

## THE RELATIONSHIP BETWEEN DIET AND RETAINED PLACENTA IN COWS \*

Krunoslav ALŠIĆ<sup>a)</sup>, Matija DOMAČINOVIĆ<sup>a)</sup>, Željko PAVIČIĆ<sup>b)</sup>, Željko BUKVIĆ<sup>a)</sup>,  
Mirjana BABAN<sup>a)</sup> and Boris ANTUNOVIĆ<sup>a)</sup>

<sup>a)</sup> Josip Juraj Strossmayer Univ. of Osijek, Fac of Agriculture, Dept. of Animal Husbandry, Trg svetog Trojstva 3, HR-31000 Osijek, Croatia, e-mail: [bantun@pfos.hr](mailto:bantun@pfos.hr)

<sup>b)</sup> Univ. of Zagreb, Faculty of Veterinary Medicine, Dept. of Animal Hygiene and Ethology, Heinzelova 55, HR-10000 Zagreb, Croatia

### ABSTRACT

Retained placenta (RP) is one of the most common complications occurring in the post-partum period in cows. Beside other reasons, many authors stress the influence of diet on the incidence of RP. However, the role of minerals and vitamins fed at this time on subsequent fertility is poorly understood. As nutritional causes of RP are due primarily to the diet fed the last 6 to 8 weeks before calving, the aim of this paper was to investigate the relationship between diet in dry and transition periods and subsequent occurrence of RP, as well as in the time of introduction to lactation in fresh cows, with special emphasis on content of minerals and vitamins in diet. The investigation has been carried out on a big dairy farm situated on the east of Croatia with 1 775 Holstein-Frisian (HF) cows in duration of one year. Diet samples from dry period (DP), from period approximately from the 21<sup>st</sup> day before calving till the day of calving (BC) and period of introduction to lactation (IL) were subjected for analysis of calcium (Ca), phosphorous (P), selenium (Se), iodine (I) and vitamins A, D<sub>3</sub> and E. The observed rate of cows with RP on the investigating farm was rather high (16.55%). High diet concentrations both for Ca and P in DP and BC periods (0.98 and 0.62% of Ca; 0.37 and 0.52% of P, respectively), combined with low concentration of vitamin D in DP and BC periods (12 800 and 14 400 IU/day, respectively), as well as low concentration of Se in DP and BC periods (0.08 and 0.07 mg/kg, respectively) combined with low concentration of vitamin E in DP and BC periods (160 and 180 IU/day, respectively) were identified as critical factors that could have contributed in influencing high incidence of RP on the investigating farm.

Key words: cattle / dairy cows / animal nutrition / minerals / vitamins / reproduction / diseases / retained placenta

## POVEZANOST PREHRANE IN ZAOSTALE PLACENTE PRI KRAVAH

### IZVLEČEK

Zaostala placenta (ZP) je ena najpogostejših motenj pri kravah v poporodnem obdobju. Številni avtorji poleg drugih vzrokov poudarjajo vpliv prehrane na pogostnost ZP. Pomen mineralov in vitaminov v prehrani v tem obdobju za nadaljnjo plodnost je malo poznan. Ker so prehranski vzroki za ZP posledica prehrane v zadnjih 6 do 8 tednih pred telitvijo, smo v pričujoči raziskavi preučevali povezanost prehrane v času presušitve in v prehodnem obdobju s pojavom ZP, pa tudi v obdobju pred prvo laktacijo pri prvesnicah, s posebnim poudarkom na vsebnosti mineralov in vitaminov v krmu. Raziskava je potekala eno leto na veliki mlečni farmi s 1 775 kravami črno-bele pasme na vzhodu Hrvaške. V vzorcih obrokov iz obdobja presušitve (OP), iz obdobja od približno 21. tedna pred telitvijo do telitve (PT) in obdobja pred začetkom laktacije (ZL) smo določili vsebnost kalcija (Ca), fosforja (P), selena (Se), joda (I) in vitaminov A, D<sub>3</sub> in E. Delež krav z ZP je bil na preučevani farmi dokaj visok (16,55 %). Kritični dejavniki, ki bi lahko

\* The article is a part of graduation thesis (justification Jun 12<sup>th</sup>, 2003), supervisor Univ. Lecturer Boris Antunović, Ph.D., D.V.M.

vplivali na visok odstotek ZP na preučevani farmi, so bili visok delež Ca in P v PT in OP (0,98 oz. 0,62 % Ca; 0,37 oz. 0,52 % P), majhne količine vitamina D v OP in PT (12 800 oz. 14 400 IU/dan), pa tudi majhne količine Se v OP in PT (0,08 oz. 0,07 mg/kg) ter malo vitamina E v OP in PT (160 oz. 180 IU/dan).

Ključne besede: govedo / krave / molznice / prehrana živali / minerali / vitamini / reprodukcija / bolezni / zaostala placenta

## INTRODUCTION

Metabolic and reproductive disorders occurring in early lactation have an important impact on the performance of the cow later in lactation (Klerx and Smolders, 1997). Retained placenta (RP) is one of the most common complications occurring in the post-partum period in cows and is generally considered as a failure of expulsion of the placenta and associated membranes within 24 h of calving. Even application of hormonal therapeutics does not have beneficial effects on resumption of ovulation and fertility after calving in cows with RP if farm management and nutrition has not been improved (Grgurić *et al.*, 2000).

Most lately data (LeBlanc, 2008) worn on RP, metritis, and endometritis as diseases of immune function in the transition period, which begin at least two weeks prepartum. A number of vitamins and trace minerals are involved in the antioxidant defense system and a deficiency of any of these nutrients may depress immunity in transition cows (Spears and Weiss, 2008). Thus, the principle for prevention is to optimize peripartum immune function, principally through management to encourage feed intake in the transition period.

Maintenance of normal uterine physiology by good nutritional management during dry and transition periods is important to reduce the incidence of RP. As nutritional causes of RP are due primarily to the diet fed the last 6 to 8 weeks before calving, the aim of this paper was to investigate relationship between diet in dry and transition period and subsequent occurrence of RP.

## MATERIALS AND METHODS

The investigation has been carried out on a big dairy farm situated on the east of Croatia. The total number of cows at the time of investigation was 1 775 and all of them were Holstein-Frisian (HF) breed. The fertility rate was 80%, with 1 450 cows in calving annually. Incidence of RP was interpreted according to the number of cows. The cows in dry, transition and post-partum period were housed in tied barns bedded with straw. Other time, the cows were housed in free-stall barns bedded with wood shavings. Diet was offered *ad libitum* as a total mixed ration (TMR) three times daily at 6.00, 14.00, and 22.00 h, respectively. After calving, cows were milked three times daily while receiving diet, and had free access to drinking water throughout the whole investigating year. At the beginning of dry period (60 days before calving), cows were receiving vitamins A, D<sub>3</sub> and E by intra muscular administration of 10 ml AD<sub>3</sub>E solution (50 000 IU of vitamin A, 25 000 IU of vitamin D<sub>3</sub> and 10 mg of vitamin E per 1 ml of solution). Selenium or other minerals were not administered to the cows.

The cows fed diets according to their reproduction phase. The ratio in the fresh cows was composed of feedstuffs with regard to maintenance feeding requirements and additional requirements adjusted for production of 30 kg/milk/day. Feedstuffs were combined in three different ways as presented in Table 1.

The diets were representatively sampled nine times, starting at the time of 6 weeks before calving, and finishing at the time of 2 weeks after calving (one sampling per week). Three sampling per each period were used to calculate average for each period. The samples were

subjected for analysis of calcium (Ca), phosphorous (P), selenium (Se), iodine (I) and vitamins A, D<sub>3</sub> and E.

Table 1. Feedstuffs present in diet in different reproduction phases of cows (dry matter kg/day/cow)

Reproduction phase / Feedstuff	Dry period (DP) – from 6 weeks till 3 weeks before calving	Before calving (BC) – from 3 weeks before calving till the day of calving	Introduction to lactation (IL) – from the day of calving till 2 weeks after calving
Green corn	2.80	2.10	2.80
Corn silage	4.20	4.20	4.20
Lucerne silage	2.40	1.20	2.40
Lucerne hay	1.76	1.76	3.52
Concentrate *	-	2.61	6.96
Brever's grain	-	1.76	2.64
Straw	1.80	1.35	0.45
Vega-Pro® †	-	0.91	0.45
Helvecia Energia® ‡	-	0.91	1.36
Fosfonal® §	0.19	0.14	0.09
Premix **	0.19	0.28	0.09
Total	13.34	17.22	24.96

The content of minerals was determined by atomic absorption spectrophotometric (AAS) method, while the content of vitamins was determined by high-performance liquid chromatography (HPLC) method.

Recommended values for nutrition requirements in dairy cattle depending on reproduction phase were set up according to the National Research Council (NRC, 2001).

## RESULTS

RP was diagnosed in 240 cows (16.55%) during the investigation year from the total number of 1 450 cows in calving. Measured contents of minerals (Ca, P, Se and I) and vitamins (A, D and E) in relationship to recommended values in different reproduction phases of cows are presented in Fig. 4–10.

\* Concentrate balanced to 18% of crude proteins, containing corn, 50%, soybean meal 20%, sunflower meal 10%, premix (Valpmix®, Valpovka d.o.o., Croatia) 5%, barley 5%, wheat flour 5%.

† Proteine additive with 330 g/kg crude proteins, Ca 3.1 g/kg, P 7.7 g/kg (Helvecia Protein Trade, Hungary).

‡ Energetic additive with 20% of protected energy, Ca 1.5 g/kg, P 3.6 g/kg (Helvecia Protein Trade, Hungary).

§ N 9%, Ca 1%, P 18%, humidity 5%, F 0.25%, Fe 8.49 g/kg, K 3.30 g/kg, Na 4.12 g/kg, S 16.48 g/kg, Mg 5.53 g/kg, Zn 371 mg/kg, Cu 39 mg/kg (Petrokemija d.o.o., Croatia).

\*\* Vit. A 200 000 IU/kg, 38 000 IU/kg vit. D, 400 mg/kg vit. E, Fe 1 200 mg/kg, Ca 60 g/kg, P 55 g/kg, Mn 1 000 mg/kg, Zn 1 000 mg/kg, Cu 200 mg/kg, Co 5 mg/kg, Se 2 mg/kg, Mg 22 000 mg/kg, antioxidant 1 000 mg/kg, Na 70 g/kg, I 15 mg/kg, arome (citrus) 200 mg/kg, flour – difference to 1 000 g.

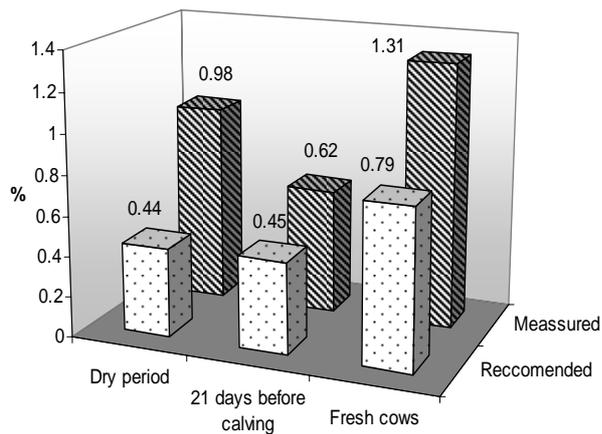


Figure 1. Concentration of Ca in diet, %.

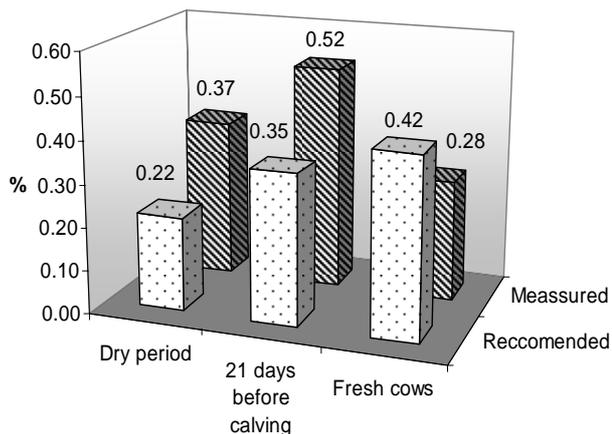


Figure 2. Concentration of P in diet, %.

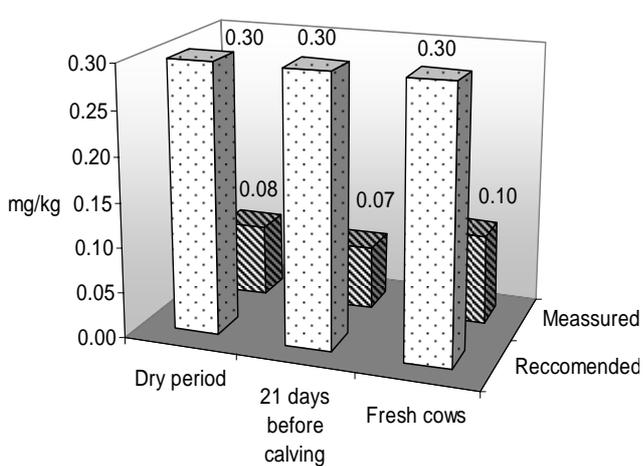


Figure 3. Concentration of Se in diet, mg/kg.

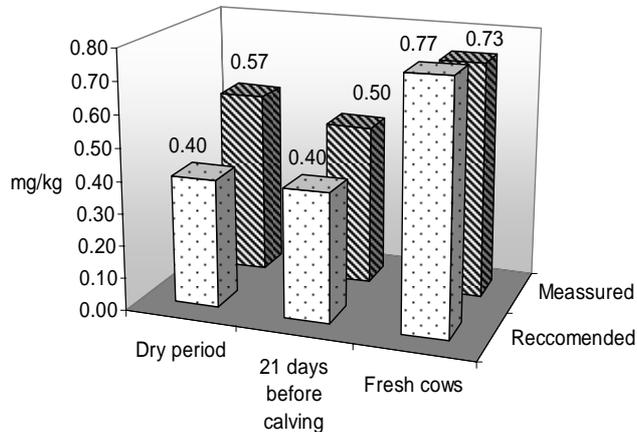


Figure 4. Concentration of I in diet, mg/kg.

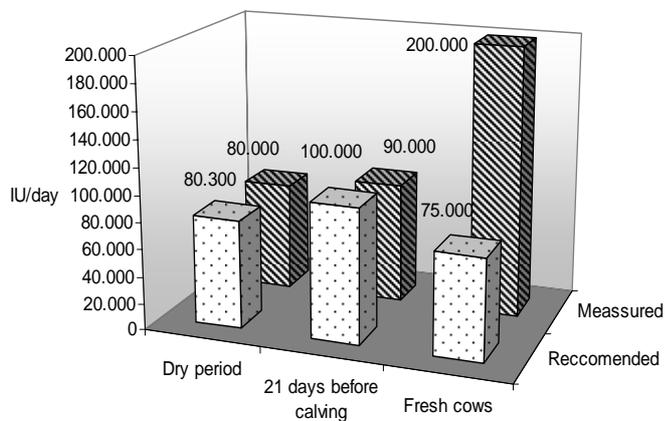


Figure 5. Concentration of vitamin A in diet, IU/day.

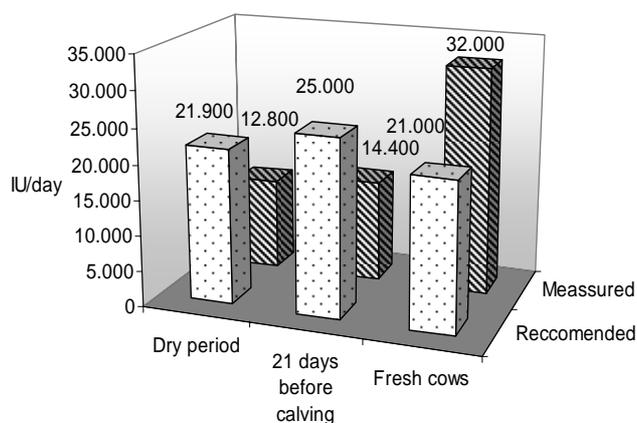


Figure 6. Concentration of vitamin D in diet, IU/day.

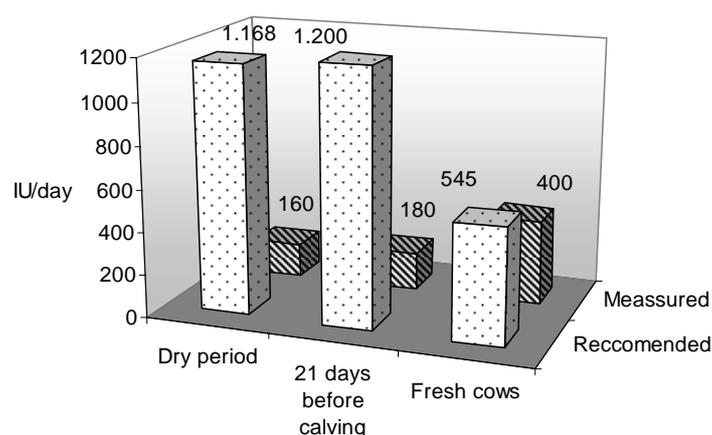


Figure 7. Concentration of vitamin E in diet, IU/day.

## DISCUSSION

Various reasons can increase incidence of RP in a herd. Gröhn *et al.* (1990) have found correlation of increased risk of RP in cows with increased parity, clinical parturient paresis, higher herd milk yield in the previous lactation and increased individual cow's milk yield. Joosten *et al.* (1991) are pointing out influence of abortion, stillbirth and multiple birth, difficult calving, caesarean section and fetotomy, as well as previous status of RP on the incidence of RP.

Beside mentioned reasons, many authors stress the influence of diet on the incidence of RP (Kelton *et al.*, 1998). Energy and protein status of cows few weeks before and after calving play important role in fertility of dairy cows. Thus, better feeding and management in high producing herds balance the negative effect of high milk production of cows on occurrence of RP (Distl *et al.*, 1989). The factors to be avoided are: high body condition score at calving, dietary deficiencies of vitamins A, D and E and deficiencies in selenium, iodine and perhaps zinc and hypocalcaemia (Han and Kim, 2005). However, the role of minerals and vitamins fed at this time on subsequent fertility is poorly understood (Wilde, 2006).

The observed rate of cows with RP on the investigating farm was rather high (in average 16.55%). Reviewing large number of references (50), Kelton *et al.* (1998) have reported average number of 8.6% cows with RP, ranging from 1.3 to 39.2%.

According to Kaneene *et al.* (1997), metabolic events associated with energy insufficiency are related to increased risk of metritis and RP. Cows in a greater degree of negative energy balance prepartum are 80% more likely to have RP (Le Blank *et al.*, 2004). Thus, higher energy consumption during the last weeks of the dry period might reduce disease risk at parturition.

Markusfeld *et al.* (1997) found out that cows that lost more body condition during the drying off period suffered more from RP. Therefore, components rich in energy should be added to diet before and after calving, which was carried out in our investigation by adding concentrate and *Helvecia energia* (Table 1). Apart from that, the rate of cows with RP was rather high in our investigation, which is in accordance with Bell and Roberts (2007), who did not find differences between cows fed low-concentrate diet and cows fed high-concentrate diet in chance of having RP.

Concerning protein nutrition, the analysis of 1 374 lactations by Curtis *et al.* (1984) is the only report that is of sufficient size to provide estimates of the effects of protein nutrition in the dry period on subsequent health. These authors found significantly lower incidence rates for RP in cows that judged to have received a high-protein diet in the dry period.

Maintenance of Ca homeostasis is critical for many functions including neuro-muscular excitability, blood clotting, and hormone secretion. Milk fever or hypocalcemia is a severe periparturient disorder characterized by lowered blood Ca concentration in high-yielding dairy cows and such cows are in increased risk of RP (Gröhn *et al.*, 1989). The metabolism of Ca is often connected with P, which means that their relationship in diet is important. Julien *et al.* (1977) found no influence of peripartum dietary P content (0.30 vs. 0.70%, dry basis) on rate of RP, and the correlation between P intake and incidence of RP was low. However, excess of Ca and P in the diet combined with deficiency of vitamin D<sub>3</sub> all affect Ca metabolism in the periparturient period and can result in hypocalcaemia. This results in loss of muscle tone in the uterus, which may contribute to the increased incidence of RP (Goff and Horst, 1997). Our results revealed higher concentrations than recommended (NRC, 2001) both for Ca and P in periparturient diet (Fig. 1 and 2), combined with deficiency of vitamin D (Fig. 6), which could have contributed to high rate of RP on the farm. As a general rule dry cows should not be fed diets which are high in Ca and P. Therefore, no legumes, especially not lucerne hay (Table 1), should be fed.

According to Wilde (2006), RP can be reduced by prevention of hypocalcaemia and also adequate Se status of the dairy cow. Se yeast is known to have higher retention in tissues and may play an important role in ensuring sufficient Se is available to the cow for reduction of RP. Vitamin E in combination with Se as antioxidants is well known for its beneficial influence on fertility in animals (Ishii *et al.*, 2002). In cows in which the prevalence of RP is high or vitamin E or Se are limited in the diet, prepartum administration of Se or vitamin E and Se reduces the incidence of RP and increases fertility after parturition (Arèchiga *et al.*, 1994). However, administration of vitamin E and Se does not reduce the incidence of RP in herds in which the incidence of RP is not elevated (Schingoethe *et al.*, 1982) or in cows consuming adequate Se (Hidiroglou *et al.*, 1987). A review of 13 studies also showed that where Se was administered or fed only 10% of cows had RP versus 32% of the control animals and 28% of animals that received a vitamin E supplement.

Although the requirement for Se is relatively low, feedstuffs produced in many areas of the world contain considerably less than 0.3 mg/kg, necessitating the need for supplementation (Spears and Weiss, 2008). Our investigation revealed that combinations of feedstuffs given to cows in different reproduction phases (Table 1) did not bring recommended quantities of vitamin E (Fig. 7) and Se (Fig. 3) in diet, which could have contributed to high rate of RP in cows. It has to be kept in mind that absorption of Se from natural feeds could be lowered by low (0.4%) or high (1.4%) percentage of dietary Ca, while 0.8% of Ca is considered to be optimal for Se absorption (Harrison and Conrad, 1984). The level of Ca in order to increase absorption of Se in our investigation was on optimal level (Fig. 1). However, diet given to cows (Table 1) was not sufficient in Se (Fig. 3) and vitamin E (Fig. 7), which raises neediness to administer Se by injection to the dry cows. This is in accordance with Harrison *et al.* (1984) who could not reduce RP and days to conception by 1 000 IU of vitamin E/cow per day given orally in cows fed diets with less than 0.06 mg/kg of Se, unless cows also were injected intramuscularly with 0.1 mg of Se/kg live weight 3 weeks before expected calving. When the diet contained at least 0.12 mg/kg of Se, 1 000 IU of dietary vitamin E/cow per day reduced the incidence of RP compared with cows not receiving supplemental vitamin E, which means that supplemental antioxidant may be less effective if another antioxidant is limiting.

As presented in Fig. 4, concentration of iodine (I) in diet was on satisfactory level in all the three phases of reproduction in cows. Controlled studies on the effects of I deficiency on the RP are lacking. In a field study with 1 572 cows in an iodine-deficient area of Finland, RP was not reduced with supplementary I (Moberg, 1961).

Content of vitamin A was on satisfactory level in our investigation, as well (Fig. 5).  $\beta$ -carotene is the major precursor of vitamin A and occurs naturally in feedstuffs. Michal *et al.*

(1994) found positive effects of  $\beta$ -carotene and vitamin A supplementation on the incidence of RP and metritis in dairy cows. However, dairy cows fed 600 mg/cow per day of  $\beta$ -carotene for 4 weeks before calving had reduced incidence of RP compared with cows fed an equivalent amount of pre-formed vitamin A (240 000 IU/day) (Michal *et al.*, 1990), which raises possible advice to swap injection of vitamin A with injection of  $\beta$ -carotene on our investigating farm.

## CONCLUSION

High diet concentrations both for Ca and P in dry period (DP) and period approximately from the 21<sup>st</sup> day before calving till the day of calving (BC) (0.98 and 0.62% of Ca; 0.37 and 0.52% of P, respectively), combined with low concentration of vitamin D in DP and BC periods (12 800 and 14 400 IU/day, respectively), as well as low concentration of Se in DP and BC periods (0.08 and 0.07 mg/kg, respectively) combined with low concentration of vitamin E in DP and BC periods (160 and 180 IU/day, respectively) were identified as critical factors that could have contributed in influencing high incidence of RP on the investigating farm. Balancing of Ca and P in diet to recommended values, administration of Se intra-muscularly and swapping injection of vitamin A with injection of  $\beta$ -carotene in prepartum period should be considered in order to reduce the incidence of RP on the investigating farm.

## REFERENCES

- Arèchiga, C.F./ Ortiz, O./ Hansen, P.J. Effect of prepartum injection of vitamin E and selenium on postpartum reproductive function of dairy cattle. *Theriogenology*, 41(1994), 1251–1258.
- Bell, M.J./ Roberts, D.J. Effect of twinning on the feed intake, performance and health of dairy cows. *Livestock Science*, 107(2007)2–3, 274–281.
- Curtis, C.R./ Erb, H.N./ Sniffen, C.J./ Smith, R.D. Epidemiology of parturient paresis: predisposing factors with emphasis on dry cow feeding and management. *J. Dairy Sci.*, 67(1984), 817–825.
- Distl, O./ Wurm, A./ Glibotic, A./ Brem, G./ Kräußlich, H. Analysis of relationships between veterinary recorded production diseases and milk production in dairy cows. *Livestock Production Science*, 23(1989)1–2, 67–78.
- Goff, J.P./ Horst, R.I. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.*, 80(1997), 1260–1268.
- Grgurić, D./ Antunović, B./ Šperanda, M./ Steiner, Z./ Florijančić, T. Effects of buserelin and human chorionic gonadotrophin on fertility of dairy cows with or without retained placenta. *Czech Journal of Animal Science*, 45(2000)8, 337–343.
- Gröhn, Y.T./ Erb, H.N./ McCulloch, C.E./ Saloniemi, H.S. Epidemiology of metabolic disorders in dairy cattle: association among host characteristics, disease, and production. *J. Dairy Sci.*, 72(1989), 1876–1885.
- Gröhn, Y.T./ Erb, H.N./ McCulloch, C.E./ Saloniemi, H.S. Epidemiology of reproductive disorders in dairy cattle: associations among host characteristics, disease and production. *Preventive Veterinary Medicine*, 8(1990), 25–39.
- Han, Y.K./ Kim, I.H. Risk factors for retained placenta and the effect of retained placenta on the occurrence of postpartum diseases and subsequent reproductive performance in dairy cows. *J. Vet. Sci.*, 6(2005), 53–59.
- Harrison, J.H./ Conrad, H.R. Effect of dietary calcium on selenium absorption by the nonlactating dairy cow. *J. Dairy Sci.*, 67(1984), 1664.
- Harrison, J.H./ Hancock, D.D./ Conrad, H.R. Vitamin E and selenium for reproduction of the dairy cow. *J. Dairy Sci.*, 67(1984), 123–132.
- Hidiroglou, M./ McAllister, A.J./ Williams, C.J. Prepartum supplementation of selenium and vitamin E to dairy cows: Assessment of selenium status and reproductive performance. *J. Dairy Sci.*, 70(1987), 1281–1288.
- Ishii, M./ Ogata, H./ Shimizu, H./ Takeuchi, Y./ Nozawa, T./ Yamamoto, Y./ Okamoto, T./ Shimamura, T./ Utsumi, A./ Jitsukawa, T./ Endo, M./ Fukuda, T./ Yamanoi, T. Effects of vitamin E and selenium administration on pregnant, heavy draft mares on placental retention time and reproductive performance and on white muscle disease in their foals. *Journal of Equine Veterinary Science*, 22(2002)5, 213–220.
- Joosten, I./ Van Eldik, P./ Elving, L./ Van der Mey, G.J.W. Factors affecting occurrence of retained placenta in cattle. Effect of sire on incidence. *Animal Reproduction Science*, 25(1991)1, 11–22.
- Julien, W.E./ Conrad, R./ Ilibbs, J.W./ Crist, W.L. Milk fever in dairy cows. VIII. Effect of injected vitamin D and calcium and phosphorous intake on incidence. *J. Dairy Sci.*, 60(1977), 431–436.

- Kaneene, J.B./ Miller, R./ Herdt, T.H./ Gardiner, J.C. The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. *Preventive Veterinary Medicine*, 31(1997)1–2, 59–72.
- Kelton, D.F./ Lissemore, K.D./ Martin, R.E. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. *J. Dairy Sci.*, 81(1998), 2502–2509.
- Klerx, H.J./ Smolders, E.A.A. Herd and cow random variation in models of interrelationships between metabolic and reproductive disorders in high yielding multiparous Holstein dairy cattle in The Netherlands. *Livestock Production Science*, 52(1997)1, 21–29.
- LeBlanc, S.J./ Herdt, T./ Seymour, W./ Duffield, D./ Leslie, K. Factors associated with peripartum serum concentrations of vitamin E, retinol, and  $\beta$ -carotene in Holstein dairy cattle, and their associations with periparturient disease. *Journal of Dairy Science*, 87(2004), 609–619.
- LeBlanc, S.J. Postpartum uterine disease and dairy herd reproductive performance: A review. *The Veterinary Journal*, 176(2008)1, 102–114.
- Markusfeld, O./ Galon, N./ Ezra, E. Body condition score, health, yield and fertility in dairy cows. *Vet. Rec.*, 141 (1997), 67–72.
- Michal, J.J./ Chew, B.P./ Wong, T.S./ Heirman, L.R./ Sandaert, F.E. Effects of supplemental  $\beta$ -carotene on blood and mammary phagocyte function in peripartum dairy cows. *J. Dairy Sci.*, 73(1990)1, 149.
- Michal, J.J./ Heirman, L.R./ Wong, T.S./ Chew, B.P./ Frigg, M./ Volker, L. Modulatory effects of dietary  $\beta$ -carotene on blood and mammary leukocyte function in periparturient dairy cows. *Journal of Dairy Science*, 77(1994), 1408–1421.
- Moberg, R. Possible influences of supplementary iodine, administered by evaporation, on reproductive performances in cattle. *Proc. 4th Int. Congr. Anim. Reprod.*, 3(1961), 682–685.
- NRC-National Research Council. *Nutrient requirements of dairy cattle*, seventh revised ed. Washington, DC, USA, National Academic Press, 2001. <http://www.nap.edu/openbook.php?isbn=0309069971>.
- Schingoethe, D.J./ Kirkbride, C.A./ Palmer, I.S. Response of cows consuming adequate selenium to vitamin E and selenium supplementation prepartum. *J. Dairy Sci.*, 65(1982), 2338–2344.
- Spears, J.W. / Weiss, W.P. Role of antioxidants and trace elements in health and immunity of transition dairy cows. *The Veterinary Journal*, 176(2008)1, 70–76.
- Wilde, D. Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle. *Animal Reproduction Science*, 96(2006)3–4, 240–249.