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Clinical and Biochemical Evaluation of Cows Occurring Severe Weight Loss After Calving

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Abstract: This study was designed to observe the biochemical and clinical changes in Holstein cows suffering from severe weight loss after calving.

Clinically, out of the 75 milking cows in the first 8 week postpartum examined, 25 cows aged 4– 8 years were found to be suffering from decreased milk production, severe body weight loss. 25 healthy cows in the same period after calving, aged 4 – 8 years, were also used as a control group. Serum biochemistry analyses including potassium (K), sodium (Na), calcium (Ca), phosphorus (P), magnesium (mg), albumin, alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), total biluribin, (TBIL) total protein (TP), creatinine (Cre), blood urea nitrogen (BUN), and β-Hydroxybutyrate (BHBA) were evaluated.

Body condition score (BCS) of 17 of 25 cows, severe weight loss after calving cows, was ≤ 2.0 and BCS of 8 cows was between 2.0 and 3.0. BCS of Animals in the control group were determined as range: 3.5 -4.0. Endometritis in 11 cows, subclinical mastitis in 8 cows, clinical ketosis in 5 cows, sub clinical ketosis in 20 cows, and left displaced abomasum in 3 cows were determined. Identical points of 25 animals were higher BHB, GGT, AST, total globulin, and total protein levels according to healthy cows and references values. In addition, these cows had hypoalbunemi and hypocalcaemia, when compared with healthy cows.

The results of the study reported here indicated that metabolic profile including serum BHBA, calcium, albumin, GGT, AST, total protein, should be evaluated to determine the causes of severe weight loss, and decreased yield in cows after calving.

Key Words: β-Hydroxybutyrate, Subclinical ketosis, Cow, weight loss, Subclinical mastitis.

Doğum Sonrası Şiddetli Zayıflayan İneklerin Klinik ve Biyokimyasal Değerlendirilmesi

Özet: Bu çalışma doğum sonrası şiddetli kilo kaybeden Holstein ırkı ineklerdeki biyokimyasal ve klinik değişimleri ortaya koymak için yapıldı. Doğum sonrası ilk 8 haftada bulunan 75 inek muayene edilmiş, yaşları 3-8 arasında değişen 25 inekte süt veriminde azalma ve şiddetli zayıflama belirlendi. Kontrol grubu olarak yaşları 3-8 arasında değişen ve aynı periyotta bulanan 25 sağlıklı inek kullanıldı. Potasyum (K), sodyum (Na), kalsiyum (Ca), fosfor (P), mağnesyum (mg), albumin, alkalin fosfataz (ALP), gamma-glutamyltransferaz (GGT), aspartate aminotransferaz (AST), total biluribin (TBIL), total protein (TP), kreatinin (Cre), kan üre nitrojen (BUN) ve β-Hidroksibutiratı kapsayan serum biyokimyasal analizler (BHBA) değerlendirildi. 17 inekte vücut kondisyon skoru 2 ve altında, 8 inekte 2 -3 arasında belirlendi. Aynı periyotta bulunan kontrol grubundaki vücut kondüsyon skoru 3.5- 4 olarak tespit edildi. Doğum sonrası şiddetli zayıflayan 25 ineğin 11'inde Endometritis, 8'inde subklinik mastitis, 5'inde klinik ketosis, 20'sinde sub-klinik ketosis, 3 inekte abomasumun sola deplasmanı saptandı. Bu inekler sağlıklı hayvanlara kıyasla yüksek BHB, GGT, AST, total globulin ve yüksek total protein

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seviyelerine sahiptiler. Ayrıca bu inekler de hipoalbunemi ve hipokalsemi mevcuttu. Burada sunulan çalışmanın sonuçları doğum sonrası ineklerdeki şiddetli kilo kaybı ve süt verimi azalmasının nedenlerini tespit etmek amacı ile serum BHBA, kalsiyum, albumin, GGT, AST, total protein seviyelerini kapsayan metabolik profilin değerlendirilmesinin gerekli olduğunu bildirmektedir.

Anahtar Sözcükler: β-Hidroksibütirat, Subklinik ketosis, İnek, kilo kaybı, subklinik mastitis.

Introduction

Mild to moderate weight loss can be occurring as physiologic conditions in the early stages of lactation in cows. However, weight loss is considered as an important clinical problem that cows have lost weight over a known period of time[22]. The most common causes of weight loss along with decreased reproductive performance, and decreased milk production in the cows are protein-calorie malnutrition, Johne's disease, tuberculosis, clinical ketosis, sub – clinical ketosis, chronic hepatic failure, parasitism, renal amyloidosis, and other chronic diseases. In addition, abomasal disorders such as left displacement, abomasal ulcer, and abomasitis are important causes of weight loss[17].

This study was designed to observe the biochemical and clinical changes in Holstein cows suffering from severe weight loss after calving.

Material and Methods

Study Population

The study was conducted in Holstein Friesian dairy cows breeding farms around Afyon, Turkey. The herd consisted of 600 dairy cows. Clinically, 25 cows aged 3– 8 years were found to be suffering from decreased milk production, severe body weight loss in the 8 week after calving. The study livestock included these 25 Holstein cows in early lactation. 25 healthy cows, aged 3 – 8 years, in the first 8 week after calving were also used as a control group for comparison to biochemical values.

Clinical Examination and Evaluation of Body Condition Score

Body temperature, pulse and respiration rates and ruminal motility were determined by clinical examination. Detailed clinical examinations were performed for left and right displaced abomasum, clinical ketosis, clinical mastitis, pneumonia, metritis, vagus indigestion, chronic traumatic reticuloperitonitis, cecal dilation; chronic enteritis, acute or chronic laminitis. Detailed history was taken from veterinary of farm about health status the study livestock within 8 week after calving. This informs were recorded. At the time of samples collection and clinical examination, body condition score of the cows was evaluated on a five-point scale (where 1 – 2 = emaciated to 5 =fat) at 0.25 unit increments[8]. The single intradermal comparative test to selected cows (test group; 25 cows; control group: 25 cows) was carried out for tuberculosis and paratuberculosis at seventh week after calving. The skin test reagents (PPD-Mycobacterium bovis, PPD-Mycobacterium avium) were injected intradermally at separate sites using 27-gauge hypodermic needles. After 72 hours, skin fold thickness was again measured. Cows suspected from an underlying infectious and non-infectious disease such as chronic pneumonia, footrot, sole abscess, renal failure, esophageal malfounctions, tuberculosis, paratuberculosis, abomasal ulcer, and traumatic reticuloperitonitis, parasitic infestations that might affect weight condition, and yield in cows were excluded from the study.

Sample Collection and Measurements

Blood samples were taken by jugular venipuncture from each cow into 10 mL evacuated tubes without anticoagulant, to evaluate routine biochemical parameters. Serum biochemistry analyses including potassium (K), sodium (Na), calcium (Ca), phosphorus (P), magnesium (mg), albumin, alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), total biluribin, (TBIL) total protein (TP), creatinine (Cre), blood urea nitrogen (BUN) were measured using by an auto analyzer (VETSCAN®, Analyzer, Abaxis Inc, USA). Serum β-Hydroxybutyrate (BHBA) was evaluated using a quantitative analyzer with multiple analyte capability for use with GDS test cards (STATSITE, GDS® diagnostics, a Division of DGS technology, Inc., USA, KetoSite®, cat no: 301000 GDS12.6, GDS12.5; STANBIO Labrotory Inc., Boerne, TX USA). Blood samples without anticoagulant were centrifuged at 3000 r. p.m. for 20 minute and sera were immediately separated and then it was stored at −20◦C until analysis. Samples with visual hemolysis were excluded from the study. To avoid a likely peak in blood BHB after a meal, the samples were

taken 6 hours after feeding. The presence of urine ketone was evaluated using a commercially available urine strip test based on sodium nitroprusside (Combur[10] test®, Ref: 0 4510089056, Roche Diagnostics GmbH, Mannheim, Germany). Pink or purple colour change was recorded as a positive for urine ketone. California Mastitis Test was also performed to subclinical mastitis, or clinical mastitis.

Statistical Analysis

The data were expressed as mean ± SE. The significance between the mean values was determined by Student's *t*-test. For all comparisons, values of $P < 0.05$ were considered significant.

Results

Total of 25 cows in lactation were below body condition score (BCS) at the time of sampling. Body condition score of 19 cows was ≤ 2.0 and BCS of 6 cows was between 2.0 and 3.0. Veterinarian of the farm emphasized that BCS in these cows had been range 4.5 -5 before calving. All animals had a normal pulse (range; 64 – 76 beats /minute, reference range 60 -80 beats/minute), body temperature (range: 38.2 – 39.1 C, reference range: 37.8 -39.2 C), and respiratory rate (range: 24- 28 breaths/minute; reference range: 10 -30 breaths/minute). While ruminal motility of 19 cows was normal limits (range: 9-11/ 5minutes; reference range: 8-12/5 minutes), 6 cows had decreased ruminal motility (range: 2 – 5/ 5 minutes). Endometritis was diagnosis in 11 of 25 cows based on palpation rectum and white pus to estrual mucus discharge from the vulva. Based on auscultation of a characteristic ping resonance during percussion on 9 -12 intercostals of the left side, it was determined that 3 of 25 cows had left displaced abomasum, which were confirmed by laparotomy. 5 cows of 25 cows, of which 3 had LDA, had clinical ketosis based on presence of ketonuria in examination of urine strip test. Sub clinical mastitis was diagnosed in 8 of 25 cows based on CMT (California Mastitis Test). Additionally, veterinarian of the farm emphasized that the other cases definitions were retained placenta (5 cows), milk fever (3 cows), and dystocia (12 cows) within 8 week after calving.

For tuberculosis and paratuberculosis, results of intradermal comparative test of all animals were negative at seventh weak after calving.

Serum BHB level was significantly ($p<0.001$) higher in cows suffering from severe weight loss after calving compared with healthy cows (table -2). Serum BHB level was determined as 1000 to 12000 µmol/L in 20 cows suffering from severe weight loss after calving. Results of BHB in these cows were evidence of subclinical ketosis. Other 5 cows had clinical ketosis, theirs BHB levels ranged from 1400 to 1445 µmol/L.

Table-1: Signalment and Healthy Status of cows suffering from severe weight loss after calving

Tablo1. Doğum sonrası şiddetli kilo kaybı gösteren ineklerin eşkali ve sağlık durumu

Cases	*Age (years)*	*BCS*	*urine keton*	*daily mean milk yield at the first 8 week/L*	*Healthy status*
1	4	3	-	18.3	metritis, subclinical ketosis, subclinical hypocalcaemia
2	5.5	3	-	15.1	subclinical ketosis,, subclinical mastitis
3	6	2	-	14.5	mastitis, metritis, subclinical ketosis, subclinical hypocalcaemia
4	4.5	3	-	15.1	subclinical ketosis, subclinical hypocalcaemia,, subclinical mastitis
5	5	3	-	19.2	metritis, subclinical ketosis
6	8	2	++	12.2	LDA, metritis, secondary ketosis,, subclinical mastitis
7	8	2	-	17.3	subclinical ketosis, subclinical hypocalcaemia
8	6.5	2	++	10.5	LDA, metritis, secondary ketosis, subclinical hypocalcaemia
9	7.5	2	-	16.7	metritis, subclinical ketosis, subclinical hypocalcaemia, subclinical mastitis
10	6	2	-	12.5	subclinical ketosis, subclinical hypocalcaemia
11	5.5	2	+	13.1	secondary ketosis, subclinical hypocalcaemia
12	6	2	-	18.7	subclinical ketosis, subclinical hypocalcaemia
13	4.5	3	+	11.6	LDA, secondary ketosis,, subclinical mastitis
14	5	3	-	20.3	subclinical ketosis, subclinical hypocalcaemia
15	7	2	-	12.3	subclinical ketosis, subclinical hypocalcaemia
16	7.5	2	++	10.4	metritis, primary clinical ketosis, subclinical hypocalcaemia
17	8	2	-	18.8	subclinical ketosis, subclinical hypocalcaemia
18	6.5	2	-	19.6	subclinical ketosis, subclinical hypocalcaemia
19	6	2	-	21.4	subclinical ketosis
20	7	2	-	17.5	metritis, subclinical ketosis, subclinical mastitis
21	7	2	-	18.9	subclinical ketosis, subclinical hypocalcaemia
22	8	2	-	17.8	subclinical ketosis, subclinical hypocalcaemia
23	8	2	-	16.4	subclinical ketosis, subclinical hypocalcaemia
24	7.5	2	+	13.7	metritis, secondary ketosis, subclinical hypocalcaemia
25	7.5	2	-	18.6	metritis, subclinical ketosis, subclinical hypocalcaemia,, subclinical mastitis

Biçimlendirilmiş: Yazı tipi: Arial Narrow, 7,5 nk, Portekizce (Brezilya)

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Table 2. Mean (± SE) biochemical findings in cows suffering from severe weight loss after calving

Tablo 2. Doğum sonrası şiddetli kilo kaybı gösteren ineklerdeki biyokimyasal değerler.

Electrolytes	*Normal Values**	*Healthy cows*	*Cows from severe weight loss after calving*	*P value*
Na^+ (mEq/L)	132 -152	140.1± 1.1	138.6 ± 1.2	NS
K^+ (mEq/L)	3.9 -5.8	5.3±0.6	4.8 ± 0.2	NS
Ca (mg/dL)	9.7 - 12.4	9.9±0.1†	8.3±0.2††	<0.05
P (mg/dL)	5.6 - 6.5	6.2±0.2	5,7±0.2	NS
Mg (mg/dL)	1.8 - 2.3	2.3±0.1	2.1±0.1	NS
Enzymes (IU/L)				
AST	43 - 127	85.3±6.7†	159.4±8.9††	<0.01
ALP	27-107	59.7±3.1	60.1±5.5	NS
GGT	15 - 39	29.2 ±3.1†	57.4± 6.1††	< 0.01
Others				
T. protein (mg/dL)	6.7-7.5	8.2±0.3†	8.7±0.2††	<0.05
Albumin(mg/dL)	3-3.6	3.1±0.2†	2.1±0.2††	<0.01
T. Globulin (mg/dL)	2.9-5.6	5.1±0.2†	6.6±0.2††	<0.05
BHBA(µmol/L)	0-865	548.8±42.7†	1311.5±131.1††	<0.001
BUN mg/dL	6 -27	16.5 ± 1.0	20.7±1.1	NS

†, †† Differences between the values involving different signals on the same row are found to be statistically significant.

*Bradford P. SMITH; Large Animal Internal Medicine, third edition, 2002

When compared with healthy cows, serum sodium, potassium, phosphate, and magnesium levels were no significant differences in cows suffering from severe weight loss after calving at the time of sampling. Serum calcium levels in cows suffering from severe weight loss after calving, in comparison with healthy cows, was significantly ($p<0.05$) low. Furthermore, veterinarian of the farm was reported that 3 cows were milk fever within 2 days after calving, and theirs recovered after calcium therapy.

With regard to statistical evaluation of serum biochemistry analyses including GGT, AST, ALP, total protein, albumin, total globulin and BUN between healthy cows and cows suffering from severe weight loss, according to healthy cows, serum GGT ($p<0.01$), AST ($p<0.01$), total protein ($p<0.05$), and total globulin levels ($p<0.05$) were significantly high in cows suffering from severe weight loss. Especially, serum GGT, total protein and total globulin were even higher than normal values. Serum albumin level in cows suffering from severe weight loss was significantly ($p<0.05$) lower than references values and healthy cows. There was not statistical difference in evaluation of serum BUN concentration between groups (table.2).

Discussion

Decreased milk production along with severe body weight loss is the most problems in dairy farms. These problems cause high cost for all countries. Therefore, it is very important to determine of causes of decreased milk production, severe body weight loss in cows, and to prophylaxis intended for these causes. In the present study was determined that it was endometritis in 11 cows, left displaced abomasums in 3 cows, clinical ketosis in 5 cows, sub clinical mastitis in 8 of 25 cows. Additionally, veterinarian of the farm emphasized that the other cases definitions were retained placenta (5 cows), milk fever (3 cows), and dystocia (12 cows) within 8 week before study. Identical points of all these animals were higher BHB, GGT, AST, total globulin, and total protein levels according to healthy cows and references values. In addition, these cows had hypoalbunemi and hypocalcaemia, when compared with healthy cows. These biochemical results (especially, hypocalcaemia, high GGT, BHB, and low albumin levels) along with clinical findings showed that cows suffering from severe weight loss after calving at the time of sampling had a serious metabolic disorder[16,18,26-28].

Elevated serum concentration of BHBA is diagnostic for clinical and subclinical ketosis[6,7,20]. To distinguish between normal cows and cows with SCK, a cut-off point of blood BHB at 1200 µmol/L has been recommended by several investigators[5-7,9]. Similarly, some authors[20,28,29] reported that BHBA for diagnosis of subclinical ketosis have ranged from 1000 to 1400 µmol/L. Therefore, in the present study, 20 of cows with BHBA levels ranged from 1200 to 1400 µmol/L (mean +SE; 1311.5±131.1 µmol/L) was defined as sub clinical ketosis (table -1). The majority of cows sampled during the first 6 week post calving were at peak risk for sub clinical ketosis as previously reported by Dohoo and Martin[5], Duffield et al.[7]. In addition, in the present study, 5 of 20 cows with BHB levels ranged from 1400 to 1445 µmol/L had clinical ketosis. Ketosis and sub-clinical ketosis are a major metabolic disorder of dairy cows. The presence of excess ketone bodies without clinical signs is defined as subclinical ketosis. Subclinical ketosis has been closely associated

with a loss in milk production, body weight and a greater incidence of left-displaced abomasum, metritis, cystic ovaries, longer calving intervals and other peri - parturient diseases[3,5,23]. Especially, clinical or subclinical ketosis have been linked with an increased risk of metritis, displaced abomasums[3,5,11,16], and mastitis, as reported in present study. Some authors[1,25] reported that elevated concentrations of BHBA have suppressed the immune response in vitro. Therefore, in the present study, determined endometritis (11 cows) and subclinical mastitis (8 cows) may be associated with occurring immunosuppression due to increased BHBA. Another reason for the endometritis is probably delayed involution due to severe negative energy balance[12,15,24,30]. Accordingly, high BHBA levels along with endometritis, subclinical mastitis, and left displaced abomasum in present study were indicated that cows in the farm had a negative energy balance.

In the present study, increased serum levels of GGT, AST, BHBA, total globulin, and decreased serum levels of albumin may regard as diagnostic indicators for chronic liver damage such as fatty liver syndrome. Albumin is the most important indicator of liver function in animals without gastro-intestinal and renal loss. There was not chronic diarrhea and proteinuria in this population of cows. Therefore, in these cows suffering from severe weight loss after calving, hypoalbunemia might be associated with a decrease in production due to chronic liver disorder. Albumin can be used to predict disease risk in close-up and fresh periods[26,27]. Van Saun[26,27] reported Fresh cows that could maintain serum albumin concentrations $\geq$ 3.5 mg/dl were less likely to have postpartum disease, and serum albumin concentrations $\leq$ 3.25 mg/dl in close-up dry cows resulted in a three-fold greater risk for postpartum disease.

In the present study, although the increases in total globulin levels in the cows suffering from severe weight loss after calving described, globulin fractions had been not determined. However, increases in beta and gamma globulins often are accompanied by hypoalbuminemia[22]. Therefore, it is though that increased total globulin is presumably associated with high beta and gamma globulins.

According to healthy cows, serum calcium values in cows suffering from severe weight loss after calving have been determined as low. Hypocalcaemia is categorized into clinical and subclinical hypocalcaemia. In the present study, cows suffering from severe weight loss at the time of sampling had not typical findings of clinical hypocalcaemia such as head shaking, stiff gait, falls easily, recumbency, head turned into flank. Therefore, these cows have been regarded as subclinical hypocalcaemia. However, in detailed history, it was emphasized that clinical hypocalcaemia was occurred in 3 cows within 48 h after calving. Subclinical hypocalcaemia is associated with many postpartum disorders such as displaced abomasums, ketosis, mastitis, retained placentas, other decreased reproductive performance, and delayed uterine involution[14]. As some investigators[13] notified, possible effects of subclinical hypocalcaemia on reproductive disorders are impaired blood flow to the uterus and to the ovaries. In addition, subclinical hypocalcaemia may be aggravating the negative energy balance of the cows due to reduction in digestive tract motility and nutrient intake[4,18,19]. High BHBA levels in cows with subclinical hypocalcaemia in the present study are support to these comments.

BUN was mild high in cows suffering from severe weight loss, according to healthy cows. But, there is not statistical difference between groups. Urea concentrations are influenced by protein intake, energy intake, and urinary excretion[2]. BUN fluctuates throughout the day. Milk urea nitrogen is slightly less volatile. Therefore, a milk urea nitrogen level is the more important than BUN for assessing nutritional status. Unfortunately, in the present study, time intervals between collecting and evaluation of milk samples were not short enough to protect the samples. In addition, 2-bromo-2-nitropropane-1,3-diol was absent to protect the samples. Thus, MUN values were not assessed.

Based on auscultation of a characteristic ping resonance during percussion on 9 -12 intercostals of the left side within 6 week after calving in 3 cows was determined to left displaced abomasum, which were confirmed by laparotomy. These animals had subclinical hypocalcaemia (ketonuria, high BHBA > 1400 µmol/L) levels and secondary clinical ketosis. LeBlanc et al.[16] reported that increased BHBA concentrations were associated with increasing risk of subsequent diagnosis of left displaced abomasums. Similarly, Geishauser et al. (1997) determined that subclinical ketosis in the first 2 weeks postpartum was associated with increased risk of LDA. In addition, subclinical

hypocalcaemia may be another cause of LDA due to reduction in abomasal motility[4,18,19].

Body condition scoring is a process used to compare the relative body fat of cows, and it is reported that body condition scoring varies during a 305 day lactation cycle. Both high body condition score in dry period and body condition loss in early lactation were important risk factors for subclinical ketosis. Especially, fat cows in dry period are greater risk than thin cows for subclinical ketosis[7,10,21]. Similarly, in detailed history of cows with subclinical ketosis in the present study, it was determined that they had high body condition score (BCS $\geq$ 4.5) in prior to calving period. However, it was reported severe body weight loss (BCS < 2) in the first 8 week after calving in these cows. Severe body condition loss after calving exhibits the negative energy balance. The negative energy balance may have long term negative effects on the milk production, calving intervals, reproductive performance, and average lifetime of cows. Indeed, high BHBA levels, severe body condition loss, left displaced abomasums, mastitis, ketosis, and reproductive problems such as endometritis after calving showed that the negative energy balance was occurred in cows in the present study.

The results of this study show that cows occurring severe weight loss after calving had negative energy balance on the basis of high BHBA levels along with subclinical hypocalcaemia, endometritis, subclinical mastitis, and left displaced abomasum. In addition, it was thought that hepatic disorder may be occur in cows due to high GGT levels, accompanied by hypoalbuminemia, and high total globulin levels. Probably, this hepatic disorder might be associated with fatty liver.

In conclusion, the results of the study reported here indicated that metabolic profil including serum BHBA, calcium, albumin, gamma-glutamyltransferase, aspartate aminotransferase, total protein with other routine clinical and laboratory findings should be evaluated to determine the causes of severe weight loss, decreased milk production in cows after calving.

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