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Changes in the blood indicators and body condition of high yielding Holstein cows with retained placenta and ketosis

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Abstract

The aim of this study was to determine the effect of changes in body condition in the dry period and the early lactation period on the incidence of retained placenta and ketosis in 94 high-yielding Holstein-Friesian cows. Body condition scoring was performed every two weeks from the beginning of the dry period until week 18 of lactation. Blood for the measuring of indicators of metabolism was sampled in weeks 1 and 2 *ante partum* and in weeks 1, 2, 3, 7 and 15 *post partum*. Retained placenta was reported in 11 cows, and ketosis was diagnosed in 18 animals. One week *ante partum*, the serum profile of cows diagnosed with ketosis during lactation revealed 0.52 mmol/l β-hydroxybutyric acid and 0.29 mmol/l non-esterified fatty acids on average. One week *post partum*, the serum profile of cows with ketosis revealed 1.59 mmol/l β-hydroxybutyric acid and 1.09 mmol/l non-esterified fatty acids and cows with retained placenta 1.65 and 1.41, respectively. From the week 5 *ante partum* to the point of lowest body condition the average body condition loss reached 1.4 points in cows with retained placenta, 1.1 points in cows with ketosis, and 0.8 points in healthy cows. Retained placenta and ketosis increased significantly conception rates by 0.47 and 0.50, respectively. Our results show that monitoring changes in the body condition and non-esterified fatty acids and β-hydroxybutyric acid blood levels in high-yielding cows in the transition period, followed by taking relevant disease-control measures, may be effective in reducing the incidence of retained placenta and ketosis in dairy cattle herds.

Dairy cows, body condition loss, serum metabolite, postpartum disease, reproductive performance

Disease prevention and maintenance of adequate production levels are the greatest challenges in high-yielding dairy herds during transition from the late dry period to the first weeks of lactation. In around 75% of cases, diseases affecting dairy cows are reported in the first month after calving (LeBlanc et al. 2006). The above mentioned can be attributed to a negative energy balance in the discussed period (Kim and Suh 2003). A significant drop in the feed intake is observed on pre-calving days (Hayirli et al. 2002). A negative energy balance causes metabolic stress which intensifies health disorders, including retained placenta that generates significant financial losses in dairy cattle herds due to high treatment costs, complications, milk loss, culling for infertility and increased mortality (Fleischer et al. 2001; Goff 2006). Ketosis is a frequent disease in high-yielding cows. It occurs when adipose tissue is used to meet energy demands and when non-esterified fatty acids (NEFA) are incompletely oxidized into ketone bodies.

Blood indicators and the body condition score (BCS) are good indicators of animals' nutritional and health status, and they are useful predictors of disease (Kaczmarowski et al. 2006). Early detection of cows prone to diseases, followed by modification of their diet and individual handling, may help reduce disease incidence. The indicators that are used to monitor imbalances in energy metabolism include glucose, NEFAs and betahydroxybutyrate (BHBA) (Ingvartsen et al. 2003). Elevated precalving cholesterol and NEFAs are associated with greater risk of retained placenta (LeBlanc et al. 2006). The body condition score and its variations are widely used to evaluate body reserves and the energy balance in cows. Gillund et al. (2001) observed that body condition loss during early lactation had negative effects on reproductive performance, while no such

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correlations were reported in other studies (Ruegg and Milton 1995; Heuer et al. 1999). Previous research studies have attempted to evaluate the effect of body condition at calving and changes therein during early lactation on the productivity, reproductive performance and health status of cows (Nogalski and Górak 2008). In view of the above, it seems interesting to analyze the influence of changes in the body condition of high-producing cows observed in the drying-off period on the overall health of animals.

The aim of this study was to investigate the associations between the incidence of retained placenta and ketosis in high-yielding dairy cows and body condition and blood profiles in the periparturient period and early lactation, and to determine whether changes in the body condition and blood indicators may help to detect cows particularly vulnerable to the above diseases.

Materials and Methods

Animals

A total of 94 animals from a herd of 330 Holstein-Friesian cows were selected for the experiment based on the following criteria: a similar calving date to eliminate the effect of calving season (the selected cows calved between 11 December 2007 and 14 March 2008), milk yield during the previous lactation (not lower than 10,000 kg of milk during 305-day lactation, for heifers milk yield was estimated based on pedigree information), lactation number proportionally for the entire herd (first lactation, second lactation, third lactation and fourth lactation with 25, 30, 24 and 15 cows, respectively). A herd of cows kept in a free-stall system was investigated in this experiment. The animals were fed a total mixed ration (TMR) based on maize silage, grass and alfalfa haylage, and brewer's spent grain. In the complete diet, the concentrate was supplemented with protein, minerals, vitamins and milk production enhancers (protein and rumen-protected fat, active yeast cultures and other energy supplements). Cows were fed a TMR formulated to produce 40 l of milk (7.15 MJ/kg net energy (lactation) and 18% crude protein). Dry cows and heifers on pre-calving days 60–45 were kept in deep litter free-stall facilities, and they were divided into two groups: 1) dry cows: 6–8 weeks a.p. (*ante partum*) to 3 weeks a.p., 2) transition cows: 3 weeks a.p. to 1 week p.p. (*post partum*). Dry cows were fed a TMR of grass and straw haylage supplemented with minerals in the dry period. Transition cows were administered lactation rations with an increasing share of concentrate. Feed was offered twice daily, using mixer wagons, and it was gathered up several times per day to ensure *ad libitum* access.

Study design and measurements

Energy reserves were estimated based on the body condition scoring (BCS) scale of 1 (thin) to 5 (obese) points with 0.25 intervals (Wildman et al. 1982). Evaluations were performed every two weeks, starting from 5 weeks a.p. to 20 weeks p.p. Blood was sampled from the external jugular vein before the morning feeding at 2 and 1 weeks a.p., and at 1, 2, 3, 7 and 15 weeks p.p. Whole blood samples were analyzed to determine glucose levels as well as plasma concentrations of β-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), albumins and aspartate aminotransferase (AST) activity. Blood tests were performed using BioSystem diagnostic kits, the Roche Hitachi 902 chemistry analyzer and the EPOLL-200 spectrophotometer. Milk yield and reproductive performance data were obtained from breeding records, Symlek system as well as based on direct observations. Energy corrected milk (ECM) and the average content of fat, protein and dry matter per kg ECM were determined for each cow during 305-day lactation.

Energy Corrected Milk, milk with standardized energy content (Sjaunja et al. 1990), was calculated based on this formula:

$$ECM\ (kg) = milk\ (\text{kg}) \cdot \frac{(0.383 \cdot fat(\%) + 0.242 \cdot protein(\%) + 0.7832)}{3.140}$$

Reproductive performance was determined by calculating the conception rate (CR, total inseminations/number of in-calf cows) and the inter-pregnancy interval (IPI, number of days between calving and successive conception).

In the herd, regular health examinations were performed by the same investigator once a week. Retained placenta was noted in 11 cows, and the clinical symptoms of primary ketosis were diagnosed in 18 animals. Retained placenta (RP) was defined as the retention of the foetal membrane for > 24 h. Ketosis (K) was diagnosed based on the following clinical symptoms: anorexia, depression and odor of acetone on the breath.

Animal handling and sampling procedures performed for the needs of this study have been fully approved by the local ethics committee in Olsztyn, Poland.

Statistical analysis

In order to compare the indicators of RP and K cows, a control group (C) of 12 cows free of the analyzed disorders was selected by the analog method. The results were processed using Statistica 9.0 software (Statsoft 2010). The effect of the analyzed disorders on blood indicators, milk yield and reproductive performance was evaluated by the least squares analysis of variance according to the below model:

$Y_{ij} = m + A_i + e_{ij}$

where: Y_{ij} – values of the analyzed indicators, m - population mean, A_i – effect of the i[th] disorder, e_{ij} – random error.

Differences between means were estimated using Tukey's test at the level of significance $P \leq 0.01$ and $P \leq 0.05$.

Results

The depth and length of the negative energy balance phase is illustrated by changes in BCS profile (Fig. 1). The lowest BCS values were noted in cows between weeks 5–7 of lactation. The energy demand and dietary energy intake were balanced at the turn of the second and third month of lactation. Between 5 weeks a.p. to the point of lowest BCS, the body condition of RP group cows deteriorated by 1.4 points, K group cows by 1.1 points, and control group cows by 0.8 points. The body condition of RP and K group cows in the dry period was higher in comparison with control group cows, and intense mobilization of fat reserves in the affected animals began already at the end of the dry period.

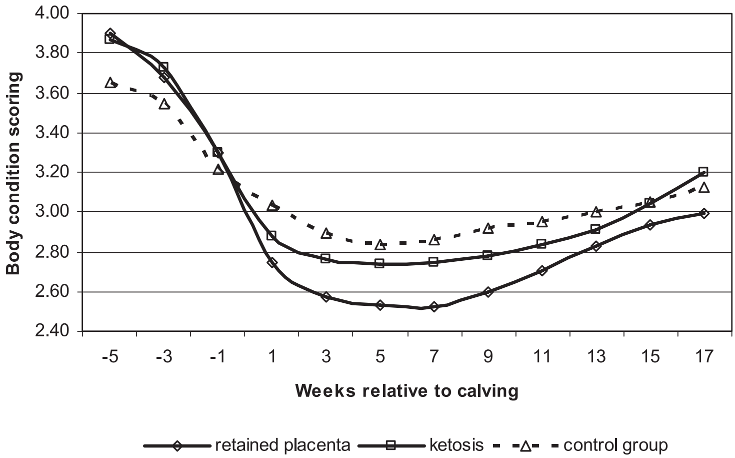

Fig. 1. Changes in body condition of cows in the dry period and the early lactation period

Significant differences in blood indicators indicative of metabolic changes were not reported in cows prior to calving (Table 1). In the first week a.p., the blood serum profile of cows diagnosed with ketosis during lactation revealed an average of 0.52 mmol/l BHBA and 0.29 mmol/l NEFA. In the second week p.p., K group animals were characterized by significantly lower glucose levels and significantly higher concentrations of BHBA and NEFAs in comparison with the remaining groups. In the RP group, high levels of ketone bodies and NEFA were observed only in the first week of lactation. In affected cows, blood cholesterol levels were significantly ($P \leq 0.05$) lower at the beginning of lactation. At the beginning of lactation, significant ($P \leq 0.05$) differences were reported in AST activity and albumin levels. In the second week p.p., AST levels in K group cows reached 218 U/l on average. In the third week p.p., albumin levels in diseased cows were lower ($P \leq 0.50$) in comparison with healthy animals.

The yield of RP cows was by 808 kg ECM lower on average, in comparison with control group cows (Table 2). Retained placenta and ketosis had a negative effect of the reproductive performance of cows. Retained placenta and ketosis group animals were characterized

Table 1. Selected indicators of metabolism (mean ± SE) in cows with retained placenta (n = 11), ketosis (n = 18) and in the control group (n = 10)

Indicator	Group	Weeks relative to calving -2	-1	1	2	3	7	15
Glucose (mmol/l)	RP	4.15 ± 0.12	4.04 ± 0.12	3.20 ± 0.12	3.45 ± 0.24[a]	3.51 ± 0.29	3.59 ± 0.14	3.47 ± 0.12
	K	3.85 ± 0.23	3.78 ± 0.13	3.23 ± 0.23	3.03 ± 0.34[b]	3.11 ± 0.25	3.42 ± 0.08	3.43 ± 0.13
	C	4.22 ± 0.09	4.23 ± 0.24	3.46 ± 0.11	3.46 ± 0.09[a]	3.48 ± 0.12	3.58 ± 0.09	3.49 ± 0.11
BHBA (mmol/l)	RP	0.42 ± 0.03	0.49 ± 0.07	1.65 ± 0.21[a]	0.93 ± 0.36[A]	0.77 ± 0.33[a]	0.59 ± 0.09	0.79 ± 0.13
	K	0.44 ± 0.04	0.52 ± 0.08	1.59 ± 0.21[a]	2.43 ± 0.37[B]	1.18 ± 0.34[b]	0.76 ± 0.10	0.66 ± 0.14
	C	0.39 ± 0.05	0.33 ± 0.08	0.75 ± 0.12[b]	0.81 ± 0.23[A]	0.72 ± 0.21[a]	0.53 ± 0.14	0.64 ± 0.06
NEFA (mmol/l)	RP	0.19 ± 0.04	0.22 ± 0.07	1.41 ± 0.27[a]	0.92 ± 0.31	0.44 ± 0.09[a]	0.21 ± 0.07	0.14 ± 0.04
	K	0.15 ± 0.05	0.29 ± 0.08	1.09 ± 0.27[a]	1.12 ± 0.32[A]	0.73 ± 0.10[b]	0.31 ± 0.08	0.20 ± 0.05
	C	0.12 ± 0.02	0.16 ± 0.08	0.78 ± 0.07[b]	0.69 ± 0.13[B]	0.41 ± 0.13[a]	0.16 ± 0.05	0.19 ± 0.02
Cholesterol (mmol/l)	RP	2.34 ± 0.09	2.23 ± 0.13	2.34 ± 0.11	3.20 ± 0.35[a]	3.49 ± 0.41[A]	5.68 ± 0.27[a]	7.82 ± 0.21
	K	2.32 ± 0.10	2.25 ± 0.14	2.30 ± 0.12	2.26 ± 0.26[b]	3.42 ± 0.41[A]	6.26 ± 0.27[a]	8.02 ± 0.22
	C	2.53 ± 0.14	2.43 ± 0.19	2.48 ± 0.23	3.08 ± 0.24[a]	4.94 ± 0.27[B]	7.11 ± 0.21[b]	7.49 ± 0.34
Ast (U/l)	RP	84.0 ± 8.19	69.5 ± 8.49	127.0 ± 7.99	142.0 ± 9.89[a]	96.8 ± 9.43	84.0 ± 6.56	91.1 ± 4.53
	K	92.5 ± 5.69	73.0 ± 5.52	126.8 ± 7.10	218.0 ± 9.90[b]	115.4 ± 9.44	98.1 ± 6.57	99.3 ± 4.54
	C	77.0 ± 5.37	88.2 ± 11.04	130,7 ± 9.81	166.2 ± 8.09[a]	113.6 ± 8.92	91.4 ± 8.57	102.2 ± 8.58
Albumins (g/l)	RP	42.7 ± 1.34	40.7 ± 0.47	38.4 ± 1.91	34.3 ± 2.84	32.9 ± 1.45[a]	38.3 ± 2.15	41.5 ± 1.26
	K	42.5 ± 1.84	41.0 ± 0.48	36.4 ± 1.78	38.4 ± 2.03	35.9 ± 1.45[a]	41.9 ± 1.25	42.7 ± 1.27
	C	40.1 ± 1.10	38.7 ± 1.34	37.0 ± 0.91	37.6 ± 0.91	40.4 ± 1.64[b]	41.5 ± 1.09	42.5 ± 1.04

RP - retained placenta, K - ketosis, C - control group, BHBA - β-hydroxybutyric acid, NEFA - non-esterified fatty acids, Ast - aspartate aminotransferase
In columns: ^{A,B} – $P \leq 0.01$; ^{a,b} – $P \leq 0.05$

Table 2. Milk yield and reproductive performance traits (mean ± SE) in cows with retained placenta, ketosis and in the control group.

Traits	Retained placenta (n = 11)	Ketosis (n = 18)	Control group (n = 10)
ECM (kg)	12204 ± 778.8	13068 ± 565.2	13012 ± 527.1
Fat (%)	4.32 ± 0.16	4.36 ± 0.13	4.18 ± 0.10
Protein (%)	3.27 ± 0.05	3.19 ± 0.06	3.18 ± 0.06
Dry weight (%)	13.06 ± 0.18	12.99 ± 0.19	12.80 ± 0.13
CR	2.28 ± 0.22[a]	2.31 ± 0.16[a]	1.81 ± 0.16[b]
IPI	135.1 ± 19.23	159.0 ± 13.12	118.6 ± 11.19

ECM - Energy corrected milk, CR - conception rate, IPI - inter-pregnancy interval
In rows: ^{a,b} – $P \leq 0.05$

by a longer inter-pregnancy period and significantly higher ($P \leq 0.50$) conception rates, compared to healthy cows.

Discussion

The optimal body condition score for dairy cows at calving is 3.5 (Nogalski and Górak 2008; Ruegg and Milton 1995). Deviations from the optimal score increase the risk of metabolic diseases and prevent the achievement of maximum milk yield values during lactation (Staufenbiel et al. 2003). Gillund et al. (2001) reported a doubling of the risk of ketosis in dairy cows with a calving BCS of > 3.5 compared with those with a calving BCS of 3.25. Their findings are consistent with a comprehensive review of periparturient morbidity and its relationship to excessive BCS mobilization (Ingvartsen 2006). In our study, retained placenta or ketosis was observed in cows undergoing intense fat mobilization, in particular in the dry period. Due to a rapid drop in the feed intake, high-producing cows mobilize energy reserves already 1–3 weeks before calving. An analysis of NEFA profiles supports an evaluation of the rate of lypolysis which is negatively correlated with the energy balance (Ingvartsen 2006). Optimal content non-esterified fatty acids and β-hydroxybutyric acid in the blood serum of healthy cows is 0.5–0.7 and 0.6–1.0 mmol/l, respectively (Whitaker et al. 2005). In our study, K and RP group cows characterized by higher body condition scores intensively mobilized their body fat reserves before calving, and the above resulted in higher NEFA and BHBA levels in their blood serum. Elevated precalving NEFA concentrations could, therefore, be a predictor of increased risk of retained placenta and ketosis. In the first week a.p., higher NEFA concentrations were observed in cows that had been diagnosed with ketosis during early lactation. Aspartate aminotransferase is responsible for protein balance, and this cytoplasmatic enzyme is a labile and sensitive indicator of changes in liver metabolism resulting from high productivity. In our study, RP and K group cows showed very high AST activity levels. The AST activity of cows affected by ketosis exceeded 200 U/l in the second week p.p., and the above results could be indicative of hepatic cell damage (Meikle et al. 2004). Ketosis was observed mainly in cows in their first and second lactation. Young animals, in particular primiparous cows, are characterized by more unbalanced metabolic and endocrinological profiles than older cows, which is why they emerge from the NEB state with greater difficulty.

Lower reproductive performance in groups K and RP could result from a high energy deficit in transition cows. High energy deficits in early lactation delayed the first detected oestrus (De Vries et al. 1999). Kim and Suh (2003) demonstrated that Holstein dairy cows with greater body condition loss in the dry period were characterized by a higher incidence of *post partum* metabolic diseases and a longer calving to first service interval. Walsh et al. (2007) noted significantly lower insemination effectiveness in cows with elevated levels of ketone bodies in the first weeks of lactation. The reproductive performance of cows should also be evaluated in view of their high productivity. For decades, Holstein-Friesian cows have been subject to intensive selection for increased milk yield. As a result, cows with inherited weaker body condition naturally prolong the *post partum* interval to stall successive pregnancies (Dechow et al. 2002). An energy deficit stimulated the release of reserve fat, and it increased milk fat synthesis in the udder, as noted in the group of cows with ketosis. Fat reserves were mobilized more readily by diseased cows, and the above should enhance productivity. In a German study, the milk yield of cows that mobilized subcutaneous fat (10–12 mm) most intensively was more than 2,000 kg higher on average than in the group of cows mobilizing 6–7 mm of fat (Staufenbiel et al. 2003). Significant correlations were not determined between disease incidence and milk yield, in particular as regards ketosis. It should be noted, however, that ketosis affects highest-yielding cows; therefore, productivity results were levelled out by the disease.

Overconditioning of dry cows and rapid fat mobilization in the transition period predisposes cows to an increased risk of metabolic disorders and lowers their reproductive performance. Maximizing feed intake in early lactation is therefore a primary goal to prevent metabolic diseases, such as ketosis. Our results show that monitoring changes in the body condition and NEFA and BHBA blood levels in high-yielding cows in the transition period, followed by taking relevant disease-control measures, may be effective in reducing the incidence of retained placenta and ketosis in dairy cattle herds.

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