CONCENTRATIONS OF ISOFLAVONES IN BLOOD PLASMA OF DAIRY COWS WITH DIFFERENT INCIDENCE OF SILENT HEAT

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Abstract

The study was carried out on 3 high-yielding dairy herds in the north-east of Poland. Blood samples were collected from 20 cows in each herd. The blood plasma concentrations of total isoflavones (free and conjugated daidzein, genistein, and their metabolites equol and p-ethylphenol) were determined using HPLC. The incidence of silent heat was 7.0% in herd I, 40.9% in herd II, and 35.0% in herd III. The total concentrations of isoflavones were significantly higher (P ≤ 0.001) in herds II (4.9 ± 0.5 µmol/l) and III (2.6 ± 0.9 µmol/l) than in herd I (1.0 ± 0.3 µmol/l). The results suggest that there might be a relationship between concentration of isoflavones in blood plasma and incidence of silent heat in cattle.

Key words: dairy cows, silent heat, isoflavones.

Phytoestrogens include a wide variety of plant products with weak oestrogenic activity. The plants shown to contain high amounts of phytoestrogens are legumes, e.g. the clovers, lucerne, and soya beans. The most important oestrogenic compounds in legumes are isoflavones, mainly genistein and daidzein (3, 7). They can bind to oestrogen receptors and cause oestrogenic effects (6). The ingestion of clover pasture, containing high levels of isoflavones, causes clinical oestrogenism in sheep, called “clover disease” (1, 9). Cattle seem to be less sensitive to isoflavones than sheep and reports of infertility in cattle on phytoestrogens are uncommon (1, 5).Isoflavones may also act as antioestrogens, depended on the ratio of phytoestrogens to endogenous oestrogens (2). Inhibitory effects of isoflavones on LH secretion (12) and aromatase activity (15) were also described. Thus, it is likely that they may play a role in the development of silent heat in cattle.

Silent heat is defined as the lack of behavioural oestrus symptoms although the genital organs are undergoing the normal cyclical changes. It is the main reason of post-partum anoestrus in dairy cows causing elongation of service period and, in consequence, substantial economical losses (13, 19). The incidence of silent heat varies from 10 to 40% among different herds. It is also influenced by heat detection, energy deficiency, housing conditions and milk yield (10, 14, 16, 17). Silent heat is a common problem in high-yielding dairy herds (14, 20). In such cows soya becomes an increasing importance for supply of protein.

Very little research has been conducted on the levels of isoflavones in blood plasma of cattle (4, 8). No studies have been carried out on the relationship between isoflavones and silent heat in cattle.

The aim of this study was therefore to determine the concentration of isoflavones in blood plasma of dairy cows in 3 herds with different incidence of silent heat.

Material and Methods

Animals. The study was carried out on 60 Polish Black and White breed cows from 3 dairy herds in the north-east of Poland. The average number of cows in the herds ranged from 60 to 100. Cows were kept indoors and fed total mixed ration, consisting grass, corn silage and concentrates or soya. The average milk yield was about 7000 kg per year. Oestrus detection was carried out 3 times daily. Cows which showed no visible oestrus signs until day 60 postpartum were clinically examined two times, at a 10-d interval.

Blood samples were collected from 20 randomly chosen cows in each herd from the tail vein into heparinised evacuated tubes. The blood samples...
were immediately centrifuged and plasma was stored at –20°C until assayed for isoflavones.

**Determination of isoflavones and their metabolites.** Plasma concentration of isoflavones and their metabolites were measured as follows: daidzein, genistein, and their metabolites, equol and p-ethylphenol, respectively, were determined on HPLC after enzymatic hydrolysis and extraction from blood plasma. Plasma samples (50 µl) were mixed with 50 µl of sulfatase type H-5 (Sigma) solution in 0.2 mol/l acetate buffer, pH 5 (the preparation contained 500 U of β-glucuronidase per 25 U of sulfatase), and the mixture was incubated at 37°C for 1 h in a shaking water bath. Isoflavones released during the incubation and their non-conjugated forms present in plasma before the hydrolysis were extracted with 900 µl of methanol/acetic acid (100:5, v/v). The mixture was vortexed for 30 s, sonicated for 30 s, again vortexed for 30 s and centrifuged for 5 min at 4°C and 5000 x g. The supernatant was diluted with 100 mmol/l of lithium acetate (1:1, v/v), centrifuged for 2 min at 4°C and 5000 x g, and 20 µl was injected onto a HPLC column (TSKgel ODS-80TS, 5 µm, 150 x 4.6 mm, TOSOH, Tokyo, Japan). The flow of the mobile phase, containing water/methanol/acetic acid (58:40:2, v/v/v) with 50 mmol/l of lithium acetate, was 0.9 ml/min. The eluate was monitored with an amperometric detector (ICA-3062, TOA, Tokyo, Japan) with the working potential set at +950 mV. When necessary, samples were diluted with the mobile phase before HPLC analysis. The result was the total plasma concentration of daidzein, genistein and their respective metabolites. Quantitative calculations were done with external standard method.

**Statistical analysis.** The differences in concentrations of isoflavones among herds were analysed using Student’s t-test.

**Results**

The incidence of silent heat was 7.0% in herd I, 40.9% in herd II, and 35.0% in herd III (Fig. 1).

![Fig. 1. Incidence of silent heat in cows the investigated herds.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Herd</th>
<th>Daidzein µmol/l</th>
<th>Genistein µmol/l</th>
<th>Equol µmol/l</th>
<th>p-ethylphenol µmol/l</th>
<th>Total µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>nd</td>
<td>nd</td>
<td>0.1±0.1c</td>
<td>0.9±0.3c</td>
<td>1.0±0.3c</td>
</tr>
<tr>
<td>II</td>
<td>0.2±0.2</td>
<td>0.1±0.1</td>
<td>1.0±0.3d</td>
<td>3.6±1.9d</td>
<td>4.9±0.5de</td>
</tr>
<tr>
<td>III</td>
<td>nd</td>
<td>tr</td>
<td>0.1±0.1c</td>
<td>2.5±0.9d</td>
<td>2.6±0.9df</td>
</tr>
</tbody>
</table>

± SD; nd – not detectable, tr – trace  
a,b – values in the same columns differ significantly (P ≤ 0.05)  
c,d; e, f – values in the same columns differ significantly (P ≤ 0.001)

The results of isoflavone determination in blood plasma are shown in Table 1. The concentrations of daidzein and genistein in herd II were 0.2 ± 0.2 µmol/l and 0.1 ± 0.1 µmol/l, respectively. In herds I and III they were very low (not detectable amounts or traces). The concentration of equol was significantly higher (P ≤ 0.001) in herd II than in herds I and III (1.0 ± 0.3 vs 0.1 ± 0.1 µmol/l). The concentrations of p-ethylphenol varied from 0.9 to 3.6 µmol/l. The total concentrations of isoflavones was significantly higher (P ≤ 0.001) in herds II (4.9 ± 0.5 µmol/l) and III (2.6 ± 0.9 µmol/l) than in herd I (1.0 ± 0.3 µmol/l).
Discussion

In the present study, incidence of silent heat ranged from 7.0 to 40.9%. This agrees with the results of previous studies, in which 10 to 40% cows were affected by silent heat (14, 20).

The total concentration of isoflavones in bovine blood plasma obtained in this study was lower than has been reported by Braden et al. (4) and Lundh et al. (8) for cattle fed red clover. The measured total isoflavones were represented mainly by the metabolite of daidzein - equol and the metabolite of genistein - p-ethylphenol. Phytoestrogens are metabolized by the rumen microorganisms (11). Equol has oestrogenic activity and according to Shutt and Braden (18) is the main substance responsible for reproductive dysfunctions in animals, whereas p-ethylphenol is non-oestrogenic.

The concentration of isoflavones was significantly higher in herds with high incidence of silent heat than in herd with low incidence of it. Our results suggest that there is a relationship between isoflavones and silent heat in cattle. Isoflavones could inhibit oestrus symptoms by influencing oestrogen synthesis and development of ovarian follicles. Pelissero et al. (15) showed that isoflavones inhibit in vitro the activity of aromatase, a key enzyme of oestrogen biosynthesis. Nwannenna et al. (12) found that phytoestrogens reduce the release of LH in response to GnRH in heifers.

The silent heat is a multifactorial phenomenon. The high rate of silent heat is mainly a result of bad oestrus detection (10, 14). Schopper et al. (17) and Ras (16) showed that negative energy balance in high yielding cows is related to the elevation of progesterone during oestrus and inhibition of oestrus behaviour. Silent heat is also influenced by milk yield and housing conditions (10, 14).

Future investigations using more animals will be needed before the hypothesis that isoflavones could depress expression of oestrus symptoms in cattle can be accepted as valid.

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References