.5 years. Moreover, such progress is related to collecting a number of milk production data not only from actually milk-recorded animals, but from their relatives as well [Sinnet-Smith *et. al.* 1987]. On the other hand, the heifers' selection based only on pedigree data is biased and does not consider the animals' direct performance. As a result, the genetic improvement on that selection pathway is slow. In light of this the attempts were undertaken at finding the physiological markers in young cattle allowing to predict their future milk productivity traits and to apply those estimates as early selection criteria [Tilakaratne *et al.* 1980, Sejrsen *et al.* 1984, Sinnett-Smith *et al.* 1987, Mackenzie *et al.* 1988; Xing *et al.* 1988, Min *et al.* 1993, Olbrich-Bludau *et al.* 1993]. In majority of investigations in that area the relationship was studied between the blood concentration of insulin in young heifers and their milk traits in the first lactation. Under standard feeding conditions as well as when reduced feeding was applied, weak correlations were identified between blood insulin content and milk

yield [Mackenzie *et al*. 1988, Xing *et al*. 1988, Staufenbiel *et al*. 1993, Skrzypek *et al*. 1999, Loevendahl *et al*. 1994]. Better results were obtained after short fasting. Moreover, the concentration of insulin was reported to be higher in the blood of animals with high genetic merit [Sejrsen *et al*. 1984, Sinnett-Smith *et al*. 1987]. Very high milk yield is often accompanied by energetic disturbances causing dangerous metabolic and fertility disorders. Therefore, the studies, very rare so far, were performed on the relationship between metabolism of exogenous glucose and insulin blood concentration [Xing *et. al*. 1993, Panicke *et al*. 2002, 2003, Sasaki *et al*. 2003]. In ruminant organism the reserve of glucose is small. Thus, its supply into cells should be uninterrupted and its metabolism be regular. The level of glucose in cow's blood is relatively constant under normal physiological conditions and ranges from 35 to 55 mg/100 ml [Kirchgessner 1997]. The milking cow requires about 3.6 kg glucose for production of 50 kg milk. The insulin plays the main role in regulation of these processes. Thus, the blood concentration of insulin and glucose are highly correlated.

Concluding, the authors are of opinion that it would be possible to predict the values of future milk production traits in cows on the basis of three GTT indicators in heifers (AINSCC, AGLUCC, combined GLIN index) and thus to perform the first selection of replacing heifers already at the age of 9-10 months. The most suitable GTT parametre to predict the milk yield and composition in lactation I seems to be combined GLIN index. Early selection of young heifers based on GTT parametres should shorten the generation interval and thus accelerate the genetic gain in milk traits as compared to traditional methods of breeding.

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Zależność między parametrami testu tolerancji na glukozę młodych jałówek a ich wydajnością i składem chemicznym mleka w pierwszej laktacji

Streszczenie

Badania przeprowadzono na 9-10 miesięcznych jałówkach hf wybranych losowo ze stada o przeciętnej rocznej wydajności 9600 kg mleka o 4,32 % tłuszczu i 3,44 % białka. W ciągu doby przed rozpoczęciem testu (*glucose tolerance test* – GTT) jałówkom podawano tylko wodę i niewielką ilość siana. Przed podaniem glukozy założono do żyły jarzmowej kaniulę, przez którą pobrano „zerowe" próbki krwi. Następnie przez kaniulę wprowadzono jednorazowo każdemu zwierzęciu 40% roztwór glukozy w ilości odpowiadającej 1 g glukozy na kg metabolicznej masy ciała (W 0.75) Dalej, w odstępach sześciominutowych, pobrano 9 kolejnych próbek krwi, w których oznaczono glukozę i insulinę, uzyskując odpowiednie krzywe. Jałówki przed ocieleniem żywiono zgodnie z obowiązującymi standardami, a po ocieleniu utrzymywano je w oborze wolnostanowiskowej, żywiąc systemem TMR według norm INRA przez całą pierwszą laktację. Obliczono współczynnik korelacji prostej między powierzchnią znajdującą się pod krzywą zawartości glukozy i insuliny oraz indeks wyrażający stosunek powierzchni pod krzywą zawartości glukozy do powierzchni pod krzywą zawartości insuliny, a także maksymalną koncentrację glukozy i insuliny. Obliczono współczynniki regresji parametrów TTG na cechy mleczności i wprowadzono je do równań regresji. Indeks wyrażający stosunek powierzchni pod krywą zawartości glukozy do powierzchni pod krzywą zawartości insuliny (GLIN *index*) okazał się najbardziej przydatny z punktu widzenia przewidywania cech mleczności jałówek w pierwszej laktacji. Indeks ten można wykorzystywać jako kryterium pomocnicze przy wstępnej selekcji remontowych jałówek hf.