Risk Assessment and Consequences of Retained Placenta for Uterine Health, Reproduction and Milk Yield in Dairy Cows

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Abstract

The objective of the study was to determine the value of metabolic indicators characterising the energy and acid-base metabolism of dairy cows for predicting the risk of retained placenta (RP). The connection between RP and different factors analysed, and the effect of RP on the development of puerperal metritis (PM) as well as on reproduction and milk production was studied. A total of 105 Holstein-Friesian cows were included and sampled between days -14–0 prepartum and then on days 4, 10–14, 28–35, 56–63 and 84–91 postpartum, for metabolic tests. From day 4, at times coinciding with the days of metabolic sampling, uterine involution and metritis were monitored by clinical examination, and from days 28–35 ovarian activity was monitored by ultrasonography. On days -14–0, the plasma non-esterified fatty acids (NEFA) concentration showed a positive Odds Ratio (OR) 102.1, \(P < 0.05\); while urinary net acid-base excretion (NABE) exhibited a negative correlation (OR 0.99; \(P < 0.05\)) with the odds of RP. NEFA and NABE were negatively correlated (Pearson’s coefficient: -0.24; \(P < 0.05\)). Ketonuria of grade ≥ 2+ increased the probability of RP (OR: Infinite; \(P < 0.05\)). On day -14–0 prepartum, elevated plasma NEFA concentration, decreasing urinary NABE and grade ≥ 2+ ketonuria indicates a higher risk of RP. The odds of RP were not influenced by parity, sex and viability of the calf, and the calving assistance. RP increased the risk of PM (OR: 27.3; \(P < 0.0001\)). The RP alone did not exert an influence on the metabolic status, reproductive performance or milk production of cows.

Risk indicators, energy balance, acid-base metabolism, milk production

In recent decades, the milk production of dairy cows has increased steadily, while their reproductive performance has declined. This is mostly due to production diseases, commonly occurring in the peripartal period (Brydl et al. 2003, 2008; Ingvartsen 2006; Thatcher et al. 2006). Retained placenta (RP) is an important disorder of dairy cows, which occurs in 4 to 18% of calvings (Han and Kim 2005). The aetiology of RP is not completely clear. Factors contributing to its occurrence include calving-related factors such as dystocia, stillbirth, twinning, length of gestation (Correa et al. 1993; Han and Kim 2005), parity (Correa et al. 1993), and certain nutritional problems (Laven and Peters 1996). In other cases, no association was found between dystocia (Curtis et al. 1985), parity (Han and Kim 2005) and the development of RP. Oxidative stress may contribute to RP as well. For this reason, the beneficial effect of adequate supplementation with known nutrients (e.g. selenium, zinc, copper, iron and vitamin E, A and C, etc.) required for antioxidant defence has been reported in some (Trinder et al. 1973; Harrison et al. 1984; Miller et al. 1993), but not all (Hidiroglou et al. 1987; Stowe et al. 1988) previous studies.

Few data are available on the role of metabolic status preceding the development of RP, more specifically, the disturbances of energy balance and acid-base metabolism as...
risk factors for RP. It is known that in the peripartal period, dairy cows have a negative energy balance (NEB). The available energy amount depends on the dry matter intake and utilisation, the latter being determined by the rumen microflora (Grummer 1995) and adaptive changes of the ruminal epithelium, which depend on the acid-base conditions within the rumen (Dirksen et al. 1999). Optimally, the majority of cows adapt to the NEB over a longer period of time during a multi-step process consisting of a series of physiological, metabolic and endocrine changes (Ingvarstsen 2006). This adaptation process can be followed with the help of indicators such as plasma non-esterified fatty acids (NEFA), acetoacetic acid (AcA), certain metabolic hormones, urinary net acid-base excretion (NABE) (Huszenicza et al. 2002; Brydl et al. 2003; Jorritsma et al. 2003). Deviations from the physiological range indicate disturbances of this adaptation mechanism and, therefore, they can be defined as risk factors or risk indicators.

NEB may develop already at the end of gestation (Brydl et al. 2003, 2008; Grummer et al. 2004) and it may contribute to the development of many production diseases such as the fatty liver syndrome and ketosis (Bertics et al. 1992), dystocia (Zamet et al. 1979), or immunosuppression (Goff 2003). Overcondition (Hayirli et al. 2002) as well as management defects and environmental factors resulting in reduced dry matter intake increase the risk of development of prepartum NEB (Grummer et al. 2004). RP has a decisive role in the development of metritis (Kaneene 1995), but ketosis occurring at the early stage of lactation is also a major risk factor (Huszenicza et al. 1998). RP increases the risk of fatty liver syndrome and ketosis (Han and Kim 2005); the latter, in turn, delays the postpartum resumption of cyclic ovarian function and prolongs the interval from calving to first ovulation (Huszenicza et al. 1998; Opsomer et al. 2000). RP and puerperal metritis (PM) prolong the interval from calving to first service and conception and decrease the conception rate (Maizon et al. 2004; Han and Kim 2005).

Few sources are available in the literature on strategies for the prevention of production diseases. Early diagnosis and prevention require a thorough knowledge of the risk factors predisposing to their development (Jorritsma et al. 2003; Ingvarstsen 2006; Mulligan et al. 2006).

The objective of this study was to obtain a better understanding of the above risk factors predisposing cows to production diseases. Certain metabolic indicators may be suitable for predicting the risk of RP and serve as a basis for the development of preventive and monitoring strategies for practice. For this reason, the correlations between RP and indicators characterising the energy balance and acid-base status of dairy cows as well as certain environmental and animal-dependent predisposing factors were studied. In addition, the effects of RP on the development of puerperal metritis as well as on metabolic status, reproductive performance, and milk production were analysed.

Materials and Methods

Characteristics of the herd studied

The farm serving as the study site had a dairy herd comprising 730 Holstein-Friesian cows. Milk production of the 305-day lactation was 8,000 kg, with 3.6–3.7% butterfat and 3.1–3.2% milk protein content. In the previous year, the calving interval was 433 days, the calving to conception interval was 168 days, the number of services per conception was 3.3, the interval from calving to first service was 97 days, and the pregnancy rate to first service was 43%. The rate of losses due to mortality, and emergency slaughter was 9.7%, and the total sorting out rate was 31.4%. The farm used loose housing system and the cows were kept in groups, in houses with deep litter and having outdoor pens. Groups were formed by considering the serial number of lactations, the milk production level, and the lactation and pregnancy status of the cows. The calving barn was of loose system, with the cows kept in small groups. In the two weeks before calving, in the calving barn and in the first month of lactation the primiparous and multiparous cows were kept together, while from the peak of lactation they were kept in separate groups. The daily ration fed to cows was formulated with the help of a ration-optimising programme (AminoCow 3.0), by taking into consideration the composition of forages determined by feed analysis, the lactation and pregnancy status, the milk production, and the body condition score. The calculated nutrient content of the total mixed ration (TMR), distributed to the cows twice a day met the requirements (NRC, 2001).
Study design
A total of 105 clinically healthy Holstein-Friesian cows two weeks before the expected calving were included in the study series. The studies comprised the following elements:

Metabolic tests
To study the energy balance and acid-base status, blood and urine samples were taken for laboratory tests. The samples were taken 3–5 h after the morning feeding. Blood samples were taken from the vena epigastrica superficialis into test tubes containing 0.05 ml sodium heparin as anticoagulant. Urine samples were obtained by catheterisation of the urinary bladder. The samples were kept at +4 °C and transported to the laboratory within 4 h. Samples were taken on day -14–0 before calving and on days 4, 10–14, 28–35, 56–63 and 84–91 postpartum.

Test indicators and methods
Energy metabolism
Blood plasma: acetoacetic acid (AcA): specific spectrophotometric determination by diazotation of the compound; non-esterified fatty acids (NEFA) by photometric method (Unicam Helios Gamma, Unicam Ltd., UK); aspartate aminotransferase (AST) activity (Human 12021); glucose (G) (Human 10121) (Autohumalyzer 900 S Plus, Human GmbH, Germany).

Urine samples: AcA: semi-quantitative determination using a reagent strip containing sodium nitroprusside (Ketostix®, Bayer Corp., USA).

Body condition score (BCS): 5-point condition scoring system (Mulvany 1977).

Acid-base metabolism: Urine pH (Radelkis OP211/1, Radelkis, Hungary); net acid-base excretion (NABE): titrimetric method (Kutas 1965).

Clinical examinations and data collection related to calving and health status
The data on parity, date of calving and the use of calving assistance (0: no; 1: yes), viability (1: normal; 2: dead calf; 3: mortality in 24 h) and sex (1: male; 2: female) of the calf were recorded with the use of codes marked in brackets here. During the first 14 days postpartum, the rectal temperature and the clinical diseases were recorded daily. Retained placenta was diagnosed if spontaneous expulsion of the foetal membranes did not occur within 24 h after calving.

Monitoring uterine health and involution
To diagnose puerperal metritis and to monitor uterine involution, rectal and clinical examinations were performed on days 4, 10–14 and 28–35 postpartum.

Diagnosis of puerperal metritis, definitions
Puerperal metritis (PM) was diagnosed if on days 4 or 10–14 postpartum the uterus contained a large volume of foul-smelling, reddish-brown, dilute discharge mostly containing necrotic tissue debris (putrid discharge) and the uterine wall had become thinner than normal. A similar diagnosis was established in cases when there was less abundant, putrid, purulent uterine discharge but the wall of the uterus was thickened and oedematous. The disease may be accompanied by fever (≥ 39.5 °C) and systemic signs (dullness, lack of appetite), as well (Sheldon and Dobson 2004; Sheldon et al 2006).

Characterisation of uterine involution
To characterise the anatomical involution of the uterus, a scoring system (U1–U3 score) was used (Becze and Weckerle 1980) on days 28–35 postpartum as follows:
U1: the uterus is of normal shape and tone, indicative of an involution of normal course; the uterus becomes erect at oestrus. U2: the uterus is slightly enlarged, has slightly descended towards the abdominal cavity, it is flaccid, pasty to touch. There may be abnormal catarrhal discharge in its lumen. This is indicative of mild subinvolution. U3: the uterus is enlarged, has descended into the abdominal cavity, it is flaccid and palpable, sometimes with abnormal content. This is indicative of severe metritis and parametritis, pyometra, and severe subinvolution. The transition forms were scored with 0.5 accuracy. Subinvolution was diagnosed if on days 28–35 postpartum the uterine score was ≥ 2.

Study of the ovaries and the oestrous cycle
In order to characterise ovarian function and the structures present on the ovaries, rectal examination of the ovaries was performed on days 28–35, 56–63 and 84–91 postpartum using an ultrasound scanner (ALOKA 7.5 MHz). The presence of follicles representing various stages of development (F1 - 3, 2–5 mm to 10–18 mm), a developing or florescent corpus luteum (CL) or a regressive corpus luteum (RCL) was studied. The presence of follicular cysts (a fluid-filled, thin-walled structure > 20 mm in diameter) and luteal cysts (a thick-walled structure containing a fluid-filled cavity) was also studied and recorded. Ovarian function was considered cyclic if a CL or RCL was present.

Data on reproduction and milk production
The dates and results of artificial inseminations (AI), the calving to conception interval, and the serial number of the successful AI were recorded. The quantity of milk produced on day 4 (kg), the quantity of milk, butterfat and milk protein (kg) and the butterfat and milk protein percentage produced during the first 100 days of lactation and, in the case of multiparous cows, the milk production in the previous 305-day lactation (kg) were also recorded.
Statistical analysis
In order to select the metabolic factors predicting the risk of development of RP, the relationship between metabolic indicators measured on days -14–0 prepartum and RP was analysed by logistic regression, where each variable was examined individually in the model (Everitt and Hothorn 2006). Differences between diseased and healthy groups of cows in terms of metabolic, reproductive and milk production indicators were studied by Mann-Whitney test (Hollander and Wolfe 1999). The correlation between BCS and metabolic indicators (NEFA) was quantified by Kendall’s rank correlation test (Valz and Thompson 1994), while the interrelationships of metabolic indicators (NABE-NEFA) were analysed by Pearson’s correlation (Reiczigel et al. 2007). The independence between the RP and parity, viability and sex of the calf, the provision of calving assistance, puerperal metritis, subinvolution and ovarian cysts was tested by Fisher’s exact test (Agresti 1990). The level of significance was set at $P < 0.05$.

Results

Metabolic status - retained placenta
Twenty-one out of the 105 cows included in the study had RP (20.0%). Of the indicators characterising energy metabolism in the two weeks (-14–0 days) before calving, NEFA concentration of the blood plasma showed a significant positive correlation with the development of RP ($P < 0.05$). A 1.0 mmol/l elevation of blood plasma NEFA concentration resulted in a 102-fold increase in the odds of RP development (Table 1, Fig. 1). The occurrence of RP did not depend significantly on prepartum blood plasma glucose and AcA concentrations and AST activity (Table 1).

A prepartum ketonuria of at least 2+ degree significantly increased the probability of RP (OR: Infinite; CI: 1.18 - Infinite; $P = 0.038$).

Decrease of the BCS between days -14–0 prepartum and day 4 postpartum increased the occurrence of RP, but the correlation was not significant (OR: 1.3; CI: 0.43 - 4.2; $P = 0.621$). At the same time, for the entire period studied (days -14–0 to days 84–91) a mild positive significant correlation was demonstrated between the BCS and the NEFA concentration (tau: 0.153; $P < 0.0001$).

Of the indicators of acid-base metabolism, urine pH and NABE measured on days -14–0 prepartum showed a negative correlation with the odds of RP; for NABE the correlation was significant (OR: 0.99; CI: 0.98 - 1.00; $P = 0.032$) (Table 1, Fig. 2). Urine NABE and blood plasma NEFA concentration showed a significant negative correlation with each other before calving.

Table 1. Relationship between metabolic indicators recorded between days -14–0 before calving and retained placenta (results obtained by logistic regression)

<table>
<thead>
<tr>
<th>Metabolic indicators</th>
<th>Retained placenta</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>OR; (95% CI); $P$</td>
</tr>
<tr>
<td>Plasma G (mmol/l)</td>
<td>0.96; (0.49–1.87); 0.90</td>
</tr>
<tr>
<td>Plasma AcA (mmol/l)</td>
<td>(0.00–4.25); 0.94</td>
</tr>
<tr>
<td>Plasma NEFA (mmol/l)</td>
<td>102.12; (1.51–6906.54); &lt; 0.05</td>
</tr>
<tr>
<td>Plasma AST (U/l)</td>
<td>1.00; (0.98–1.03); 0.84</td>
</tr>
<tr>
<td>Urine pH</td>
<td>0.81; (0.46–1.41); 0.46</td>
</tr>
<tr>
<td>Urine NABE (mmol/l)</td>
<td>0.99; (0.98–1.00); &lt; 0.05</td>
</tr>
</tbody>
</table>

OR: odds ratio, CI: confidence interval, $P$: significance, G: glucose, AcA: acetoacetic acid, NEFA: non-esterified fatty acids, AST: aspartate-aminotransferase, NABE: net acid-base excretion
The indicators characterising the energy balance (plasma glucose, AcA, NEFA, AST and BCS) and acid-base balance (urine pH, NABE) of RP+ and RP- animals were not significantly different when studied on days 4, 28–35, 56–63 and 84–91 postpartum. Details are not shown here.

Animal-related and environmental factors - retained placenta

The odds of RP were not influenced significantly by parity ($P = 0.85$), sex (OR: 0.95; CI: 0.32 - 2.77; $P = 1$) and viability ($P = 0.83$) of the calf, and the provision of manual calving assistance (OR: 0.63; CI: 0.24 - 2.19; $P = 0.75$).

Retained placenta - reproductive performance

RP did not exert a significant influence on whether the CL/RCL structures indicative of cyclic ovarian function could first be found on the ovaries on days 28–35, 56–63 or 84–91 postpartum. RP had no significant influence on the rate of re-conception (OR: 1.10; CI: 0.35 - 3.93; $P = 1$) or on the number of services required for pregnancy. The interval from calving to first service was about 3–7 days shorter in healthy cows than in cows with RP. For the multiparous cows and for all cows taken together, the interval from calving to first service was shorter in healthy animals. At the same time, primiparous cows with RP had a longer interval from calving to first insemination. These differences were not significant (Table 2). RP did not significantly affect the occurrence of subinvolution, follicular cyst or luteal cyst (Table 3). At the same time,
it significantly and greatly increased the odds of development of PM (OR: 27.3; CI: 4.0 - 126; \( P < 0.001 \)) (Table 3).

Forty-seven out of the 105 cows included in the study had PM (44.8%).

### Retained placenta - milk production

The effect exerted by RP on milk production was inferred from the comparison of RP+ and RP- groups. During the data analysis, primiparous and multiparous cows were evaluated both separately and collectively (Table 2). Milk production (kg) on day 4 postpartum of both primiparous and multiparous RP+ cows was lower than that of the RP- animals, but the difference was not significant. The milk production (kg) during the first 100 days of lactation of primiparous RP- cows was lower than, while that of the multiparous RP- cows exceeded that of the RP+ animals. For the primiparous cows the difference was significant \( (P < 0.05) \). Collective evaluation of the groups did not demonstrate significant differences in the 100-day milk production. The 100-day butterfat % of RP- cows was 0.2–0.4% higher than that of the RP+ animals, but the difference was not significant. As compared to RP+ cows, primiparous RP- cows had a lower, while multiparous RP- cows a higher, 100-day butterfat production; in this case the difference was significant for the multiparous cows \( (P < 0.05) \). Collective evaluation of the primiparous and multiparous cows did not reveal a significant difference between RP- and RP+ cows in 100-day butterfat production.

In milk protein production there was no major difference between the RP- and the RP+ groups. For the 100-day milk protein kg and milk protein % similar trends were observed as for milk and butterfat production (Table 2).

### Discussion

NEB developing at the end of gestation markedly increased the odds of developing RP. Elevated plasma NEFA concentration and \( \geq 2+ \) ketonuria measured on days -14–0 prepartum are in all probability suitable metabolic indicators for characterising the increased risk of RP because of NEB. The acidotic shift in acid-base balance before calving was indicated by the decreasing NABE in the urine. This acidotic shift might also have contributed to the prepartum development of NEB, which is supported by the significant negative correlation found between plasma NEFA and urine NABE. It is known that the amount of energy available for the body depends, besides dry matter intake, on the rumen microflora (Grummer 1995) and on adaptive changes of the ruminal epithelium determining the utilisation, which depend on the acid-base status (Dirksen et al. 1999). In the last two weeks before calving, the decreasing urinary NABE in itself indicated the increasing risk of development of RP. Prepartum feed rich in starch and concentrate and poor in fibre increases the risk of ruminal acidosis (Brydl 1995) and, consequently, NEB (Holtenius et al. 2003). The conclusion presents itself that although at the end of gestation the dry matter intake decreases also under physiological conditions (Bertics et al. 1992), factors negatively affecting dry matter intake and/or disturbing the process of ruminal adaptation increase the chance of development of prepartum NEB and, consequently, RP. In addition to nutrition inducing ruminal acidosis, further risk factors may include overcondition (Hayirli et al. 2002), numerous management failures, certain environmental factors such as discomfort (Grummer et al. 2004) or heat stress (De Rensis and Scaramuzzi 2003).
The course of the solving of the junction between maternal and foetal placenta starts about two weeks before parturition. The phagocytic and resorptive capacity of polymorphonuclear (PMN) neutrophils present in the crypts plays an important role in this mechanism. Decreased neutrophil function is one of the possible causes of RP (Kimura et al. 2002). The NEB manifested in increased plasma levels of NEFA and beta-hydroxybutyrate impair the migration, phagocytic and killing activity and/or the oxidative burst of PMN and other leukocytes (Goff 2003; Sartorelli et al. 1999, 2000; Suriyasathaporn et al. 2000; Zerbe et al. 2000).

When studied alone, RP usually exerted a negative influence on the reproductive indices but significant correlations were not found, which is consistent with the findings reported by other authors (Muller and Owens 1974; Coleman et al. 1985; Halpern et al. 1985; Kaneko et al. 1997). In our study - in agreement with others (Paisley et al. 1986; Hussian 1989; Levis 1997; Sheldon and Dobson 2004; Han and Kim 2005) - the RP presents an outstanding risk for the development of PM. Therefore, the negative effects of RP may become significant primarily because of their role in facilitating the development of PM, rather than on their own (Stevenson and Call 1988; Laven and Peters 1996; Han and Kim 2005; Könyves et al. 2009) The odds of the development of ovarian cysts was not affected by RP which is consistent with previous reports (Gröhn and Rajala-Shultz 2000; Han and Kim 2005). However, Erb et al. (1985) found an indirect association between the occurrence of ovarian cysts and retained placenta mediated by endometritis.

Our finding that the odds of developing RP were not affected substantially by parity, is consistent with some (Han and Kim 2005) but not other previous studies (Curtis et al. 1985; Erb et al. 1985; Gröhn et al. 1990; Correa et al. 1993) which reported an increased incidence of RP with advancing parity. The relatively low sample size could be a possible explanation of our findings. In our study the manual calving assistance did not increase the risk of RP. An explanation may be the appropriate assistance practice performed in the examined herd. Similar results were reported by Curtis et al. (1985) as veterinary assisted dystocia was not associated with higher incidence of RP.

The effects exerted on milk production are not explicit either. An explanation of the modest influences exerted on the reproductive and production indices may be that at the early stage of lactation studied by us RP did not significantly affect the metabolic status. However, others reported that RP is a predisposing factor for metabolic disorders in dairy herds (Curtis et al. 1985; Markusfield 1986; Han and Kim 2005). The NEB manifested in elevated plasma NEFA in the first 30 days of lactation predisposes to the delayed anatomic involution of the uterus (Könyves et al. - in press). The indirect negative effect of RP on milk yield mediated by puerperal metritis is reported by Könyves et al. (2009).

In conclusion, the odds of developing RP markedly increased in cows developing NEB and showing an acidotic shift in acid-base balance before calving. On days -14–0 prepartum, the elevated plasma NEFA concentration and ≥ 2+ ketonuria indicative of NEB and the decreasing urinary NABE suggestive of the acidotic shift of acid-base balance are metabolic indicators highly suitable for characterising the risk of development of RP. RP in itself did not markedly affect the metabolic status between days 4 and 84–91 of lactation, nor did it influence reproductive performance and milk production in the given lactation. RP greatly increased the odds of development of puerperal metritis.

Hodnocení rizika retence placenty a jejích důsledků pro stav dělohy, reprodukci a mléčnou užitkovost dojnic

Cílem této studie bylo stanovení metabolických ukazatelů charakterizujících energetický metabolismus a acidobazickou rovnováhu dojnic pro zhodnocení zdravotního rizika spojeného s retencí placenty (RP). Zjištěna byla spojitost mezi RP a různými analyzova-
nými faktory, podobně jako vliv RP na vývoj puerperální metritidy (PM), na reprodukci a produkci mléka. Pro metabolické testy bylo vybráno celkem 105 holštýnsko-fríských krav, jimž byly odebírány vzorky krve v intervalech vyjádřených ve dnech před/po porodu: -14–0 prepartum, 4.den po porodu, 10–14, 28–35, 56–63 a 84–91 po porodu. Od 4. dne po porodu provázelo odběr vzorků krve pro metabolické ukazatele i klinické vyšetření - diagnostiku involuce dělohy popř. metritidy, a následně od období 28–35. dne p.p. byla taktéž ultrasonograficky sledována aktivita vaječníků. V předporodním období -14–0 se přímo úměrně zvýšila koncentrace nenasycených mastných kyselin (NEFA) v krevní plasme s pozitivní pravděpodobností výskytu RP (OR) 102.1, \( P < 0.05 \); zatímco acidobazický výluček moči (NABE) vykazoval ve vztahu k RP zápornou korelaci (OR 0.99; \( P < 0.05 \)). NEFA je nepřímo úměrná NABE (Pearsonův koeficient: 0.24; \( P < 0.05 \)). Přítomnost ketonů v moči dosahující úroveň ≥ 2+ zvýšovala pravděpodobnost RP (OR: \( \infty \); \( P < 0.05 \)). V předporodním období -14–0 signalizují zvýšené riziko zadržení placenty pozměněné ukazatele jako je zvýšená plasmatická koncentrace nenasycených mastných kyselin (NEFA), nižší hodnota acidobazického výlučku moči (NABE) a ketonurie ≥ 2+. Pravděpodobnost výskytu RP nebyla ovlivňována paritou matky, pohlavím nebo životností mláďat, ani přítomností odborné asistence u porodu. Retence placenty zvýšila riziko vzniku puerperální metritidy (OR: 27.3; \( P < 0.0001 \)). V této studii nebyl prokázán vliv RP na metabolické ukazatele, reprodukční potenciál ani produkci mléka u dojnic.

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