Determination of selected acute phase proteins during the treatment of limb diseases in dairy cows

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ABSTRACT: The purpose of this study was to assess the diagnostic value of fibrinogen, haptoglobin, and serum amyloid A determination in the monitoring of the treatment of limb diseases in dairy cows. Fourteen lame cows were examined, while 10 clinically healthy cows constituted the control group. Blood samples from the ill animals were collected on three occasions: (1) upon arrival at the clinic, (2) between the third and sixth day after arriving, and (3) upon return to the owner. Blood samples from the control cows were collected once. Plasma levels of fibrinogen, haptoglobin, serum amyloid A, and total serum protein and its fractions (albumin, α-, β-, γ-globulins) were measured. Significantly higher fibrinogen, haptoglobin, and serum amyloid A levels were observed in the affected cows upon arrival at the clinic than in the control cows. Based on the changes in fibrinogen, haptoglobin, and serum amyloid A concentrations, the cows were divided into those with a systematic decrease in acute-phase protein levels during treatment (Group I, n = 6) and those which showed an increase in one or more acute-phase proteins despite treatment (Group II, n = 8). A stepwise decrease in the examined acute-phase proteins was observed in the first group and indicated an uncomplicated course of treatment; however, treatment of the second group did not appear to be wholly successful. A majority of the cows under treatment (n = 13) exhibited abnormal levels of the examined acute-phase proteins upon return to the owner. This indicates that these patients did not recover completely. The monitoring of plasma acute-phase protein concentrations can be a valuable complement to the clinical assessment of the treatment course and in the early detection of disease complications.

Keywords: fibrinogen; haptoglobin; serum amyloid A; cattle; lameness; disease monitoring

Limb pathology is a serious problem in dairy herds, as lameness considerably affects milk production. High-yield milk cows are more predisposed to lameness (Green et al., 2002) and larger herds seem to have more problems, with 23.7% to 54.6% of dairy cows reported as being affected (Clarkson et al., 1996; Whitaker et al., 2000; Booth et al., 2004). Lameness means that these animals take longer to return from the milking parlor and it also causes decreases in milk and protein production (Juarez et al., 2003). Moreover, the milk yield sometimes starts to decrease before clinical symptoms can be observed, and negative effects are especially apparent in cows during the first lactation. Milk loss ranges from 1.2 to 2.8 kg/day during the first two weeks after diagnosis (Rajala-Schultz et al., 1999). A lame cow loses on average 350 kg of milk (from 160 to 550 kg) during its lactation; lower milk production can persist for as much as four months before lameness is diagnosed and treatment applied, and may even last up to five months after treatment (Green et al., 2002). The risk of culling due to lameness increases with each lactation (Esslemont and Kossaibati, 1997). More

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than 60% of lameness occurs before the 120th day of lactation (Booth et al., 2004) and the resulting losses are considerable enough to draw attention to the necessity of early diagnostics of lameness (Green et al., 2002).

The acute-phase response is nonspecific and it develops after an injury, regardless of the pathogenic factor. In an acute-phase reaction, the organism responds in a multifactorial way to inhibit/restrict the inflammatory process and to repair damaged tissues. One of the mechanisms involved is the production and secretion of acute-phase proteins (APPs) by the liver (Eckersall and Conner, 1988). This is effected by pro-inflammatory cytokines (IL-1, IL-6, TNF-α), regardless of the inflammation-causing factor (Godson et al., 1995; Alsemgeest et al., 1996; Yoshioka et al., 2002). With the aid of this nonspecific reaction it is possible to use changes in the concentrations of APPs universally to monitor the course of the disease, independently of its nature (Jawor and Stefaniak, 2006). The proteins whose concentrations increase due to inflammatory factors are termed positively reacting (e.g. haptoglobin – Hp, serum amyloid A – SAA, fibrinogen – Fb) and those whose concentrations decrease negatively reacting (e.g. albumin, transferin) (Prasse and Fayer, 1981; Murata et al., 2004).

Diagnosing inflammatory processes in cattle presents numerous problems, as clinical symptomatology is quite poor; alterations in the classical parameters of the inflammatory response are relatively slight and not very specific. Moreover, cattle are kept together in a group, which makes the observation of individuals more difficult (Stefaniak, 2003). APPs are sensitive factors enabling early and precise detection of inflammation in ruminants (Kent, 1992). Horadagoda et al. (1999) found that in order to differentiate between an acute and a chronic state in cattle, APPs are much more useful than classical hematological tests (leukocyte count, neutrophil percentage, or band neutrophil percentage). This is consistent with Schalm's results (cited by Jain, 1986), where leukocyte and fibrinogen reactions were analyzed simultaneously during serious inflammatory states in cattle. The proteins most often examined in cattle are Fb, Hp, and SAA. Determining Hp and SAA has proved useful not only in detecting inflammatory processes, but also in monitoring health and disease (Gruys et al., 1993). Hirvonen and Pyorala (1998) showed the possibility of using Hp and Fb in the postoperative monitoring of infectious complications, but they stressed that the values obtained before and after operation should be compared, as increases in Hp values have been reported in the latter case. Because surgical trauma has not been shown to increase Fb concentrations, evaluating Fb may be helpful in the postoperative monitoring of infectious complications, e.g. peritonitis. Plasma Hp and Fb concentrations were accurate parameters for differentiating traumatic reticuloperitonitis from other gastrointestinal disorders (Hirvonen and Pyorala, 1998). The usability of Hp and SAA levels in blood serum and milk in the diagnostics of mastitis has been demonstrated in detecting acute, chronic, and subclinical states as well as in distinguishing between mild and moderate states (Eckersall et al., 2001; Gronlund et al., 2003, 2005; Nielsen et al., 2004; Hiss et al., 2007). So far there is no consensus on the normal concentration range of SAA in clinically healthy animals, and these values differ according to the author and the cattle examined (in dairy breeds they are higher than in beef cattle). Hp and SAA levels have also been found to be higher in animals suffering from acute inflammation than in those affected by non-acute inflammation (Stefaniak, 2003). Ganhaim et al. (2003) proposed the following acceptable values of haptoglobin, serum amyloid A, and fibrinogen in monitoring the health of the calves in a herd: Hp: 0.13 g/l, SAA: 25.6 mg/l, and Fb: 6.45 g/l. When determining several acute-phase proteins simultaneously, e.g. Hp, SAA, and $\alpha_1$acid glycoprotein, with the aim of assessing their usefulness in differentiating between acute and chronic inflammatory states, Horadagoda et al. (1999) found that SAA showed the highest sensitivity, while Hp had the highest specificity.

Measuring the concentrations of acute-phase proteins has been applied in viral infections, in identifying calves requiring anti-inflammatory treatment, in making health management decisions (Heegaard et al., 2000; Ganheim et al., 2003; Berry et al., 2004; Humblet et al., 2004), and in monitoring postoperative complications in cows (Hirvonen and Pyorala, 1998). However, little is known about these proteins with regard to limb diseases.

The objective of this study was to determine whether an evaluation of APPs at selected time points during the treatment of inflammatory states of limb disease could be used to monitor the course of treatment and as an early predictive agent of possible complications. We sought to characterize the relationship between changes in concentrations

of the investigated proteins and the clinical course of treatment.

MATERIAL AND METHODS

Study animals

The investigation was carried out on 14 cows which were patients of the Clinic for Ruminants of the University of Veterinary Medicine in Vienna, Austria, in the years 2005–2006. The control group consisted of 10 clinically healthy dairy cows which came from a private breeder and were in various lactation phases and in a dry period. The cows under study were divided into two groups based on changes in the dynamics of positively reacting acute-phase proteins.

Group I: in which systematic decreases in APP levels in subsequent blood collections were recorded: six heads.

Group II: in which an increase in the level of one or more APPs in the second or third blood collection was recorded: eight heads.

Group III: the control group.

All the patients of the clinic are listed in Table 1 according to the diagnosis of the main disease and group membership (I or II).

Sampling

Blood samples had been collected once from the control cows for other diagnostic purposes (permission No. 91/04 II by the Local Ethics Commission for Experiments on Animals at the Agricultural University in Wroclaw). Blood for analysis was collected during the necessary examinations for diagnosing the cases and took place on three separate occasions which were timed so as to allow the most accurate determination of the investigated dynamics:

– on the day of the patient's arrival at the clinic,
– between the third and sixth day of stay,
– when the animal became healthy enough to be sent back to its owner for further treatment.

In some cases, these time points were not strictly adhered to: for patients arriving at the clinic in the evening or at night, when it was not possible to schedule the examination and blood collection together, and in cases where the animal was returned to the owner before the third planned blood collection. Altogether, three blood samples were missing.

Methods

All cows were examined upon arrival at the clinic and on every day of their stay at the Clinic according to the method of Baumgartner (2005). Each cow was assigned a clinical number and a case history card was created and updated according to Clinic regulations. The orthopedic examinations were performed according to the method of Stanek (1997).

Blood was collected from the jugular vein using two types of sterile test tubes of the Vacutainer™ System (one for blood serum and the other with the anticoagulant K_2EDTA). Blood collected to obtain serum was left for two hours and then centrifuged at 2 000 × g for 10 min at room temperature. The blood serum was collected in test tubes and stored at –20°C until it was assayed. The concentration of Fb was determined in the whole blood according to the method of Millar et al. (1971) as modified by Brugmans et al. (1998). In the serum samples the concentrations of Hp by the guaiacol method according to Jones and Mould (1984), SAA by ELISA (Tridelta Development), total serum protein (TSP) by the Biuret method, and TSP fractions (albumin, α-, β- and γ-globulins) by paper electrophoresis were determined.

Statistical analysis

The data underwent unidirectional analysis of variance in a non-orthogonal system using the statistical package STATISTICA 7.1. The significance of differences between groups was estimated using the Tukey test for unequal sizes of groups. The values between the groups at subsequent collections were compared each time.

RESULTS

In the examined cows, arthritis, sole ulcer, and white line disease (WLD) were the most often diagnosed diseases. All cases of sole ulcers and white line disease were localized on the hind limbs in the outer claw. Arthritis affected only the lateral hoof joints and hind limbs, except for one cow which suf-

Table 1. Examined limb pathology cases

Patient No.	Qualitative diagnosis	Experimental group	Degree of lameness/body temperature at arrival °C/main symptoms	Treatment	Other disorders found at hospitalization
1	**sole ulcer**, white line disease, interdigital hyperplasia	II	slight/38.8/bedsore on hind limb with purulent discharge	wound cleaning	n.d.
2	**interdigital hyperplasia**, dermatitis digitalis	I	no signs/38.3/swelling around pastern joint	resection of interdigital hyperplasia, antibiotic *i.m.*	n.d.
3	**sole ulcer**, necrosis distal sesamoid bone and flexor tendon	II	moderate/38.8/swelling around pastern joint	first 13 days conservative therapy, then amputation of the distal phalanx, antibiotic and anti-inflammatory drugs *i.m.*	n.d.
4	**arthritis pedal joint**, sole ulcer, necrosis distal sesamoid bone	I	moderate/39.2/swelling auround medial interphalangeal joint	amputation of distal phalanx, anti-inflammatory drugs i.m.	n.d.
5	**arthritis pedal joint**, sole ulcer, tenosynovitis	I	severe/38.8/swelling above fetlock joint	amputation of distal phalanx, tendon resection, antibiotic *i.m.*, anti-inflammatory drugs *i.m.*	n.d.
6	**arthritis pedal joint, phlegmone of the bulbs**	I	moderate/39.2/painful swelling above hoof	amputation of distal phalanx, tendon resection and sesamoid bone, antibiotic and anti-inflammatory drugs *i.m.*	n.d.
7	**arthritis pedal and pastern joint with osteolisis**, signs of periostitis on pedal and short pastern joint, white line disease, double sole, septic tendovaginitis and necrosis medial deep and superficial flexor tendon	II	moderate/37.9/painful swelling around the coronary band	amputation of distal phalanx, tendon resection, antibiotic *i.m.*, anti-inflammatory therapy *i.m.*	respiratory tract infection
8	**arthritis pedal joint**, necrosis deep digital flexor tendon come from white line disease	II	moderate/39.0/swelling to fetlock, reluctance to bear weight on limb	amputation of distal phalanx, anti-inflammatory drugs *i.m.*	n.d.
9	**arthritis pedal joint with osteolysis**, signs of periostitis on pedal bone, phlegmone of the bulbs, white line disease	I	severe/38.7/reluctance to bear weight on limb	amputation of distal phalanx, antibiotic *i.m.*, anti-inflammatory drugs *i.m.*	n.d.
10	**open fracture pedal bone and purulent arthritis coffin joint**, heel-horn erosion	II	severe/39.1/reluctance to bear weight on limb, painful swelling to carpus	amputation of distal phalanx, antibiotic *i.m.*, anti-inflammatory drugs *i.m.*	bronchitis
11	**arthritis pedal joint**, septic tendovaginitis, urovagina	II	severe/39.0/painful swelling to proximal interphalangeal joint	amputation of distal phalanx, antibiotic *i.m.*, anti-inflammatory drugs *i.m.*	n.d.
12	**abscess on amputation wound** (after former resection of superficial and deep flexor tendon)	II	slight/37.9/swelling in the region of the amputation wound	abscess opened and flushed for 4 days, antibiotic *i.m.*	n.d.
13	**fibrinous arthritis pedal joint**, interdigital hyperplasia	II	severe/38.3/reluctance to bear weight on limb	joint drainage, resection of interdigital hyperplasia, antibiotic *i.m.*, anti-inflammatory drugs *i.m.*	n.d.
14	**arthritis pedal joint**, white line disease with necrosis of tuberculum flexorium and deep flexor tendon, distal sesamoid bone, bursitis at the tarsus with abscess	I	unable to bear weigth/38.7/painful swelling around fetlock joint	amputation of distal phalanx, antibiotic *i.m.*, anti-inflammatory drugs *i.m.*	n.d.

The major disease is bolded; n.d. = not detected

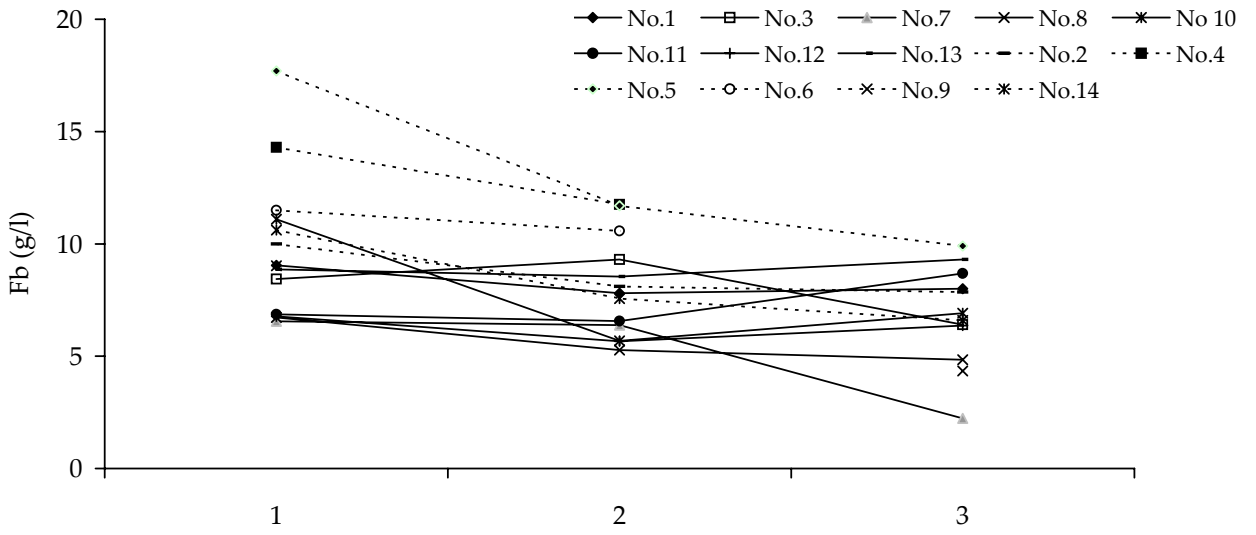

Figure 1. Course of fibrinogen concentration in the cows' plasma during treatment.

The numbers (1, 2, 3) refer to subsequent blood collecting, dashed line – Group I, continuous line Group II

fered from inner coffin and pastern joint arthritis. At the time of the cows' admission, body temperature values of (37.9–39.2°C, average: 38.7°C), heart rate (60–96 bpm, mean: 79 bpm), and breathing (16–80/min, average: 34/min) were recorded. The individual courses of Fb, Hp, and SAA concentration in examined cows are shown in Figures 1, 2, and 3, respectively. Mean values and standard errors of the positively reacting acute-phase proteins are shown in Figures 4, 5, and 6 and the medians and their ranges in Table 2.

In nine control cows there was no detectable Hp, while the Hp concentration in one amounted to 0.77 g/l. Fb concentration did not exceed the normal range in any of the cows (Jain, 1986). SAA concentration was lower than 76.7 μg/ml, which is within the range of values found by Tourlomoussis et al. (2004) in clinically healthy dairy cows.

The mean values of Fb, Hp, and SAA in all the ill animals exceeded the normal range upon their admission to the clinic (Figures 4, 5, 6). These values were higher in Group I than in II, and the difference

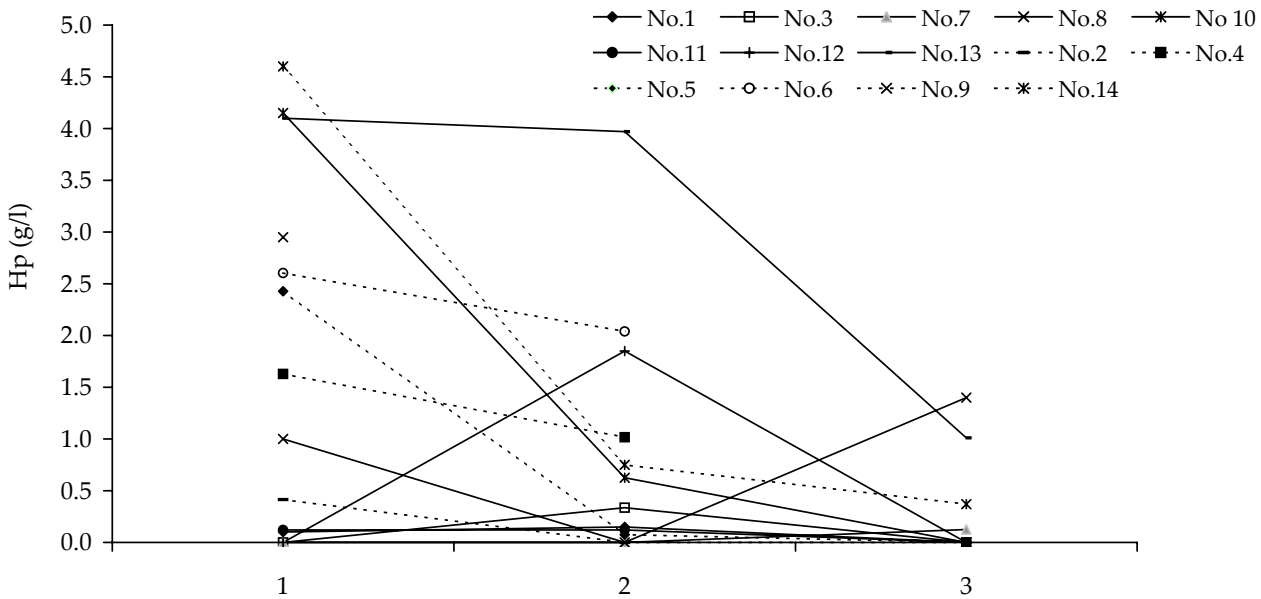

Figure 2. Course of haptoglobin concentration in the cows' serum during treatment.

The numbers (1, 2, 3) refer to subsequent blood collecting, dashed line – Group I, continuous line Group II

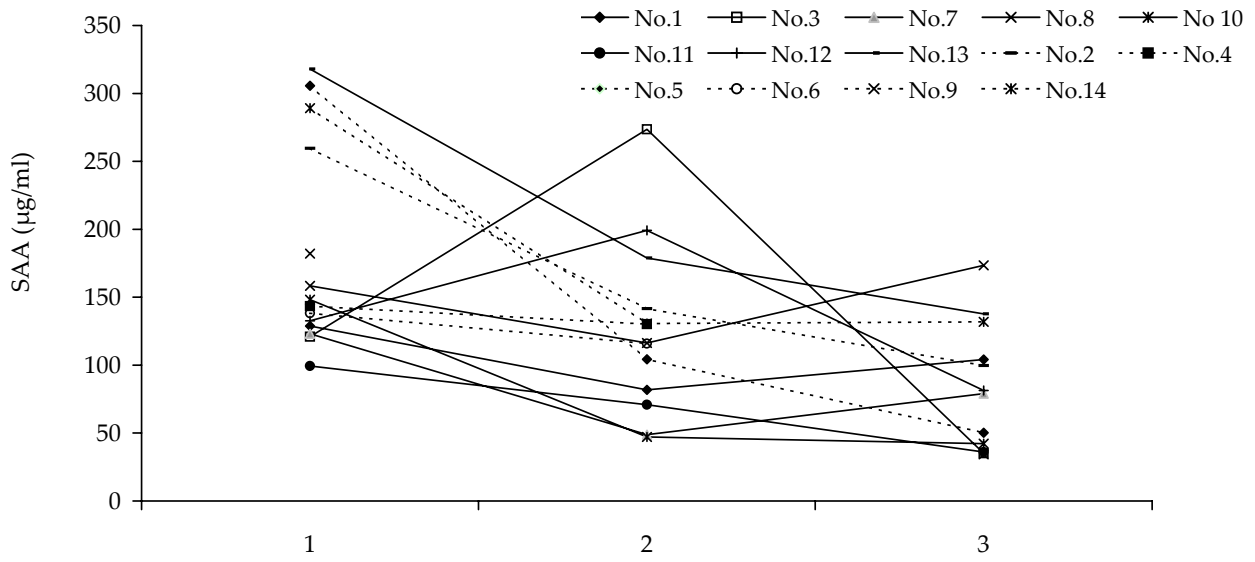

Figure 3. Course of serum amyloid A concentration in the cows' serum during treatment.

The numbers (1, 2, 3) refer to subsequent blood collecting, dashed line – Ggroup I, continuous line Group II

was of high statistical significance for Fb (Figure 4). In Group I, all animals had concentrations of APPs considerably exceeding the upper limit of the normal range, while in Group II the Fb concentrations in four cows corresponded to the upper limit of the norm. Hp was not detectable in three animals. In the course of treatment, decreases in the investigated protein concentrations were recorded, which seemed to result from the reduction of the inflammatory processes. The highest concentrations of the proteins under examination were recorded at the time of admission to the clinic. At each blood collection, group I had the highest average Fb concentration. However, the initially very high Fb concentration in Group I considerably decreased (as a consequence of treatment) and the relative decrease between the first and the last examination was more than 41%, while in Group II it amounted to only 18%. It is noteworthy that Group II showed a lower mean Fb upon arrival at the Clinic than Group I, but that at the second examination the mean was within the normal range. Mean SAA values at the first collection in Group I were 66 µg/ml higher than in Group II, while in the subsequent collections their mean values were even lower than in Group II (Figure 6). Similarly, the Hp concentration at the first collection was higher in Group I, but lower at the second and third collections than in Group II (Figure 5). The decreases in Hp and SAA levels were on average much more apparent in cows undergoing treatment without complications. Between the first and third blood collections

Table 2. Medians and ranges of Fb, Hp, and SAA concentrations in the examined groups of animals

Group	Fb (g/l)			Hp (g/l)			SAA (µg/ml)		
	sample			sample			sample		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
I	11.1 9.02–17.7	10.6 7.56–11.76	7.2 4.34–9.9	2.5 0.41–4.6	0.8 0–2.04	0.0 0–0.37	220.8 143.4–289.1	130.1 104.2–141.6	75.0 34.3–131.9
II	7.6 6.55–11.1	6.5 5.27–9.3	6.7 2.23–9.3	0.1 0–4.15	0.2 0–3.97	0.0 0–1.01	130.7 99.4–317.9	99.1 47.1–273.6	80.2 35.2–173.4
III		5.0 4–6.2			0.0 0–0.77			16.6 0–76.7	

Table 3. Mean concentrations and standard deviations of total serum proteins (TSP) and albumin (Alb) in the examined groups of animals

Group	TSP (g/l)			Alb (g/l)		
	sample			sample		
	1st	2nd	3rd	1st	2nd	3rd
I	78.13 ± 7.19	77.75 ± 6.34	72.81 ± 6.4	26.1^{B} ± 3.3	26.3^{B} ± 1.7	25.9^{B} ± 2.8
II	74.38 ± 5.51	69.84 ± 5.65	70.63 ± 5.43	30.8 ± 5.4	28.0^{B} ± 5.1	28.6^{B} ± 2.8
III		75.6 ± 3.64			34.5^{A} ± 3.2	

Data marked by different letters were statistically different – capital letters $P \leq 0.01$

Table 4. Mean concentrations and standard deviations of α-, β-, and γ-globulin in serum

Group	α-globulin (g/l)			β-globulin (g/l)			γ-globulin (g/l)		
	sample			sample			sample		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
I	11.6^{A} ± 1.4	11.5^{A} ± 1.0	11.1^{A} ± 1.2	10.4 ± 1.8	10.5 ± 3.1	8.4 ± 2.0	30.0 ± 5.0	29.5 ± 4.18	27.4 ± 6.7
II	10.9^{A} ± 1.4	9.8 ± 1.5	9.6 ± 1.1	10.0 ± 2.3	9.0 ± 1.2	8.9 ± 1.9	22.8 ± 6.8	23.0 ± 5.4	23.5 ± 5.4
III		8.4^{B} ± 1.0			9.7 ± 1.8			22.9 ± 3.8	

Data marked by different letters were statistically different – capital letters $P \leq 0.01$

the Hp concentration decreased by 96.3% (Group I) and 73.7% (Group II) and for SAA the decreases amounted to 64% and 44%, respectively. TSP concentrations decreased slightly in the course of treatment in both groups of cows (Table 3). Albumin concentration (Table 3) at the time of admission was lowest in Group I (below the lower limit of the norm, with a considerable statistical difference in comparison with the control group). In Group II the values oscillated in the region of the lower limit of the normal range (Smith, 1996). In contrast to positive APPs, which in the course of treatment tended to approach the normal value, the albumin concentration underwent further decreases in both groups of treated cows (Table 3). Average α-globulin concentration (Table 4) in the control

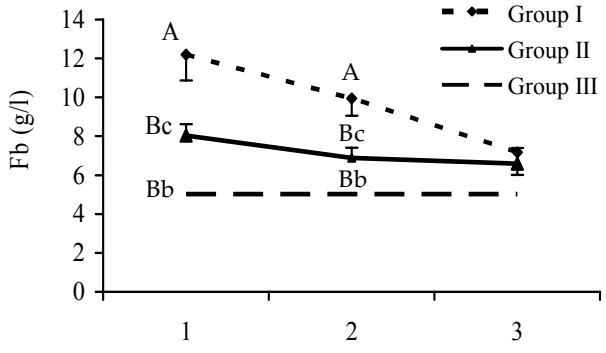

Figure 4. Concentrations of fibrinogen in plasma of the healthy controls and patients. The points show the means and the whiskers denote standard error. The numbers (1, 2, 3) refer to subsequent blood collecting. Data marked by different letters were statistically different: small letters $P \leq 0.05$, capital letters $P \leq 0.01$

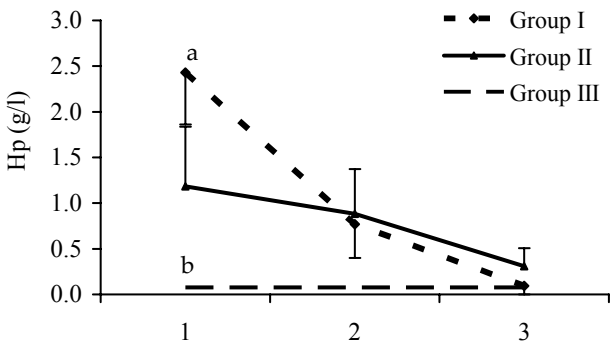

Figure 5. Concentrations of haptoglobin in serum of the healthy controls and patients. The points show the means and the whiskers denote standard error. The numbers (1, 2, 3) refer to subsequent blood collecting. Data marked by different letters were statistically different- small letters $P \leq 0.05$

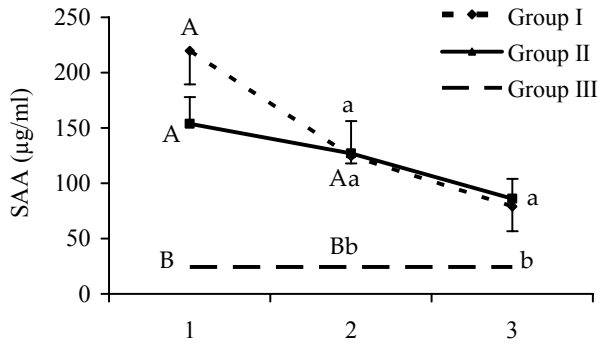

Figure 6. Concentrations of serum amyloid A in serum of the healthy controls and patients. The points show the means and the whiskers denote standard error. The numbers (1, 2, 3) refer to subsequent blood collecting. Data marked by different letters were statistically different: small letters $P \leq 0.05$, capital letters $P \leq 0.01$

group remained within the norm (Smith, 1996), in contrast to both groups of treated animals, where considerably higher α-globulin concentrations (a large, statistically significant difference compared with the control group) were noted at the first collection. When treated, both groups of cows with limbs in inflammatory states showed a diminishing tendency with regard to the globulin fraction. The average concentrations of β-globulin (Table 4) in all of the groups under examination did not exceed the normal range (Smith, 1996). It should be noted that in group I the average concentration of γ-globulin considerably exceeded the upper limit of the norm (Table 4), while in Group II and in the control group these values were similar and oscillated in the region of the upper limit of the normal range.

DISCUSSION

From earlier investigations (Murray et al., 1996; Green et al., 2002) it can be seen that the most frequent lesions within the area of the hoof horn and limb skin have been ulcers, white line disease, interdigital necrobacillosis, and digital dermatitis. In our own investigations, a different frequency of occurrence of limb diseases was observed. This was probably due to the fact that the study was not carried out on animals from a farm, but from a clinic where animals with advanced and complicated inflammatory states are usually hospitalized. Clarkson et al. (1996) reported that changes in the outer claw were nearly four times more frequent than those in the inner; in our own investigation we only observed lesions in the outer claw. According to Toussaint Raven, (cited by Clarkson et al., 1996), such a frequency can be explained by the fact that greater weight is placed on the outer hind claw.

The diagnosis of an elevated Hp concentration in one of the control cows was a surprising finding. Different normal Hp ranges for ruminants, ranging from undetectable, so-called "0" levels to 0.1 g/l are described in the literature (Stefaniak, 2003). The level found in the above-mentioned animal could have been caused by an advanced undiagnosed inflammatory state. The highest SAA concentration in the examined control group was 76.7 µg/ml, while the lowest SAA level in ill cows at the moment of admission to the clinic exceeded 99.4 µg/ml. Since the SAA concentrations in healthy dairy cows recorded by Tourlomoussis et al. (2004) did not exceed the range of 7–124 µg/ml and were higher than those obtained from the control cows in our own investigation, the question arises as to what SAA levels in cows without any pathological symptoms should be regarded as worrying.

In the treated cows there were extensive inflammatory states upon admission to the clinic; however, the concentrations of positive APPs were higher in Group I than in Group II. Low albumin levels in Group I (Table 3) and, at the same time, high concentrations of α- and γ-globulins (Table 4) suggested a serious inflammatory state, probably due to a bacterial agent. It seemed that the increase in the α-globulins was caused by the fact that the majority of APPs occurred in that fraction. Based on the decreasing levels of particular proteins, we suppose that an appropriate course of treatment was undertaken (without complication) in Group I. The decrease in the concentrations of all three positively reacting proteins between the first and second blood collection in Group II (case Nos. 7, 8, 10, 11, and 13) attests to the fact that the applied treatment was appropriate and contributed to eliminating the inflammatory processes in the cows. As a consequence of further complications (e.g. wound infections, bronchitis, occurrence of other inflammatory states of the limbs), increases in one or two of the examined APPs took place at the third blood collection. The slight Hp increase from 0.1 to 0.15 g/l and the simultaneous decreases in Fb and SAA in cow No. 1 at the second collection probably resulted from the surgical intervention undertaken, which consisted of resection of the infected tissues. An Hp increase with a normal Fb

level was observed by Hirvonen and Pyorala (1998) after abdominal surgery. In the same cow, slight increases in the Fb and SAA values at the third collection (seven days after the second collection) were recorded which were probably due to wound infection. No relationships between the initial concentrations of Hp, Fb, and SAA and prognosis were found. The concentrations of the particular proteins were dependent on the severity of the inflammatory lesions, and similar conclusions were drawn by Skinner et al. (1991).

In the cows investigated, the highest Hp concentrations (> 4 g/l) occurred in cows Nos. 10, 13, and 14, in which the inflammatory states where especially serious (Table 1). The highest SAA concentration (317.9 μg/ml, Table 2) was recorded in cow No. 13. Abnormal levels of the examined proteins (Fb, Hp, SAA, albumin) were observed in the majority of the treated cows at the time of their return to their owners. This indicates that most of the treated cows had not completely recovered, but that their disease state was such that the treatment could be continued by a local veterinary surgeon.

APP monitoring can facilitate the verification of the correctness of the decisions made. Because the agreement of the owner was not received, amputation of the distal phalanx in cow No. 3 was not performed and increases in all three positively reacting proteins, in the case of SAA by 126%, occurred. As there was no progress in healing, after 12 days the decision was made to amputate the claw, and 14 days later the clinical state of this animal considerably improved and the examined protein levels attained the normal range.

The triple determination of APPs we made during the treatment seems to be a valuable supplement to clinical findings. For practical purposes we recommend at least two APP estimations. The first should be made at the beginning of the treatment, because concentrations of APP reflect the extent of underlying tissue damage and are often used as indicators of disease severity (Deignan et al., 2000; Heegaard et al., 2000). It is noteworthy that the ability to produce SAA and Hp may be an innate characteristic of the individual (Jacobsen et al., 2004). The second should be made immediately before returning the cow to its owner. This estimation, in addition to other clinical parameters (a decrease in lameness, an increase in appetite and milk yield), is a good measure of the efficiency of the therapy. We suggest an additional estimation during treatment in the following cases: when there is doubt regarding the correctness of the treatment, when there is very little or no clinical improvement, or before making critical decisions, such as amputation.

APP determination should also be useful during the recovery period because, for example, the time between dressing changes after claw resection is gradually prolonged. In that period, apart from the assessment of the leaning of a cow on an affected limb and general behavior in field conditions, there is no possibility to assess the healing process.

The results of our investigation show that the estimation of both the levels and the dynamics of changes in the concentrations of APPs can be a valuable supplementation to the clinical assessment of the course of treatment and in detecting complications rapidly. The results obtained encourage employing Fb, Hp, and SAA assays during the clinical treatment of dairy cows.

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