EFFECTS OF LOW GLUCOSINOLATE RAPESEED MEAL IN SOW PREGNANCY DIET

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INTRODUCTION

Meal from french rapeseed cultivars with a low glucosinolate level (LGR) can be used up to 20 % in the diet of growing pigs (Bourdon and Aumaitre, 1990). Results obtained with the canadian LGR "Tower" show that it can be fed to sows without any detrimental effect (Flipot and Dufour 1977). However, Danielsen (1985) found that when sows were fed a diet with 20% of LGR containing 3.6 to 12.2 µM glucosinolates/g, litter size at farrowing and at weaning tended to be reduced. Etienne and Dourmad (1987) showed that high glucosinolate rapeseed (HGR, cv. "Jet Neuf" containing 147 µM glucosinolates/g) induced an important thyroid enlargement in the fetuses when introduced at the 20% level in the pregnancy diet. This effect was reduced by substituting HGR with LGR (cv. "Tandem") containing 49 µM glucosinolates/g. However, with both kinds of rapeseed, survival of the embryos measured at 40 days post-coïtum was decreased. It was thus important to verify if these detrimental effects could be alleviated by using LGR levels lower than 20% in the diet.

MATERIALS AND METHODS

Animals

A total number of 160 Large White gilts were inseminated around 260 days of age and 149 kg liveweight. They were then allocated in four groups fed different diets based on cereals. The diets were isonitrogenous (16.4% protein and 0.65% lysine) and soybean oil meal was replaced by 0, 7, 14 or 20% LGR (cv. "Darmor" containing 37.2 µM glucosinolates/g). Gilts were fed 2.3 kg/day of their respective diet until slaughter at 111 days of pregnancy.

Samplings and Measurements

A blood sample was obtained by venipuncture of the gilts at 7 days of pregnancy. A second one was taken at slaughter. Immediatly after bleeding, the gilts were hysterectomized, and 10 ml blood samples were obtained from the umbilical chord in 8 fetuses/litter chosen at random, or on the whole litter if littersize was lower than 8 fetuses. Hematocrit, leucocyte and erythrocyte counts were measured in all blood samples. Differential leucocyte count was also determined on dam's blood. Triiodothyronin (T3) and thyroxin (T4) were measured in blood plasma of gilts by radioimmunoassay and thiocyanates (SCN) by the method of Bowler (1944). In each litter, three plasma samples were analyzed for T4: one obtained by pooling the plasmas of the eight sampled fetuses (mean T4) and the others corresponding to the plasmas of the fetuses that had the smaller (mini T4) and the biggest (maxi T4) thyroid weight corrected for liveweight. Iodine was also measured in plasma of gilts and on the pooled samples of each litter by using a specific electrode.

Fetuses and placentas were counted and weighed. Ovulation rate was measured by counting the number of corpora lutea dissected from the ovaries. After dissection from the genital tract, the two uterine horns of the gilts were weighed and measured. Thyroid, adrenals, liver and kidneys of gilts and of all fetuses were dissected and weighed.

Statistical analysis

Results were compared by General Linear Model least squares analysis of variance (SAS, 1988). Differences among treatment means were determined by Duncan's multiple range test. The data related to several fetuses in each litter were analyzed according to a split-splot design with litter being subplot. Covariates were also used for some data: net weight of the gilts for their organ weight and ovulation rate for littersize and embryonic mortality.

RESULTS

Hematocrit, erythrocyte and leucocyte counts in gilt blood were not affected by the diet at 7 days nor at 111 days of pregnancy (mean values: 42.6%, 7.66 x $10^6/mm^3$ and 14.58 x $10^3/mm^3$ at 7 days and 38.1%, 6.68 x $10^6/mm^3$ and 11.63 x $10^3/mm^3$ at 111 days for hematocrit, erythrocyte and leucocyte counts, respectively). Differential leucocyte count was also similar in the four groups (neutrophils, 29.1%; eosinophils, 3.2%; basophils, 0.7%; lymphocytes, 65.4%; monocytes, 1.6% as means for the two pregnancy stages). Like for the dams, hematocrit, erythrocyte and leucocyte counts in fetus blood were similar in the four groups, respectively 34.4%, 4.58 x $10^6/mm^3$ and 4.39 x $10^3/mm^3$.

Table 1. Blood parameters of the gilts and their fetuses

RSM level	0%	7%	14%	20%	Rsd(1)	Sign(2)
Gilts, 7 days of pregnancy:						
SCN, µg/ml	3.07a	5.96b	7.15c	7.39c	1.26	***
Iodine, µg/ml	0.37a	0.31b	0.29b	0.29b	0.04	**
T3, ng/ml	1.67	1.92	1.84	1.96	0.60	NS
T4, ng/ml	49.2	51.3	51.7	50.5	7.3	NS
Gilts, 111 days of pregnancy:						
SCN, µg/ml	3.45a	5.43b	5.98b	7.68c	1.50	***
Iodine, µg/ml	0.38a	0.27b	0.26b	0.29b	0.10	***
T3, ng/ml	1.00	1.28	0.99	1.38	0.66	NS
T4, ng/ml	26.8	29.7	27.4	28.7	6.1	NS
Fetuses:						
Iodine, µg/ml	0.42a	0.42a	0.35b	0.40a	0.09	*
T4 mini, ng/ml	82.7a	74.0b	72.8b	68.7b	12.5	***
T4 maxi, ng/ml	81.3a	77.4a	76.5a	66.2b	11.5	***
T4 mean, ng/ml	81.6a	74.0b	73.8b	66.5c	9.2	***

(1) Residual standard deviation.
(2) Statistical significance; ***, P<0.001; **, P<0.01; *, P<0.05; ·, P<0.10; Values on a same line indexed with the same letter are not statistically different.

Plasma parameters measured in gilts and their fetuses are presented in Table 1. Thiocyanates in the gilts increased linearly with the level of LGR of the diet at 7 days as well as at 111 days of pregnancy. At these two stages, iodine level was significantly decreased in the gilts fed LGR compared to the control ones. On the opposite, T4 was not affected by the treatment at any stage. Iodine was decreased in the fetuses from gilts fed 14% LGR only, whereas T4 mini, T4 maxi and T4 mean were significantly lower in the fetuses from the gilts in the three groups fed LGR.

Table 2. Weight of the organs of the gilts and fetuses(1)

RSM level	0%	7%	14%	20%	Rsd	Sign
Gilts(2):						
Thyroid, g	12.96a	13.13a	15.81b	16.29b	4.7	***
Adrenals, g	6.90	7.22	7.25	6.85	1.35	NS
Liver, kg	2.10a	2.44b	2.68b	2.84c	0.37	***
Kidneys, kg	0.38ab	0.37a	0.39ab	0.40b	0.04	*
Fetuses:						
Thyroid, g	0.23a	0.27ab	0.32b	0.39c	0.13	***
Adrenals, g	0.20	0.19	0.18	0.17	0.04	NS
Liver, g	37.04a	32.68b	32.88b	31.46b	6.82	**
Kidneys, g	7.43	6.95	7.20	7.20	1.45	NS

(1) See Table 1.
(2) Organ weights of the gilts are corrected for net weight.

Gestation rate was not affected by the diet: 127 of the 160 gilts were pregnant at slaughter (30 to 34/group). Their liveweight at slaughter and their carcass net weight were also similar. Thyroid and liver weight in the gilts increased linearly with the LGR level in the diet, whereas that of adrenals was not affected (Table 2). The effect of LGR on kidney weight was less obvious. All the fetuses were dissected (1306 fetuses, i.e. 320 to 342/group). The weight of their thyroid increased linearly with LGR level. The weight of the liver from fetuses of gilts fed rapeseed meal was lower than that from fetuses of control gilts, whereas adrenal and kidney weight was not affected.

Prolificacy of the gilts was not affected by the introduction of LGR in the pregnancy diet. The number of normal or mummified fetuses and the embryonic mortality measured at the end of pregnancy were similar in the four groups (Table 3). On the opposite, fetus mean weight decreased linearly as LGR level in the diet increased, from 1103 g in the control group to 986 g when the dams were fed 20% LGR. Measurements of the uterus were also affected: total uterus length increased linearly with LGR level whereas uterus weight was lower. As a consequence, the uterus weight/length ratio was lower in the gilts fed RSM compared to the control ones.

Table 3. Reproductive performance and uterus measurements (1)

RSM level	0%	7%	14%	20%	Rsd	Sign
Ovulation rate	17.9	16.7	18.0	17.8	2.9	NS
Live fetuses	10.1	10.2	10.6	10.3	2.9	NS
Mummified fetuses	0.5	0.7	0.7	0.6	1.1	NS
Embr. mortality %	41.3	40.9	40.0	40.5	15.9	NS
Fetus weight, g	1103	1035	1027	986	182	·
Placenta weight, g	234	225	231	219	69	·
Uterus length, m	3.05a	3.28ab	3.37b	3.55b	0.55	**
Uterus weight, kg	4.06a	3.35b	3.82ab	3.53ab	1.08	*
Ut. weight/length	1.34a	1.04b	1.16b	1.01b	0.34	***

(1) See Table 1.
Numbers of fetuses alive or mummified and embryonic mortality are corrected for ovulation rate.

DISCUSSION

The method used for the measurement of thiocyanate levels is not specific, as indicated by the SCN found in the plasma from control sows. However, the linear SCN increase with LGR level in the diet suggests that the amount of thiocyanates which cross the digestive tract is related to the quantity of dietary glucosinolates. Moreover, they can act early in pregnancy since plasma levels at 7 days of pregnancy are similar to those measured 15 weeks later. Thyroid weight of the gilts increases in the same way as SCN level, and this effect is significant from 14% LGR in the diet. In growing pigs, Bourdon and Aumaître (1990) also found a linear relationship between the thyroid weight and the total quantity of glucosinolates ingested. They also showed a similar relationship for liver weight similar to that obtained in this experiment. Liver hypertrophy could be linked to the detoxification processes which occur in this organ. Plasma level of T3 and T4 in the gilts was similar in the four groups. Thyroid hypertrophy, which is proportional to LGR intake, enabled to compensate for its dysfunction even when gilts were fed diets with 20% LGR during 16 weeks. The lower iodine level in the plasma from gilts fed rapeseed meal however suggests that due to the metabolites derived from glucosinolates which are absorbed, more iodine is needed by the enlarged thyroid in order to synthetize thyroid hormones in a normal range. Dourmad and Etienne (1987) found similar results in gilts fed diets with 20% HGR or LGR and concluded that the sows themselves seem not very affected by rapeseed meal intake.

In the fetuses as in their dams, thyroid hypertrophy is related to the LGR level in the diet, but this effect is more important in the progeny: compared to the control group, thyroid weight was multiplied by 1.7 in the fetuses from the 20% group instead of 1.3 in the gilts. The comparison with the weight of adrenals, which is taken as a control for variation of endocrine gland weight, strengthens this conclusion. In a given group, T4 variation between the fetuses with the biggest and the smaller thyroid is limited in comparison with the variation between groups. As indicated by plasma T4, thyroid secretion appears then relatively independant from its size. When comparing the fetuses in the four groups, thyroid function however is affected by rapeseed meal intake: as LGR percentage in the diet increases, thyroid becomes more hypertrophied and plasma T4 is more decreased. Plasma T4 is not related to plasma iodine in the fetuses, suggesting that iodine is not limiting for T4 synthesis. Since plasma levels of thyroid hormones in the gilts are similar in the four groups, it can be inferred that maternal T4 transfer to the fetus is limited in the pig. Similar conclusions were drawn in the rat, sheep and human (Thomas and Nathanielsz, 1983). On the opposite, some of the goitrogenic compounds derived from glucosinolates seem able to cross the placental barrier as shown by the effects on thyroid and T4. The fetuses appear more sensitive than gilts to these compounds since they are hypothyroidic. This effect is evident even at the 7% LGR level and becomes more important when higher levels are used in the pregnancy diet. Liver weight in fetuses decreased when LGR increased, contrarily to gilts. This effect was mainly related with the decrease of fetus weight. Compounds responsible for liver hypertrophy in gilts may not cross the placental barrier or detoxification processes could be different or non existant in fetuses.

Whatever the LGR level in the diet, no effect was found on the number of fetuses alive or mummified nor on embryonic mortality. This contrasts with our earlier results which showed an increased embryonic mortality in the gilts fed 20% HGR or LGR (Etienne and Dourmad, 1987). In that previous experiment, there was no relationship between embryonic mortality and thyroid hypertrophy. It was then suggested that embryonic survival was affected beyond a glucosinolate threshold level or that the compounds implicated were different from those responsible for thyroid dysfunction. An explanation of the discrepancy between the two experiments should be that the LGR variety used here is different and has a lower glucosinolate content than that used earlier. Furthermore, fetus and placenta weight decrease linearly between 0 and 20% LGR, the difference in fetal body weight being 11% between the extreme groups. It is noticeable that in gilts fed LGR, weight/length of uterus is decreased, which means that uterus thickness is smaller. More information should be necessary in order to know which tissue is concerned and the mechanisms involved. However, such an effect could mean that the nutritional exchanges between the dams and their fetuses were decreased, or that the secretory activity of the uterus, which is greatly important for embryo survival and development during early pregnancy (Pope et al., 1989), is lowered. Another explanation for the decrease of fetus weight could lie in their altered thyroid function. Thyroid hormones have been shown to affect fetal development. For instance, thyroidectomy in fetal sheep at mid-pregnancy decreases by 33% its birthweight (Hopkins and Thornburn, 1972). Moreover, due to their impaired thyroid function, iodine-deficient fetal mammals have lower prenatal and perinatal survival and retardation in brain and body development (Hetzel and Mano, 1989). The same mechanism should be involved in the present case since fetal growth decrease parallels to that of T4 and to the amount of glucosinolates ingested by the gilts.

CONCLUSIONS

Fetal pigs are highly sensitive to the goitrogenic properties of rapeseed meal: their thyroid hypertrophy is greater than in their dams and their thyroid function is impaired. These effects are evident even at the 7% level of LGR in the diet. At this level, body weight of fetuses aged 111 days is decreased by about 70 g. Such varieties of LGR then cannot be advised in sow pregnancy diets. It appears now of importance to know if the new french varieties of LGR with a very low glucosinolate content can be used without such detrimental effects.

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