Artykuł przeglądowy *Review*

Prevention of negative energy balance in the transition period – implications for plasma metabolites, production and reproduction of cows

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Summary

The authors present methods to prevent a negative energy balance taking into consideration the effect of the most commonly applied energy preparations on the level of plasma metabolites, as well as on the productivity and fertility of cows. The first studies indicating the potential application of glucoplastic compounds, particularly synthetic glycerol (GLY), propylene glycol (PG) and propionic compounds in the prevention of the effects of energy deficit after calving, including ketonemia, were published approx. 50 years ago. Monopropylene glycol (1,2-propandiol) is particularly useful in the prevention of problems resulting simultaneously both from acidosis and ketosis. The administration of 500 ml PG from day 7 after calving did not have a significant effect on the dynamics of ovarian follicles, the mean number of days to the emergence of the first cohort of ovarian follicles, dominant follicles or the duration of the follicular wave. Due to supplementation of PG between day 7 and day 42 after calving an improvement of conception rate from 33 to 57% was obtained, the date of the first ovulation post partum was accelerated from day 44.5 to day 32.3 and the first luteal phase after calving was extended from 7.3 to 13.1 days. Currently a new trend may be observed in replacing synthetic PG by qualitatively more universal and effective substances contained in plants. The best known substance of this kind is 1,2,3-propanetriol, i.e. glycerol, obtained during biofuel production.

Fats are most typically fed between week 4 and week 12 of lactation. In cows receiving an increased fat content with their feed no deterioration of yields was observed, or uptake of dry matter and starch for the production of blastocysts, found in cows receiving a diet poor in fat. Fatty acids, irrespective of their value, may constitute a threat for the ruminal environment due to the modification of the flora colonising it and metabolic indexes. An increased amount of unsaturated acids in the feed ration results in their enhanced availability in the duodenum. Cows receiving an addition of fat showed more evident estrus symptoms. Fat had a positive effect on hormone metabolism thanks to improved ovarian function. A higher probability of embryo implantation and their low mortality were also demonstrated. A diet rich in calcium salts of long-chain fatty acids (Ca-LCFA) fed in the preparturition period considerably reduced the frequency of postpartum complications, such as placenta retention, metritis as well as mastitis. Similarly, an addition of fat had an advantageous effect on the stimulation of ovaries as well as the development and diameter of corpora lutea.

When feeding grain with an addition of Ca-LCFA after calving an increase was observed in the number of medium-sized and large follicles during the first 25 days after parturition and during the synchronized estrus cycle. Supplementation of the feed ration with calcium salts of fatty acids had an advantageous effect on the function of corpora lutea, conception rate and the length of pregnancy interval. Administration of wet crushed corn grain instead of dry grain results in enhanced ruminal fermentation. It also causes a more balanced energy and protein metabolism, a better state of health, a higher daily milk production, a higher protein concentration in milk as well as a better reproduction performance replacement of triticale with corn in the transition period as well as during lactation; it improved the conception rate, reduced the insemination index, and it also reduced calving interval. A considerable number of publications concerning the advantageous effect of an addition of corn on parameters of the energy metabolism, metabolic indexes, production traits and the milk composition does not change the fact that the body of data concerning reproduction in cows fed in this way is insufficient.

Keywords: negative energy balance, prevention, blood metaboilites, production, reproductive performance, cows

Energy deficit in the transition period is a metabolic disorder of considerable intensity, significantly affecting productivity and fertility of high-producing cows (10, 26, 28, 32). The problem of energy deficit may be reduced by the administration of energy additives to feed in the feeding of cows and by organizational changes aiming at an increase in dry matter uptake during lactation. Applied prophylactic supplements both increase the energy value of feed and reduce energy deficit, a typical phenomenon in cows during the first trimester of lactation. The most commonly administered substances include glucoplastic compounds, dietary fat and other supplements, such as niacin or live yeast strains. This study presents methods to prevent a negative energy balance taking into consideration the effect of the most commonly applied energy preparations on the level of plasma metabolites, as well as productivity and fertility of cows.

Glucoplastic substances

The potential to enhance blood glucose concentration using an increased carbohydrate uptake with feeds is limited due to ruminal fermentation of almost the entire amount of glucose to volatile fatty acids. In order to avoid this adverse phenomenon the so-called glucose promoters may be used in the feeding of cows (8). The first studies indicating the potential application of glucoplastic compounds, particularly synthetic glycerol (GLY), propylene glycol (PG) and propionic compounds in the prevention of the effects of energy deficit after calving, including ketonemia, were published approx. 50 years ago. Propionic acid stimulates the secretion of insulin, which inhibits mobilization of free unsaturated fatty acids (FUFA). It also exhibits strong antiketogenic properties. What is important, it is the basic source of glucose formation, which modulates energy balance. Cows receiving compounds of propionic acid consumed greater amounts of feed after calving (58). In turn, a reduced uptake of concentrates shortly before and after calving is directly connected with an enhanced predisposition to ketosis (30, 57). Production of propionic acid was also stimulated by the administration of an ionophore, monensin sodium, with feed. Monensin produced by strains of *Streptococcus cinnemonensis* is used in cattle feeding as a growth stimulant, changing ruminal fermentation (8). Consequently, this also enhances the synthesis of glucose from propionic acid and energy metabolism (17).

Propylene glycol (PG) as a potential source of exogenous energy attracted the attention of researchers as early as 1939 (19). A repeated increase in the interest in this substance was observed in the 1970's. Monopropylene glycol (1,2-propandiol) is particularly useful in the prevention of problems resulting at the same time both from acidosis and ketosis (8, 18, 21). Its supply with feed in an amount ranging from 184 to 513 g a day resulted in a markedly reduced risk of ketosis (21). An addition of 311 and 500 g PG led to an over 3.5-fold decrease in the frequency of ketosis and a significant increase in blood glucose concentration (9, 34). Already after a week of glycol administration the level of glucose increased from approx. 40 to over 50 mg% (mg/100 mg) in comparison to the control cows. The glucoplastic effect of PG was confirmed by studies, in which at day 7 after calving in both experimental groups having received different additions of glycol a higher concentration of glucose was recorded than that found in the control (31). In the opinion of some researchers, a considerable increase in blood glucose level is provided thanks to the administration of 500 and 750 g PG in the loose form, while the application of 250 g does not cause such as effect. On the other hand, concentrations of other metabolites (urea nitrogen, triglycerides and cholesterol) did not change (40, 56).

A surprising phenomenon observed in cows receiving an addition of PG was connected with a definite increase in blood insulin content (25, 37). In contrast, it results from more recent data that a diet stimulating the secretion of insulin administered during the first 30 days after calving has an advantageous effect on the percentage of pregnant cows by day 120 of lactation (22, 27). Moreover, a significant reduction was also observed in the level of ketone bodies in milk and in the blood, as well as FUFA – β-hydroxybutyric acid – at the simultaneous increase in the blood concentration of insulin-dependent growth factor I (IGF-I) (28, 38). Glycol as a supplement to feed rations in the periparturient period caused an increase in milk yield; however, it did not have a direct effect on body weight (16, 35). Milk yield in the first four weeks of lactation was highest in the group that received 500 g/day/head of this preparation in comparison to the group of cows which did not receive such a supplement (8). However, this increase seems rather unlikely in herds with a good feeding program (29). Supplementation with 769 g of 65% PG in the period from day 21 before to day 21 after parturition did not have a significant effect on the date of the first ovulation post partum and it did not cause a significant increase in the contents of 13,14 dihydro, 15-keto $PGF_{2\alpha}$ (PGFM) (11). Moreover, no advantageous effect was observed for an addition of 250 ml monopropylene glycol supplied from mid-gestation until calving on the length of postpartum *anoestrus* (13). An addition of glycol did not influence the periparturition concentration of progesterone, androstendion and estradiol in the fluid of ovarian follicles aspirated at day 15 after calving (43). Administration of monopropylene glycol to dairy cows after calving at 6 weeks before mating in the amount of 200 ml in the morning, or in the morning and in the evening, did not have a significant effect on the appearance of estrus after calving, while it had only a slight effect on the conception rate between week 12 and week 16 after calving (14). Similarly, the administration of 500 ml PG from day 7 after calving did not have a significant effect on the dynamics of ovarian follicles, the mean number of days to the emergence of the first cohort of ovarian follicles, dominant follicles or the duration of the follicular wave. Moreover, no effect of PG was found on the date of the first ovulation, dimensions of the preovulation follicle or its effect on the quality of oocytes measured by the capacity to form a blastocyst after *in vitro* fertilization (IVF) (48). In other studies at supplementation of PG between day 7 and day 42 after calving an improvement of conception rate from 33 to 57% was obtained, the date of the first ovulation post partum was accelerated from day 44.5 to day 32.3 and the first luteal phase after calving was extended from 7.3 to 13.1 days (38).

It is assumed that glycol should be administered in the dry-off period approx. 2 weeks before calving at a dose of 100-150 g/day, while after calving it should be from 300 to 750 g daily (39, 56). In view of the properties of PG and the common negative effects of energy deficit in high-producing cows it has been suggested not to treat glycol solely as an energy supplement, but rather as an important therapeutic preparation.

At present a new trend may be observed to replace synthetic PG by qualitatively more universal and effective substances contained in plants. The best known such substance is 1,2,3-propanetriol, i.e. glycerol, obtained during biofuel production. Its occurrence in all plants and animal fats has been confirmed experimentally. Glycerol is administered to cows in a liquid, less palatable form or as loose powder. It is absorbed mainly in the rumen and metabolized by microorganisms to propionic acid. It considerably reduces FUFA and ketone compounds, while elevating blood levels of glucose, β-hydroxybutyric acid and insulin (20, 31, 47).

Dietary fat

At present increasing the proportion of fat is applied with increasing frequency as a method to enhance the energy level in the feed ration for high-yielding cows in the first period of lactation. Advantages of fat supplied in the diet include a two-fold increase in the concentration of energy (over 9 EB kcal/g) in comparison to the other nutrient components of feed, as well as a high digestibility frequently exceeding 85% (42). Its positive effect on technological processes, e.g. feed pelleting, has been shown (7). An addition of fat to the diet of ruminants is considered by conservationists as a method to reduce CH_4 and CO_2 emissions to the environment (41). In the feed ration for ruminants fat may be supplied in the natural form or as a fat supplement. Dry matter of bulk feed contains from 1 to 5% crude fat. Cereal grain contains from 1 to 4% crude fat, while seeds of oil crops from 15 to 40%. Natural vegetable fats are most frequently found in the form of tri-, di- and monoacylglycerols. All fats supplied with the feed ration undergo lipolysis in the rumen. End products of lipolysis include free fatty acids and glycerol, which are transformed into volatile fatty acids (41). In cows receiving an increased fat content with their feed no deterioration of yields was observed, or uptake of dry matter and starch for the production of blastocysts, found in cows receiving a diet poor in fat (15). Hydrogenated triglycerides (HTG) added to the feed ration based on corn silage had a more advantageous effect on lipid metabolism and the metabolism of glycogen than an addition of calcium soaps of fatty acids (30).

Fats are most typically fed between week 4 and week 12 of lactation. We need to assume that animals should be gradually adapted to the uptake of a greater amount of fat. The duration of the application of an enriched feed ration depends on the persistence of lactation, body condition of cows and reproduction performance (7, 50).

Two forms of dietary fat are commercially available, i.e. unprotected, such as vegetable oils, oil cake, fish oil, and the protected form.

Oils are rich sources of mono- and polysaturated fatty acids. Linseed and rapeseed oils added at approx. 10% of the feed ration are commonly used. An addition of fish oil should not exceed approx. 4% of the feed ration. It was also determined that its addition in a maximum amount of 200 g/head/day does not have a negative effect on milk taste (43, 49). Supplying fats in the diet or a modulation of ruminal fermentation modifies the composition of fatty acids in animal products and all body fats in dairy cows (46). An addition of fish oil and vegetable oils to the feed ration of cows resulted in an increased milk content of fatty acids, having a positive effect on human health, including trans-11 C18:1 vaccenic acid, cis-9 trans-11 conjugated linolic acid and trans-10 cis-12 CLA as well as n-3PUFA, formed in the rumen (5, 42). Some researchers are of an opinion that very small amounts of the above mentioned substances may also considerably affect the metabolism of glucose and fats in the liver, peripheral metabolism and resistance of cows consuming it (44). Thus a certain potential is perceived in the use of specific fatty acids as feed additives in order to improve their health and yields of dairy cows in the transition period.

In feeding of cattle an addition of unprotected fat is applied at 2-2.5% dry matter. The total amount of fats in the feed ration should not exceed 7.5%, with fat in the basal diet amounting to 3.0%. Fat in the unprotected form undergoes the process of lipolysis, which leads to hydrogenation of fatty acids. Such fat does not constitute a source of energy for ruminal microorganisms and may cause its acidification. The negative effect of unprotected fat on ruminal fermentation may be preventively limited by increasing the concentration of calcium in the feed ration to approx. 1.1%, thanks to an addition of calcium sources readily soluble in the rumen, e.g. calcium lactate or propionate (41). Moreover, in order to reduce the negative effect of unprotected fat on ruminal metabolism an addition of protected fats is used. These fats are neutral for ruminal fermentation, since they are digested in the abomasum.

Fatty acids, irrespective of their value, may constitute a threat for the ruminal environment due to the modification of the flora colonising it and metabolic indexes. An increased amount of unsaturated acids in the feed ration results in their enhanced availability in the duodenum. In this way they may be introduced to the fat of milk or meat in ruminants. The use of fish oil and the resulting specific smell may reduce total feed uptake and influence the taste of obtained animal products. This problem was solved by specific processing of fish oil. Although the addition of fat causes an increase in the concentration of energy and its uptake, at the same time dietary (unprotected) fat, by deteriorating digestibility of structural carbohydrates and negatively affecting ruminal fermentation – primarily as a result of a reduced cellulolytic activity of ruminal microorganisms – may reduce energy utilisation in the entire feed ration (42, 49). The higher the content of structural carbohydrates in the feed ration, the more advisable such an addition is and the lower the threat of destabilisation of ruminal fermentation (42).

Protected fat is most frequently applied in the form of calcium soaps of fatty acids, seeds of oil crops, encapsulated fats and fatty acid amides. As analyses have indicated, a significant disadvantage of protected fat is its negative effect on the consumption of dry matter. However, in the feeding of high-producing cows, after balancing the feed ration, the addition of protected fat is one of the methods to reduce energy deficit (42).

It is rather commonly assumed that the use of fat as a feed additive results in an improvement of reproduction indexes (23, 58). Cows receiving an addition of fat showed more evident estrus symptoms. Thanks to improved ovarian function fat had a positive effect on the hormone metabolism. A higher probability of embryo implantation and their low mortality were also shown (23, 44, 51). A diet rich in calcium salts of long-chain fatty acids (Ca-LCFA) fed in the preparturition period considerably reduced the frequency of postpartum complications, such as placenta retention, *metritis* as well as *mastitis* (24, 25). Similarly, an addition of fat had an advantageous effect on the stimulation of ovaries as well as the development and diameter of corpora lutea (43, 46). When feeding grain with an addition of Ca-LCFA after calving, an increase was observed in the number of medium-sized (6-9 mm) and large follicles (> 15 mm) during the first 25 days after parturition and during the synchronized estrus cycle (33). Supplementation of the feed ration with calcium salts of fatty acids had an advantageous effect on the function of corpora lutea, conception rate and the length of pregnancy interval (52). An interesting observation was connected with a higher concentration of progesterone at 2-4 days before insemination and the fact of an increasing percentage of cows that became pregnant at less than 150 days after calving. Feeding of heifers with a diet containing 6% calcium salts of palmitic acid increased the content of fat in cumulus-oocyte complexes (COC); however, the composition of fatty acids did not change depending on the content of PUFA in the diet, leading to the selective accumulation of saturated fatty acids in oocytes (49, 60).

Moreover, in many studies a more advantageous effect of diet enrichment with polyunsaturated fatty acids (UFA) than monosaturated acids was shown in relation to the dimensions of follicles observed on ovaries (6, 7, 23). Polyunsaturated fatty acids stimulated the level of insulin, cholesterol and IGF-I in the fluid of large ovarian follicles; however, their effect on the level of progesterone was not observed (23, 45, 55). It was also shown that in cows receiving a higher than average addition of UFA the diameter of the ovulation follicle was larger in comparison to females receiving their lower addition (57). Administration of n-3 FA, LNA; C18:3 n-3, or either EPA or DHA, reduced considerably the percentage of aborted pregnancies in the first period after fertilization, since (particularly n-3 FA) has a suppressive effect on intrauterine secretion of $PGF_{2\alpha}$ (1, 45, 47, 49). A significant effect of n-3 FA supplementation was reported during the dry-off period and early lactation on an increase in ovarian follicles (49). In more recent studies an advantageous effect of a diet rich in UFA on the quality of oocytes aspirated *in vivo* was shown. The percentage of oocytes reaching *in vitro* metaphase II and the level of apoptosis was higher than that in control cows (6). In turn, the addition of 50 μM linolenic acid (LNA; 18:3) directly to the medium for *in vitro* oocyte maturation resulted in an increase of the percentage of embryos undergoing cleavage, also contributing to an increase in the percentage of embryos developing to the phase of blastocysts with a higher inner cell mass (ICM) and a higher number of trophoectoderm (TE) cells (34). An increased number of blastomeres was observed when superstimulated embryo donor cows were fed diets enriched with either linoleic or α-linolenic acid (C18:3n-3) compared with a saturated source of FA (53).

There are also reports on a lack of a significant effect of dietary fat on reproduction indexes (1). Such a situation frequently occurs in case of a considerable increase in milk production. Fat supplementation of cows in late pregnancy reduced lactation and had a negative effect on the status of the metabolism in cows, which was reflected in the concentration of blood serum metabolites (38). Similarly, the addition of fat in the form of pelleted fat (PrFA) with a low proportion of unsaturated fatty acids or calcium soaps of long-chain fatty acids (CaLFA) to the diet of cows, supplied from day 256 of pregnancy, did not have a significant effect on the level of progesterone and estradiol in the fluid of follicles with a diameter of over 6 mm, aspirated in the early postpartum period

(38). The addition of fermentation-neutral fatty acids (47% fat) supplied from the day of calving to day 100 of lactation did not have a significant effect on the diameters of the first dominant follicle and the level of estradiol between day 7 and day 14 pp, as well as the number of ovarian follicles of different classes (3-5, 6-9 and 10-15 mm) (4).

Starch as an additional source of glucose after calving

The only source of by-pass starch digested in the small intestine is dried corn grain. Starch is found in other cereal grains and it is degraded in 100% in the rumen. Some studies indicate (59, 61) that this metabolic process is more effective than in case of synthesis of propionic acid. Administration of wet crushed corn grain instead of dry grain results in enhanced ruminal fermentation, it causes a more balanced energy and protein metabolism, better health state, a higher daily milk production, higher protein concentration in milk, as well as better reproduction performance (10). Feeding crushed corn before calving was connected with a higher body condition score (BCS), or the blood serum level of leptin and IGF-I. After calving a more considerable increase was observed in milk production, an increase in the concentration of FUFA and insulin, as well as a significant delay in ovarian activity than was the case in control cows (12, 36). Irrespective of the type of applied diet, its supplementation with steam-flaked corn (SFC) improved productivity to a higher degree than after feeding ground corn (GC). Cows fed SFC consumed less dry matter and produced more milk with a lower percentage fat content. Blood glucose concentration and urea nitrogen concentration in milk were similar at different feeding regimes, but blood urea nitrogen level was higher in cows fed GC than those receiving an addition of SFC (18). Feeding cows with a mixture of silages from annual ryegrass with an addition of corn (BLEND) was more advantageous than the application of a diet based solely on annual ryegrass silage (RS). Cows fed with BLEND consumed more dry matter (DM), organic matter (OM), neutral detergent fiber (NDF) as well as acid detergent fiber (ADF) than cows fed with RS. At the same time these animals tended to produce greater amounts of milk with a lower fat content than cows fed RS (18). However, a disadvantageous characteristic of this feeding regime includes a considerable reduction of the available carbohydrate pool, constituting a significant source of energy for ruminal microorganisms. In a study by Mikuła et al. (37) replacement of triticale with corn in the transition period as well as during lactation improved the conception rate (0.45 vs. 0.78), reduced the insemination index (1.26 vs.1.91), and it also reduced calving interval (115 vs. 130 days).

The considerable number of publications concerning the advantageous effect of an addition of corn on parameters of energy metabolism, metabolic indexes, production traits and milk composition does not change the fact that the body of data concerning reproduction in such fed cows is insufficient.

Other methods of preventing energy deficit and their effect on reproduction

It has recently been speculated that a considerable shortening or even elimination of the dry-off period improves dry matter uptake in the periparturient period, reduces milk production, at the same time improving energy balance after calving and reducing the number of days to the onset of postpartum ovarian activity (29). Similarly, stimulation of IGF-I production by supplementation of somatotropin (bST) post partum has an advantageous effect – particularly under heat stress – on the reproduction and productivity of cows (3, 54). A high blood IGF-I concentration is significantly correlated with a high supply of exogenous energy (2).

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