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Effect of glucose supplemented diet on natural and gonadotropin induced puberty attainment in gilts*

A.J. Zięcik[1], W. Kapelański[2], M. Zaleska[1] and J. Riopérez[3]

[1]Institute of Animal Reproduction and Food Research, Polish Academy of Sciences
Tuwima 10, 10-746 Olsztyn, Poland
[2]Department of Pig Breeding, University of Technology and Agriculture
Mazowiecka 28, 85-084 Bydgoszcz, Poland
[3]Intituto de Nutrición y Bromatologia, Faculdad de Farmacia, Ciudad Universitaria
280-40, Madrid, Spain

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ABSTRACT

The studies were designed to assess whether a glucose-enriched diet could affect natural and/or PMSG/hCG induced puberty attainment in gilts.

In Experiment 1, 145 day old crossbred gilts were randomly allocated to receive a control-standard (n=25) or glucose-supplemented diet (60 g/kg feed; n=25) for 25 days in three equal portions at 8.00, 13.00 and 18.00 h .Gilts were observed morning and afternoon for the detection of oestrus.

In Experiment 2, fifty 145-155 day old prepubertal gilts were divided into two equal groups and fed for 25 days as in Experiment 1. On Day 26 of the experiment, all gilts were injected with 750 IU PMSG and 72 h later with 500 IU hCG. Three days after the last injection of gonadotropin, the gilts were slaughtered at a commercial abattoir and their reproductive tracts and carcasses were examined.

Based on observations of vulval development and behavioural signs of oestrus, gilts given the glucose-enriched diet in Experiment 1 reached oestrus earlier (170.1±0.9 day) than the control group (175.9±1.6 day; $P<0.05$). Also gilts given the glucose-supplemented diet showed more intense signs of oestrus than control gilts (2.8±0.4 vs 2.5±0.7 points, respectively; $P<0.05$). In Experiment 2 three gilts fed the glucose-enriched diet and three fed the control-standard diet did not respond to PMSG/hCG treatment. In the remaining animals glucose supplementation of feed increased ovulation rate (8.5±0.7 vs 6.2±0.6; $P<0.05$) as well as ovary weight (4.9±0.5 vs 3.6±0.3 g; $P<0.05$). Similarly, weight (356.5±23.8 vs 264.7±20.2 g) and capacity (723.0±65.8 vs 511.0±35.7 cm^3) of uterus were significantly ($P<0.01$) higher in for gilts fed glucose-enriched diet than for controls.

The results show that the addition of glucose to the standard diet stimulated puberty attainment and/or ovarian and reproductive tract receptiveness to PMSG/hCG in prepubertal gilts.

KEY WORDS: pig, glucose diet, puberty, oestrus

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INTRODUCTION

Dietary energy source affected the postprandial plasma insulin level in mature gilts when the diet was divided into two parts daily (Van den Brand et al., 1997). The same effect was observed when the diet of prepubertal gilts was given in three, but not four, equal portions (Zięcik et al., 2002). Since several studies have shown that insulin is positively related to reproductive processes (Matamoros et al., 1990; Tokach et al., 1992; Cox et al., 1997), we anticipated that three times daily increase of insulin concentration in blood samples during 25 days affect ovarian and follicular development in prepubertal gilts. However, stimulation of ovarian development was not achieved in our earliest experiment possibly due to young age and low body weight and/or genotype of used gilts (Zięcik et al., 2002).

The present studies have been designed to assess whether a glucose-enriched diet could affect natural and/or PMSG/hCG induced puberty in crossbred gilts.

MATERIAL AND METHODS

In Experiment 1, 145 day old crossbred (Polish Large White x Polish Landrace) gilts were randomly chosen to receive a control-standard diet (n=25) or one supplemented with glucose (60 g/kg feed; n=25) for 25 days (2.5 kg daily) in three equal portions at 8.00, 13.00 and 18.00 h. Composition of standard and glucose enriched diet respond to starch and glucose diet described recently (Zięcik et al., 2002). Gilts were housed in groups of two to three animals per pen (3.7 x 2.0 m) with concrete floors covered with wood shavings and access to feed and water. After completion of the period of experimental nutrition, gilts were fed twice daily with the standard diet and then oestrus was observed by the same worker (at 7.00, 11.00, 16.00 and 18.00 h). The signs were rated according to Karalus et al. (1990):

3 - vulva red and swollen, standing oestrus, sometimes including vaginal discharge
2 - vulva red and swollen but not accompanied by standing oestrus
1 - some redness and slight swelling of vulva
O - no outward physical or behavioural signs.

Oestrus checks were carried out at least for 30 days after completion of the experimental feeding. During the last 15 days of experimental feeding gilts had contact with a mature boar for 20 min, twice daily at 7.00 and 18.00 h.

In Experiment 2, fifty prepubertal crossbred gilts about 155 days of age were assigned to two groups and fed for 25 days as in Experiment 1. On Day 26 the gilts were injected i.m. with 750 IU PMSG (Werfasser Alvetra - Werfft Chemie, Austria) in 1.5 ml saline and 72 h later with 500 IU hCG (Werfachor Alvetra - Werfft Chemie, Austria) in 1.0 ml saline. Four days after the last injection of gonadotropin gilts

were slaughtered at a commercial abattoir and the reproductive tracts and carcasses were examined. Capacity of uterus was measured according to method of Kwaśnicki (1951) originally used for intestine volume measurements. Briefly, uteri were placed in the calibrated vessel with water. A cannula was inserted through the cervix to the uterus and filled with water up to the internal pressure of 10 cm of water post. The capacity of the uterus was expressed in cm^3 on the base of water displacement.

All data were presented as mean ± SEM and compared between treatments with Student's t-test. All calculations were performed using the statistical package GraphPad PRISM (GraphPad Software, San Diego, CA, USA).

RESULTS

Body weight at the start of the Experiment 1 was similar for glucose and standard diets (64.0 ± 1.9 vs 63.9 ± 1.4, respectively), but glucose gilts were heavier at the end of experimental feedings (86.5 ± 1.5 kg) than standard gilts (81.5 ± 1.6 kg). Age at the end of experimental feeding was the same (glucose - 170.5 ± 0.5 vs standard - 170.7 ± 0.5 days). Results of the observations for oestrus are presented in Table 1. Two gilts in each group failed to show any signs of oestrus up to

TABLE 1

Results of oestrus signs observations in gilts (± SEM) in Experiment 1

Parameters	Diet	
	glucose-enriched	control
No of gilts showing signs of oestrus until 30 days after the period of experimental feeding	23	23
Days from the start of experiment to occurrence of oestrus	$26.6^a \pm 1.5$	$31.4^b \pm 1.4$
No of gilts showing oestrus during experimental feeding	9	6
No of gilts showing signs of oestrus during experimental feeding and until 7 days after the end of experimental feedings	19 (82.6%)	16 (69.6%)
*Intensity of oestrus (Karalus et al., 1990)	$2.83^a \pm 0.39$	$2.48^b \pm 0.73$
Age of puberty attainment (occurrence of the first oestrus), days	$172.1^a \pm 1.2$	$177.1^b \pm 1.6$

a>b, P<0.05

* scale 0-3, see material and methods

30 days after completion of the experimental feeding, and are not represented in this analysis. Signs of oestrus appeared about 5 days earlier in gilts fed glucose-enriched diet than in control group ($P<0.05$) as well as more gilts fed glucose diet showed oestrus before day 25 of experiment (Table 1). In this group 82.6% gilts exhibited signs of oestrus compared to 69.5% in control group. Also, the intensity of heat was higher in glucose than control group ($P<0.05$; Table 1).

In Experiment 2, three gilts receiving glucose-enriched diet and three in control-standard diet did not respond to PMSG/hCG treatment. In remaining animals the glucose-supplemented diet caused increased ovulation rate (8.5 ± 0.7 vs 6.2 ± 0.6; $P<0.05$) as well as increased ovary weight (4.9 ± 0.5 g vs 3.6 ± 0.3 g; $P<0.05$) when compared to control, respectively (Figure 1). Similarly, weight (356.5 ± 23.8 vs 264.7 ± 20.2 g; $P<0.01$), capacity of uterus (723.0 ± 65.8 vs 511.0 ± 35.7 cm^3; $P<0.01$) and length of cervix (16.9 ± 0.5 vs 14.2 ± 0.2; $P<0.05$) were significantly higher in glucose-enriched than in standard fed gilts. Supplementation with glucose did not affect other carcass parameters, except slightly higher lean meat percent (55.6 ± 0.8 vs 53.3 ± 0.8; Table 2).

TABLE 2

Age, body weight and carcass evaluation of gilts in Experiment 2 (± SEM)

Parameters	Diet	
	glucose-enriched	standard
No of animals	25	25
Age of gilts, days		
at the start	155.0 ± 3.6	154.4 ± 5.2
at the end of experiment	184.0 ± 3.6	183.4 ± 5.2
Body weight, kg		
at the start	69.2 ± 6.5	69.3 ± 8.6
at the end of experiment	95.0 ± 6.7	94.4 ± 7.8
Carcass evaluation		
back fat, mm	13.4 ± 2.2	14.6 ± 2.7
loin eye, mm	53.4 ± 5.9	52.6 ± 8.3
lean meat, %	55.6 ± 3.8	53.3 ± 4.0

DISCUSSION

Our Experiment 1 results indicate that gilts fed with glucose-enriched diet reached oestrus earlier than gilts of the control group and showed more intense signs of oestrus. However, it should be underlined that both experimental groups in this study reached puberty relatively early (at 170 - 176 days) without any gonadotro-

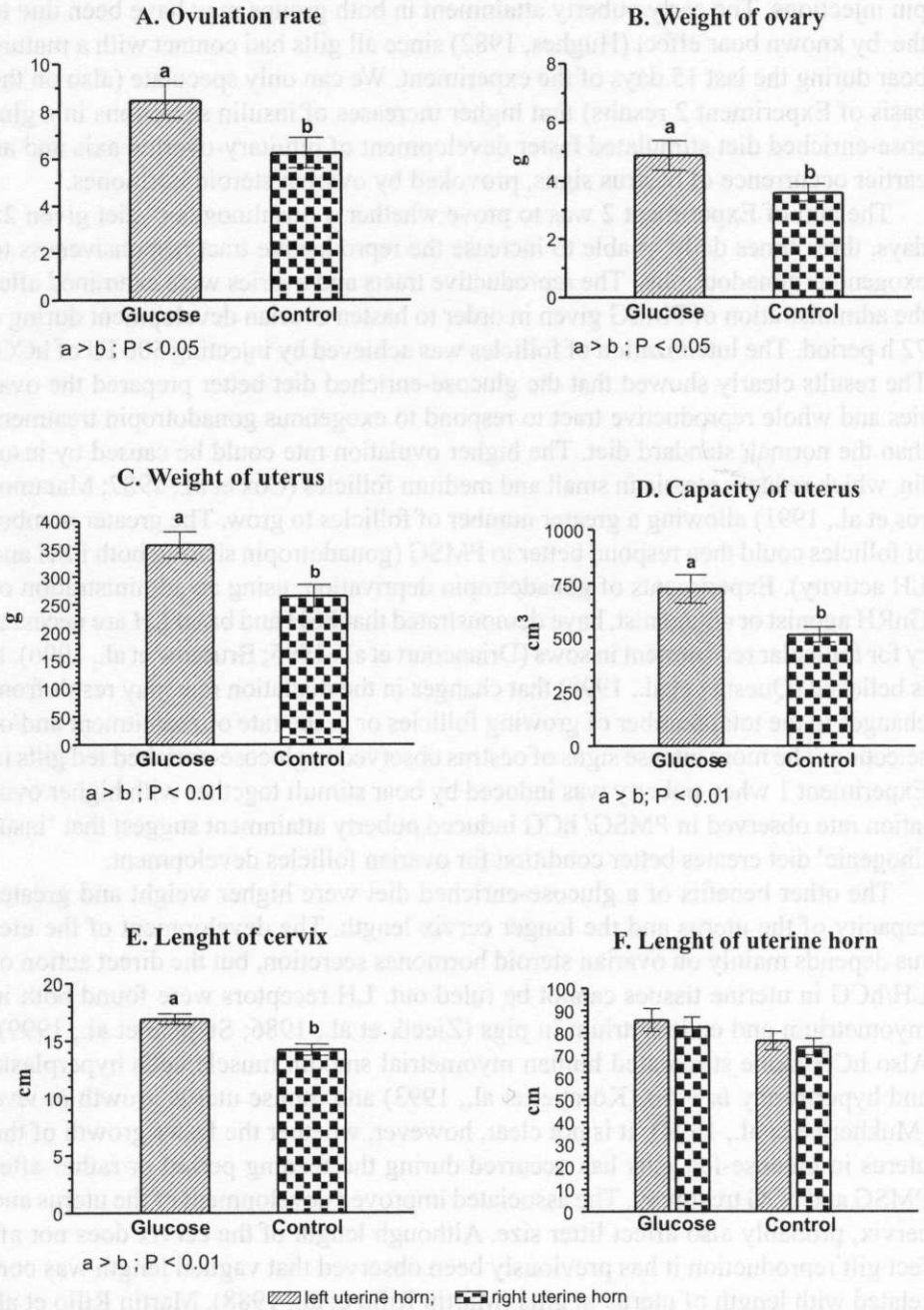

Figure 1. Effect of PMSG and hCG on ovarian (A, B) and reproductive tract (C, D, E, F) responses in prepubertal gilts fed glucose-enriched and standard-control diet (± SEM)

pin injections. The early puberty attainment in both groups may have been due to the by known boar effect (Hughes, 1982) since all gilts had contact with a mature boar during the last 15 days of the experiment. We can only speculate (also on the basis of Experiment 2 results) that higher increases of insulin secretions in a glucose-enriched diet stimulated faster development of pituitary-ovarian axis and an earlier occurrence of oestrus signs, provoked by ovarian steroid hormones.

The aim of Experiment 2 was to prove whether a 'insulinogenic' diet given 25 days, three times daily, is able to increase the reproductive tract responsiveness to exogenous gonadotropins. The reproductive tracts and ovaries were examined after the administration of PMSG given in order to hasten ovarian development during a 72 h period. The luteinization of follicles was achieved by injecting 500 IU of hCG. The results clearly showed that the glucose-enriched diet better prepared the ovaries and whole reproductive tract to respond to exogenous gonadotropin treatment than the normal, standard diet. The higher ovulation rate could be caused by insulin, which reduces atresia in small and medium follicles (Cox et al., 1987; Matamoros et al., 1991) allowing a greater number of follicles to grow. The greater number of follicles could then respond better to PMSG (gonadotropin sharing both FSH and LH activity). Experiments of gonadotropin deprivation, using an administration of GnRH agonist or antagonist, have demonstrated that FSH and basal LH are necessary for follicular recruitment in sows (Driancourt et al., 1995; Brussow et al., 1996). It is believed (Quesnel et al., 1998) that changes in the ovulation rate may result from changes in the total number of growing follicles or in the rate of recruitment and/or selection. The more intense signs of oestrus observed in glucose-enriched fed gilts in Experiment 1 when puberty was induced by boar stimuli together with higher ovulation rate observed in PMSG/ hCG induced puberty attainment suggest that 'insulinogenic' diet creates better condition for ovarian follicles development.

The other benefits of a glucose-enriched diet were higher weight and greater capacity of the uterus and the longer cervix length. The development of the uterus depends mainly on ovarian steroid hormones secretion, but the direct action of LH/hCG in uterine tissues cannot be ruled out. LH receptors were found both in myometrium and endometrium in pigs (Ziecik et al., 1986; Stepien et al., 1999). Also hCG alone stimulated human myometrial smooth muscle cells hyperplasia and hypertrophy *in vitro* (Környei et al., 1993) and mouse uterus growth *in vivo* (Mukherjee et al., 1994). It is not clear, however, whether the faster growth of the uterus in glucose-fed gilts has occurred during the feeding period or rather after PMSG and hCG treatment. The associated improved development of the uterus and cervix, probably also affect litter size. Although length of the cervix does not affect gilt reproduction it has previously been observed that vaginal length was correlated with length of uterus in gilts (Martin Rillo et al., 1988). Martin Rillo et al. (2001) concluded that the litter size at first farrowing is positively associated with vagina-cervix catheter penetration depth during insemination of the gilt.

Gilts fed with glucose-enriched diet in Experiment 1 were heavier at the end of experimental feedings than fed with standard diet. On the other hand supplementation with glucose increased percent of lean. It is difficult to conclude if higher growth rate and leanness affected reproductive performance of these gilts. It has been found that growth rate was positively correlated with age at puberty (Rydhmer et al., 1992) but animals with improved lean tissue feed conversion resulted in increased age at puberty (Kerr and Cameron, 1997). However, in Experiment 2 the improved responsiveness of the gilts to gonadotropin-stimulated ovulation in the absence of an effect on liveweight implies a specific glucose effect on the reproductive axis.

CONCLUSIONS

Our results showed that the addition of glucose to a standard diet affected the attainment of puberty and/or ovarian and reproductive tract responses to gonadotropins treatment in prepubertal gilts. We suggest that the described methods can become very useful tools in overcoming delayed puberty in many pig herds.

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STRESZCZENIE

Wpływ wzbogaconej w glukozę diety na osiągnięcie naturalnej i indukowanej przez PMSG/hCG dojrzałości płciowej przez loszki

Celem badań było sprawdzenie czy dieta wzbogacona w glukozę może wpływać na osiągnięcie zarówno naturalnej jak i indukowanej przez PMSG/hCG dojrzałości płciowej u loszek.

W Doświadczeniu 1, 145-dniowe loszki mieszańce otrzymywały standardową dietę kontrolną (n=25) lub dietę z dodatkiem glukozy (60 g/kg paszy; n=25) przez kolejne 25 dni w trzech równych porcjach o godz. 8,00, 13,00, i 18,00. Objawy rui obserwowano rano i wieczorem i oceniano ich nasilenie.

W Doświadczeniu 2, pięćdziesiąt loszek mieszańców w wieku 145-155 dni podzielono na dwie grupy i żywiono przez 25 dni, jak w Doświadczeniu 1. W 26 dniu doświadczenia wszystkim loszkom podano 750 IU PMSG i w 72 godziny później - 500 IU hCG. Cztery dni po ostatniej iniekcji loszki podano ubojowi, a narządy rozrodcze oraz tusze przebadano i oceniono.

Na podstawie obserwacji wyglądu sromu i behawioralnych objawów rui stwierdzono, że loszki żywione dietą z dodatkiem glukozy w Doświadczeniu 1, osiągnęły ruję wcześniej (170,1 ± 0,9 dnia) niż grupa kontrolna (175,9 ± 1,6 dnia; P<0,05). Loszki otrzymujące dodatek glukozy w diecie wykazały również bardziej intensywne objawy rui niż loszki w grupie kontrolnej (2,83 ± 0,39 vs 2,48 ± 0,73 punktów, odpowiednio; P<0,05). W Doświadczeniu 2, trzy loszki otrzymujące dietę z glukozą i trzy żywione dietą standartową nie zareagowały na podanie PMSG/hCG. U pozostałych zwierząt dodatek glukozy w paszy spowodował wzrost liczby owulacji (8,47 ± 0,73 vs 6,25 ± 0,65; P<0,05), jak również masy jajników (4,86 ± 0,51 g vs 3,59 ± 0,29 g; P<0,05) w porównaniu ze zwierzętami kontrolnymi, odpowiednio. Podobnie masa (356,5 ± 23,85 g vs 264,7 ± 20,21 g) i pojemność macicy (723,0 ± 65,77 vs 511,0 ± 35,68 cm^3) były istotnie (P<0,01) większe u loszek żywionych dietą wzbogaconą w glukozę niż karmionych dietą standardową.

Uzyskane wyniki wskazują, że wzbogacona w glukozę dieta wpłynęła korzystnie zarówno na przyspieszenie osiągnięcia dojrzałości płciowej jak i receptywność układu rozrodczego na PMSG/hCG u niedojrzałych płciowo loszek.