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Effect of diet composition and frequency of feeding on postprandial insulin level and ovarian follicular development in prepubertal pigs*

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ABSTRACT

Several studies have shown that insulin is positively related to reproductive processes. The objectives of the present study were to investigate the effect of three diets, consisting of different major energy sources (starch, glucose or fat) given in 2, 3 or 4 portions daily on postprandial glucose and insulin levels, and to assess the influence of 3 times a day feeding on follicular development in prepubertal gilts.

In Experiment 1, Polish Landrace gilts aged 142 days (n=18) were randomly divided into three equal groups (n=6) fed with starch, glucose and fat diet. The same animals received their diet in three frequencies (2, 3 or 4 times daily). Each period started with three days of adaptation to the of the same diet. On day 4, blood samples were taken before, during and after feeding. The next day the frequency of feeding was changed without change of diet. The plasma glucose and insulin concentrations were determined by enzymatic- and radioimmunoassay, respectively.

In Experiment 2, forty-four 140 day old gilts were divided into three treatment groups and given starch, glucose and fat diets in three equal portions at 8.00, 13.00 and 18.00 h. After 25 days of experimental feeding gilts were slaughtered the day after treatment to assess: ovarian weight, follicular development and the total number of LH receptors in one ovary.

Basal plasma glucose concentrations were similar for the three diets given 3 and 4 times a day (3 times daily: 50.8±4.9, 56.8±4.0, 56.3±7.4 mg/dl; 4 times daily: 53.3±1.7, 65.8±5.9, 58.3±7.5 mg/dl), but differed ($P<0.05$) for the three diets given twice daily (71.4±10.4, 40.8±3.9, 53.6±8.6 mg/dl, starch, glucose, fat, respectively). The basal plasma insulin concentration was similar for the three diets and different frequency of feeding (2 times daily: 7.8±1.4, 9.3±1.0, 9.5±0.9 μIU/ml;

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3 times daily: 6.2±1.4, 10.3±1.0, 8.6±1.0 μIU/ml and 4 times daily: 8.5±1.6, 7.9±0.8, 8.2±2.7 μIU/ml, starch, glucose and fat, respectively). After feeding, the insulin concentration in gilts fed the glucose or starch diet showed a faster increase compared with the fat diet in all studied periods of feeding. The area under the total curve for the plasma insulin level was greater ($P<0.05$) for the glucose diet than for fat diet (6870±656 vs 4028±751 arbitrary units, respectively for twice daily feeding; 6314±718 vs 3274±472, respectively for three daily feeding), whereas the starch diet was intermediate (6104±762 and 3855±729, respectively) and not significantly different from the other diets. Four daily feeding diet not caused significant differences for starch, fat and glucose diets in area under curve for insulin level. Feeding the starch, glucose and fat diets for 25 days (in three equal portions) did not affect the weight (g) of the ovary (2.8±0.1, 2.8±0.2 and 3.0±0.1, respectively), the number and size of healthy and atretic follicles nor LH receptor concentration (9.0±1.2; 10.5±1.7 and 9.6±1.2 fM/mg protein, respectively).

The results confirmed earlier observations that the dietary energy source affects the postprandial plasma insulin concentrations when the diet is divided into two parts. A similar effect was observed when the diet was given in three, but not four, equal portions each day. However, three times daily increase of insulin concentration in blood samples for 25 days did not affect ovarian and follicular development in prepubertal gilts of pure Polish Landrace breed.

KEY WORDS: insulin, glucose, puberty attainment, gilts

INTRODUCTION

It is desirable to induce early puberty attainment in the gilt as she is a costly non-productive animal until the initiation of her first pregnancy. There are many factors which can be manipulated in order to reduce age of puberty and hence minimize the cost of introducing replacement gilts. The major ones are growth rate, nutrition, genetics, climate and the animals' social environment (Hughes, 1982). Increasing dietary energy intake of gilts' four or more days before oestrus has been reported to increase their ovulation rate (Robertson et al., 1951; Zimmerman et al., 1960; Dailey et al., 1972). The hormonal mechanisms involved in these increases in ovulation rate remain largely undefined. The metabolic hormone, insulin has been implicated as necessary for reproductive processes in some studies (Siegel and Wade, 1979; Kirchick et al., 1982). Several studies have shown that insulin can be positively related to follicular development (Matamoros et al., 1990; Tokach et al., 1992; Cox et al., 1997). Plasma insulin levels can be altered by feeding level (Booth et al., 1996) or exogenous insulin injections (Cox et al., 1997). Van den Brand et al. (1997) showed that the nature of the dietary energy source can affect the postprandial plasma insulin concentration.

The objectives of the present study were to investigate the effect of three different diets consisting of different energy sources (starch, glucose and fat) given 2, 3 or 4 times daily on postprandial insulin concentrations, and to determine if three times daily feeding influenced follicular development in prepubertal gilts.

MATERIAL AND METHODS

In Experiment 1, Polish Landrace gilts aged 142 days (n=18) were randomly divided into three equal groups (n=6) fed with starch, glucose and fat diet (see below). The same animals received their diet in three frequencies (2, 3 or 4 times daily). Each period started with three days of adaptation to the diet and frequency (2: 8.00 and 18.00 h; 3: 8.00, 13.00 and 18.00 h; 4: 8.00, 11:30, 15:00 and 18:30 h). Gilts were housed individually in 9 m^2 pens. The day before the experiment began all gilts were surgically fitted with a permanent jugular catheter according to the method previously described by Kotwica et al. (1978). On day four of each frequency-feeding period, blood samples were taken before *via* the catheter, during and after each feed. Following sampling, the feeding frequency was changed. Three experimental diets were prepared (Table 1) and all consisted of a basal diet with sufficient protein, vitamins and minerals (according to Polish Norms of Pig Nutrition, 1993). The carbohydrate-rich-diet (starch) contained 391.3 g/kg starch and

TABLE 1

Composition of experimental diets

Ingredients	Diet		
	glucose	fat	starch
	g	g	g
Barley	240	240	587
Wheat middlings	55	55	100
Toasted soyabean	57	57	–
Extracted soyabean	115	115	140
Supplement of extracted sunflower seed*	178	178	–
Extracted rape seed	40	40	–
Meat-and-bone meal	50	50	30
Lucerne meal	2	2	20
Wheat bran	–	–	100
Maize starch	178	–	–
Glucose	60	–	–
Tallow	–	81	–
Chalk	8.1	8.1	5
Monocalcium phosphate	7.1	7.1	10
Salt	2.4	2.4	3
L-Lysine	1.2	1.2	–
DL-Methionine	1.2	1.2	–
Premix	5	5	5
Calculated content	g/1000 g	g/843 g	g/1000 g
crude protein	200	200	190
starch	364.5	186.4	391.3
MJ NE (for pig daily)	12.3	12.3	12

* ingredients: barley-15%, extracted soyabean-25%, meat-and-bone meal-30%, lucerne meal-15%, extracted rape seed-15%

12 MJ of net energy (NE). In the carbohydrate/glucose-enriched diet (glucose) 27 g of starch from the starch diet was replaced with 60 g of glucose. The glucose diet additionally contained 178 g of maize starch and less barley (Table 1). The fat-enriched diet (fat) contained 96.1 g/kg of tallow as the major energy source. The glucose and fat diets were isocaloric (12.3 MJ NE daily according to Hoffman et al., 1972).

At the beginning and end of the experiment gilts were weighed. Blood samples were taken at -60, -48, -36, -24, -12, 0, 12, 24, 36, 48, 120, 150, 220 min relative to the 8.00 h feeding. Blood samples were collected in ice-cooled polypropylene tubes containing 0.3 M EDTA and 1% aspirin (pH 7.4), then centrifuged at 1500 x g for 15 min at 4°C. The plasma was stored at -20°C until analysis. The plasma insulin concentration was quantified in one radioimmunoassay using the commercial kit Coat-A-Count Insulin (Diagnostic Products Corporation, Los Angeles, CA, USA). The maximum binding for ^{125}I-insulin was 46%. The sensitivity was 4 μIU/ml at 80% binding. The intraassay coefficient of variation (CV) was 5.1%, whereas interassay coefficient of variation was 7.1%. The plasma glucose concentration was measured by oxidase test using the commercial kit (Alpha-Diagnostic Ltd., Warsaw, Poland).

In Experiment 2, forty-four 140 day old Polish Landrace gilts were divided into three treatment groups and given starch (n=14), glucose (n=15) or fat (n=15) diets in three equal portions at 8.00, 13.00 and 18.00 h as described in Experiment 1. After 25 days of experimental feeding, gilts were slaughtered the next day after treatment to assess: ovarian weight, follicular development and the total number of LH receptors in one ovary. Ovaries collected from each gilt were weighed and then one ovary was immediately frozen in liquid nitrogen for further determination of LH receptors. The other ovary was placed in Bouin's fluid, embedded in paraffin and serially sectioned. Every fiftieth section (6 μm) was stained with haematoxylin-eosin. In each section all antral follicles were microscopically counted. All measured follicles were morphologically classified as healthy or atretic on the basis of degenerative changes as the presence of pycnotic nuclei and/or local destruction of the basement membrane. The follicles were divided into three size classes: small <1mm, medium 3-6 mm and large >6 mm in diameter.

LH receptor levels were determined, as previously described, and validated (Ziecik et al., 1989; Jana et al., 1996). Briefly, ovaries were thawed, homogenized in six volumes (v/w) of 25 mmol/l Tri HCl buffer pH 7.4 containing 2.50 mmol/l of sucrose at 4°C with an Ultra-Torax homogenizer. The homogenate was then filtered through four layers of cheesecloth, and the filtrate was centrifuged for 20 min at 800 g at 4°C. The supernatant was centrifuged further for one hour at 25 000 x g at 4°C and the sediment suspended in 3 ml of ice-cold 25 mmol/l Tris-HCl buffer, pH 7.2 containing 0.1% BSA and 5 mmol/l$MgCl_2$. Finally, obtained membrane fractions were assayed for protein determination by the method of Lowry et al. (1951).

hCG (CR-127) was labelled using the chloramines-T method (Greenwood et al., 1963; Catt and Dufau, 1975). Non-specific binding was measured by the addition

of 1 ng unlabelled hCG and was less than 2% of the total ^{125}I-labelled hCG added. The concentration of LH/hCG, unoccupied binding sites and association constants (Ka) for hormone binding to receptors were determined using the EBDA computer program (Elsevier BIO-SOFT, Cambridge, UK). Seven subsaturating quantities of unlabelled hCG (0.15-5 ng) were used for each receptor preparation.

All data were presented as mean ± SEM. Glucose and insulin concentrations were compared by one-way analysis of variance (ANOVA). Both basal glucose and basal insulin concentrations were calculated per gilt per period by repeated measures ANOVA as the mean value of the samples taken at 60, 48, 36, 24, 12 and 0 min before feeding. The area under the curve during the sampling period (0-240 min) was calculated as the area corrected for basal glucose and insulin concentrations.

RESULTS

Experiment 1

The gilts body weight (kg) at the start of experiment was 63.3±1.4 on starch diet, 63.2±1.2 on glucose diet and 64.7±2.0 on fat diet, and at the end of experiment was 83.6±2.9, 85.7±1.5 and 86.1±3.6, respectively.

Basal plasma glucose concentrations were similar in the three diets given 3 and 4 times a day (3 times: 50.8±4.9, 56.2±4.0, 56.3±7.4 mg/dl; 4 times: 53.3±1.7, 65.8±5.9, 58.3±7.5 mg/dl), but differed in the three diets given twice daily (71.4±10.4, 40.8±3.9, 53.6±8.6 mg/dl, starch, glucose, fat, respectively; $P<0.05$ glucose vs starch). Pre-feeding basal plasma glucose concentrations in gilts fed twice daily were lowest for glucose, intermediate for fat and highest for starch group. All groups showed a similar increase of glucose concentration in plasma after feeding which resulted in the same rate of glucose concentrations 120-240 min after feeding (Figure 1). There were also differences in plasma glucose concentration 120-240 min after feeding in diets given twice daily ($P=0.06$, glucose vs starch).

The basal plasma insulin concentration was similar for three diets fed 2, 3 and 4 times daily (2 times: 7.8±1.4, 9.3±1.0 9.5±0.9 μIU/ml; 3 times: 6.2±1.4, 10.3±1.0, 8.6±1.0 μIU/ml and 4 times: 8.5±1.6, 7.9±0.8, 8.2±2.7 μIU/ml, starch, glucose and fat, respectively; Figure 2). After feeding, the insulin concentration in gilts fed the glucose or starch diet showed a faster increase compared with the fat diet in all studied feeding periods. This resulted in a significantly ($P<0.05$) differed plasma insulin level 24-48 min after feeding ($P<0.05$ glucose vs starch and fat in gilts fed 3 times daily; $P<0.05$ glucose vs fat in gilts fed twice daily; Figure 2). The highest differences in insulin concentration among starch, glucose and fat diets 24-48 min after feeding were found in gilts fed 3 times daily (53.8±7.7 vs 22.3±1.6 and 27.5±2.7, respectively; $P<0.05$). In gilts fed twice daily the significant differences

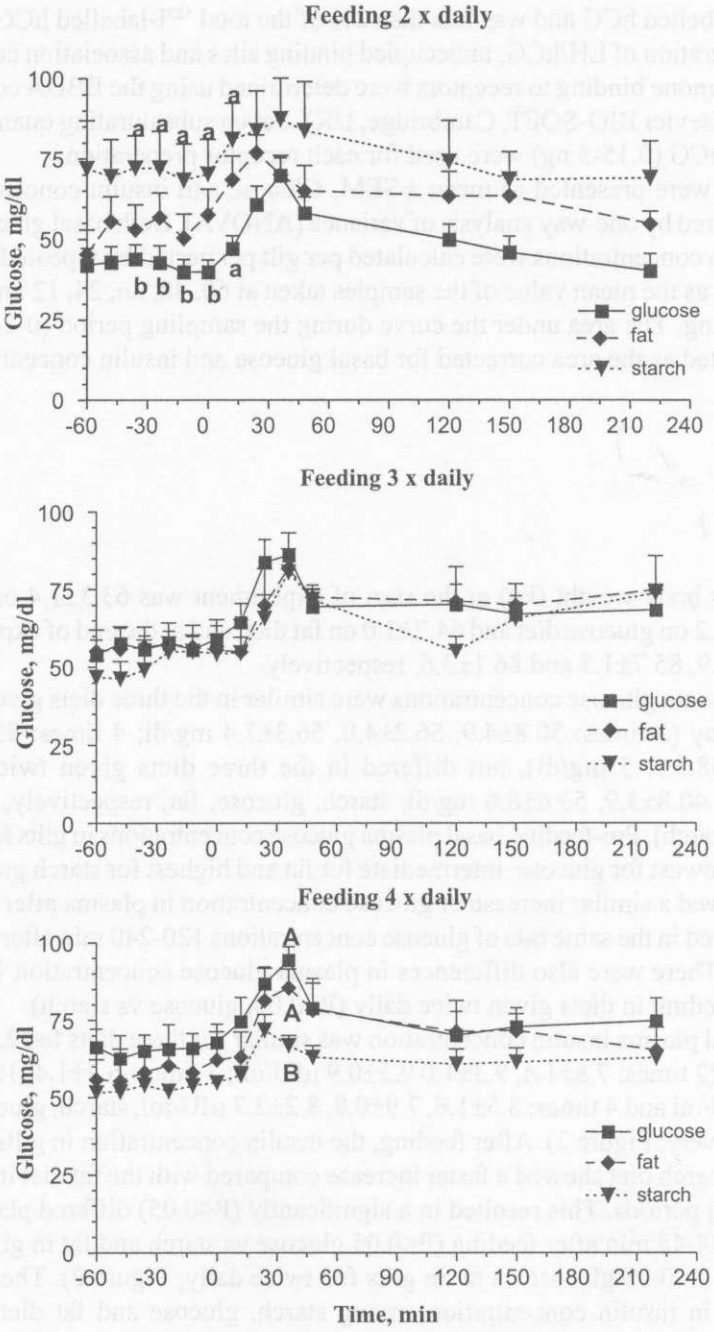

Figure 1. Pre- and post-feeding plasma glucose concentration (means ± SEM) for gilts given starch, glucose and fat enriched diets in two, three or four equal feed per day (a>b, $P<0.05$; A>B, $P<0.01$)

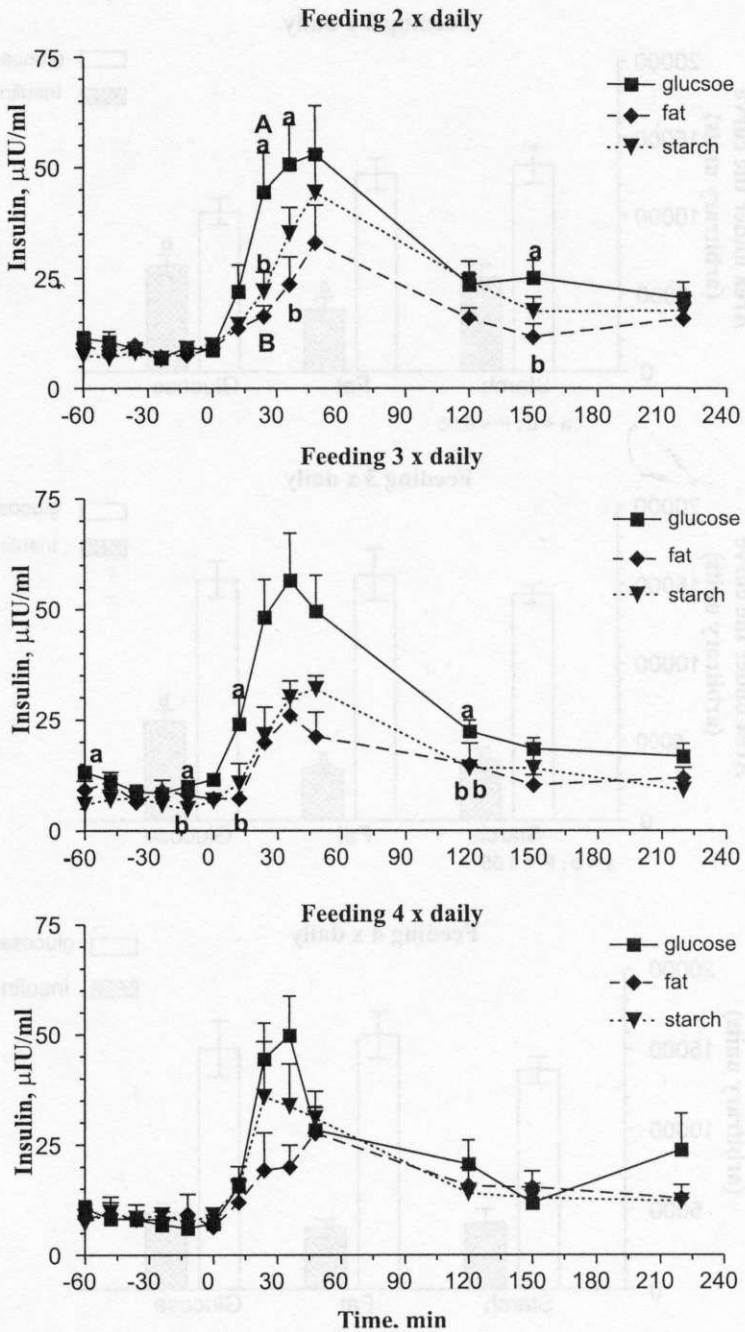

Figure 2. Pre- and post-feeding plasma insulin concentration (means ± SEM) for starch, glucose and fat groups (a>b, P<0.05; A>B, P<0.01)

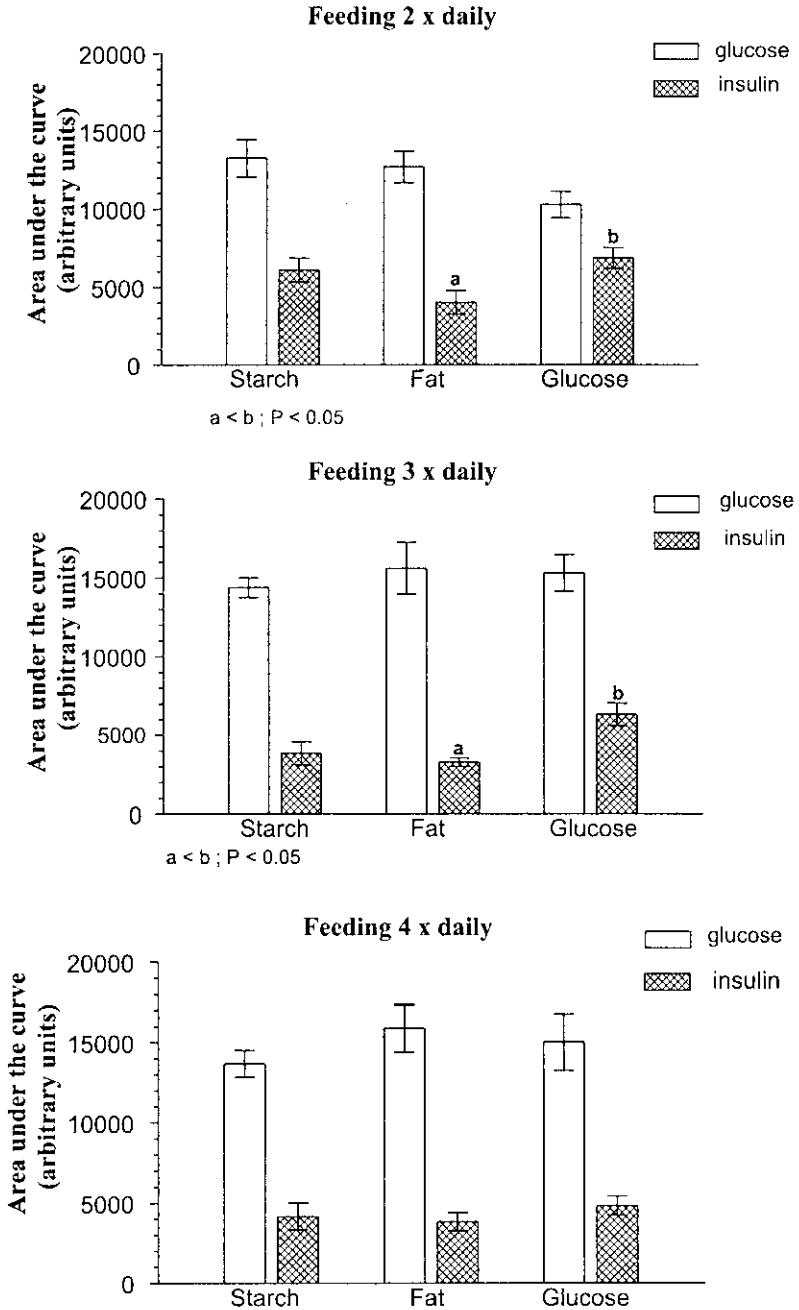

Figure 3. Area under the curve for plasma insulin and glucose during the period of 240 min after providing feed in relation to feeding schedule for gilts given starch, glucose and fat diets (means ± SEM)

were between glucose and fat diets (48.8±6.3 vs 24.3±5.0; P<0.05). The plasma insulin level 120-240 min after feeding differed between diets when they were given three times daily (P=0.07, fat vs starch). The maximum and minimum insulin concentrations occurred when gilts were given the glucose and fat diets in all feeding periods. Those for the starch diet were intermediate and did not differ significantly from the other two diet types.

The area under the total curve for plasma insulin level was greater for the glucose diet than for the fat diet (2 meals per day: 6870±656 vs 4028±751; 3 meals per day: 6314±718 vs 3274±472 arbitrary units, respectively), whereas the starch diet was intermediate for 2 and 3 meals per day (6104±762 and 3855±729, respectively; Figure 3) and not significantly different from the other diets. Four daily feeding did not caused significant differences for starch, fat and glucose diets in area under curve for insulin level (4165±840, 3840±574 and 4837±602, respectively).

Analyses within diets and the feeding period showed a significant maximum insulin and minimum glucose (basal glucose - G_{BASAL}) in gilts fed twice daily. The maximum plasma insulin level and the maximum plasma glucose concentration ratio (I_{MAX}/G_{MAX}, both 24-48 min after feeding) significantly decreased in starch and fat diets when compared to the glucose diet in 2 and 3 frequency periods (glucose>starch>fat; Table 2). Differences in the ratio of the maximum plasma insulin level (I_{MAX}, 24-48 min after feeding) and the basal plasma glucose concentration (G_{BASAL}, [-60]-0 min after feeding) were similar to I_{MAX}/G_{MAX} in those same frequencies of feeding. However, in gilts fed twice daily this relationship was different (glucose>fat>starch, Table 2). There were also differences in the ratio of insulin and glucose area under the total curve (I_{AREA}/G_{AREA}; Table 2).

TABLE 2

Values of ratio I_{MAX}/G_{MAX}, I_{MAX}/G_{BASAL}, I_{AREA*}/G_{AREA*} in three diets and frequencies of feeding

Frequency of feeding	Energy source (diet)	I_{MAX}/G_{MAX}	I_{MAX}/G_{BASAL}	I_{AREA*}/G_{AREA*}
2	G	0.8 ± 0.1[a]	1.3 ± 0.2[a]	0.7 ± 0.1[a]
	F	0.4 ± 0.1[b]	0.6 ± 0.2[b]	0.3 ± 0.1[b]
	S	0.4 ± 0.1	0.5 ± 0.1[b]	0.5 ± 0.0
3	G	0.6 ± 0.0[a]	0.9 ± 0.1[a]	0.4 ± 0.0[a]
	F	0.3 ± 0.1[b]	0.4 ± 0.1[b]	0.2 ± 0.0[b]
	S	0.4 ± 0.0[b]	0.6 ± 0.1	0.3 ± 0.0
4	G	0.5 ± 0.1	0.7 ± 0.1	0.4 ± 0.1
	F	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.0
	S	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.1

* under total curve

S - starch, G-glucose, F-fat; a>b, P<0.05 in columns for 2 and 3 frequency of feeding

I_{MAX} - maximum insulin, G_{MAX} – maximum glucose, G_{BASAL} - basal glucose levels, respectively

I_{AREA}, G_{AREA} - area under the curve of insulin (I) and glucose (G) levels

Experiment 2

The twenty-five day glucose, starch and fat diets divided into three equal portions daily did not affect the weight of ovary (2.8±0.1, 2.8±0.2 and 3.0±0.1 g, respectively). The number and size of healthy and atretic follicles did also not change (Table 3).

The LH receptor concentration (fM/mg protein) was similar in all the glucose, starch and fat groups (9.0±1.2; 10.5±1.7 and 9.6±1.2, respectively).

TABLE 3

Summary of ovarian weights, number of follicles and concentration of LH receptors per on ovary in gilts fed 25 days (3 times daily) with starch, glucose and fat diets (± SEM)

Group	Weight of ovary g	Total number of follicles						LH receptors fM/mg protein
		<3 mm		3-6 mm		>6 mm		
		healthy	atretic	hearty	atretic	healthy	atretic	
Starch (n=14)	2.8	25.8 ± 2.3	12.4 ± 14	1.3 ± 0.3	0.6 ± 0.3	0.2 ± 0.2	-	10.5 ± 1.7
Glucose (n=15)	2.8	27.9 ± 6.3	12.9 ± 2.0	0.9 ± 0.2	0.6 ± 0.3	0.2 ± 0.2	-	9.0 ± 1.2
Fat (n=15)	3.0	26.4 ± 3.8	15.0 ± 1.7	1.1 ± 0.3	0.7 ± 0.2	-	-	9.6 ± 1.2

DISCUSSION

The aim of this study was to investigate the effects of specific dietary sources on the plasma glucose and insulin concentrations, and the reproductive tract and ovarian development in prepubertal gilts of pure Polish Landrace breed. The composition of experimental diets (glucose and fat) was based on that proposed by Van den Brand et al. (1997) with slight modifications (supplementation of extracted sunflower seed in glucose and fat diets, and an addition of wheat bran to the starch diet). Van den Brand et al. (1997) used their experimental diets for mature gilts fed twice daily. In our experiment, diets were additionally divided into three and four portions, and the plasma glucose and insulin profiles were determined. The postprandial patterns of the plasma glucose concentration in our study were similar to those found by Ponter et al. (1991) and Van den Brand et al. (1997) only when gilts were fed 3 and 4 times daily. In these cases there were no differences between diets in basal plasma glucose concentrations before feeding in gilts fed three various diets. However, in gilts fed the glucose diet twice daily, the basal glucose level was the lowest and significantly different when compared to the diet rich in starch. It can be explained by the highest insulin release and maintenance at high level after feeding in gilts fed the glucose-enriched diet.

It is interesting that such a relationship between plasma glucose and insulin concentrations was not found in gilts fed 3 times daily, but gilts fed the glucose-enriched diet maintained the highest postprandial insulin levels in blood. The highest basal pre-feeding level of plasma glucose in the fat-enriched diet can be explained by the relatively low insulin release after feeding.

Generally, our results obtained in prepubertal gilts confirmed the data of Van den Brand et al. (1997) that a dietary energy source affects the postprandial plasma insulin levels when the isocaloric diet is divided in two parts in mature gilts. Additionally, we were able to show the same effect when three but not four equal portions were applied. However, three times daily increase of insulin concentration in blood samples during a period of 25 days has not influenced follicular development in purebred prepubertal gilts.

The failure of the glucose-enriched and insulin generating diet to influence follicular development could be caused by two factors: (1) too young and low body weight gilts used in experiments and (2) the use of pure-bred gilts. Age and body weight are very important factors for the attainment of puberty in gilts (Hughes, 1982; Britt et al., 1989). On the other hand, pure breeds reach puberty approximately 3-4 weeks later than hybrids (Hughes, 1982).

To confirm our hypothesis we used in the next experiments with the glucose-enriched diet, slightly older (10-15 days) and crossbred gilts to study natural and PMSG/hCG induced puberty attainment in gilts. In those studies the addition of glucose to a standard diet given in three portions daily speeded up the attainment of puberty and increased ovarian and reproductive tract responses to PMSG/hCG treatment in pubertal crossbred gilts (Zięcik et al., 2002).

CONCLUSIONS

Our results are in agreement with earlier observations, dietary energy source affects the postprandial plasma insulin levels in mature gilts, when their diet is given in a twice-daily feeding regimens. Additionally a similar effect was observed in prepubertal gilts, when their diet was given in three but not four equal portions. However, three times-daily feeding increased insulin concentrations in blood samples during a 25 day feeding period had no significant effect on ovarian and follicular development in prepubertal gilts of the Polish Landrace breed.

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STRESZCZENIE

Wpływ diety i częstotliwości karmienia na poziom insuliny oraz rozwój jajnika u niedojrzałych płciowo loszek

Insulina może korzystnie wpływać na procesy rozrodcze. Celem badań było określenie wpływu diet zawierających różne główne źródła energii (skrobię, glukozę, tłuszcz) oraz podzielonych na 2, 3 i 4 porcje dziennie, na poposiłkowy poziom insuliny, jak również oszacowanie wpływu 3-krotnego karmienia na rozwój pęcherzyków jajnikowych u niedojrzałych płciowo loszek.

W Doświadczeniu 1, 18 loszek mieszańców w wieku ok. 142 dni otrzymywało trzy diety (n=6) w trzech częstotliwościach karmienia (2, 3 i 4 razy dziennie). Doświadczenia poprzedzono trzydniowym okresem adaptacji do diety i częstotliwości karmienia. Czwartego dnia krew pobierano przed, w trakcie i po karmieniu. Następnego dnia zmieniano częstotliwość karmienia tą samą dietą i procedurę powtarzano. Poziom glukozy i insuliny we krwi oznaczano odpowiednio metodą enzymatyczną i radioimmumologiczną.

W Doświadczeniu 2, loszki PBZ (n=44) podzielono na trzy grupy i podawano dietę skrobiową (S), glukozową (G) i tłuszczową (T) w trzech jednakowych porcjach o godzinie 8:00, 13:00 i 18:00. Po 25 dniach karmienia loszki ubito w celu określenia masy jajników, rozwoju pęcherzyków jajnikowych i ogólnej liczby receptorów LH w jajniku.

Podstawowy poziom glukozy był podobny przy skarmianiu wszystkich diet podawanych 3- i 4-krotnie w ciągu dnia (3 razy dziennie: 50,8±4,9, 56,2±4,0, 56,3±7,4 mg/dl; 4 razy dziennie: 53, ±1,7, 65,8±5,9, 58,3±7,5 mg/dl), lecz różnił się gdy świnie żywiono 2-krotnie (71,4±10,4, 40,8±3,9, 53,6±8,6 mg/dl; odpowiednio skrobia, glukoza, tłuszcz; glukoza vs skrobia P<0,05). Podstawowy poziom insuliny we krwi był podobny przy skarmianiu trzech diet i przy różnych częstotliwościach podawania paszy (2 razy dziennie: 7.8±1.4, 9.3±0.99, 9.50±0.9 μIU/ml; 3 razy dziennie: 6.2±1.4, 10.3±1.0, 8.6±1.0 μIU/ml i 4 razy dziennie: 8.5±1.6, 7.9±0.8, 8.2±2.7 μIU/ml; odpowiednio skrobia, glukoza, tłuszcz). Po karmieniu poziom insuliny u loszek otrzymujących dietę skrobiową lub z glukozą wzrastał szybciej w porównaniu z dietą tłuszczową, niezależnie od częstotliwości podawania paszy. Pole pod krzywą dla poziomu insuliny we krwi było większe w przypadku diety z glukozą niż z tłuszczem (2 razy dziennie: 6870±656 vs 4028±751; 3 razy dziennie: 6314±718 vs 3274±472 jednostek umownych, odpowiednio), i miało wartość pośrednią przy podawaniu diety skrobiowej (6104±762 i 3855±729, odpowiednio) i nie różniło się istotnie od wartości uzyskanych przy dwóch pozostałych dietach. Podawanie wszystkich diet przez 25 dni nie miało wpływu na masę (g) jajników, liczbę i rozmiar zdrowych oraz atretycznych pęcherzyków, ani na koncentrację receptorów LH.

Uzyskane wyniki potwierdzają wcześniejsze obserwacje uzyskane dla dojrzałych loszek, że rodzaj źródła energii w diecie wpływa na poposiłkowy poziom insuliny, gdy paszę podawano w dwóch porcjach. Podobny efekt zaobserwowano u niedojrzałych płciowo loszek, gdy dietę podawano w dwóch i trzech, lecz nie czterech jednakowych porcjach. Trzykrotne zwiększenie zawartości insuliny w krwi w ciągu doby w ciągu 25 dni nie miało wpływu na rozwój pęcherzyków jajnikowych i jajników u niedojrzałych płciowo loszek rasy PBZ.