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Alterations of growth hormone, cortisol, luteinizing hormone, and insulin concentrations in early-postnatal calves affected with diarrhea

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Abstract

The aim of the study was to investigate the influence of diarrheic infections during the early postnatal phase of calves on the concentrations of hormones controlling reproduction and metabolism. Blood samples were collected from 20 male and female calves via jugular vein catheters every 15 min for 6 hr at Days 3, 9, and 21 of life. The animals were classified into three groups. Group 1 (controls): healthy calves ($n = 9$). Group 2: calves affected with diarrhea at Day 9 ($n = 7$). Group 3: calves with diarrhea at Days 3 and 9 ($n = 4$). Infections occurred spontaneously and were mainly due to *E. coli* infections. All affected calves had recovered at Day 21. Mean GH concentrations in the calves in Groups 2 and 3 compared to control calves had increased by Day 3 ($P < 0.01$; $P < 0.001$). Cortisol levels of calves in all groups were highest at Day 3 and decreased thereafter ($P < 0.001$). Cortisol concentrations were lower at Day 3 in animals in Groups 2 ($P < 0.001$) and 3 ($P < 0.05$) than in controls. Pulsatile LH release was detectable at Days 9 and 21 only in healthy calves. Insulin increased at Day 9 during diarrhea. The results indicate that cortisol concentrations decreased whereas GH concentrations were increased before diarrhea was observed. The onset of pulsatile LH release was delayed in diarrheic calves. It is concluded that diarrhea exerts effects upon the release of reproductive and metabolic hormones in early postnatal calves.

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1. Introduction

There is a lack of information on the interactions between hormone concentrations and diseases of the gastrointestinal tract in early postnatal calves. The early postnatal time is a period of dramatic changes. In particular, the gastrointestinal tract is suddenly being responsible for nutrient absorption. The intestine is the most rapidly developing tissue during this time [1]. In the initial first few days, transport from lumen of the gut to the animal's blood stream is facilitated by a high permeability of the gut. The permeability is later decreased, which may protect from bacterial infection.

Studies of cortisol and growth hormone have shown that these hormones may play a role in regulation of gut permeability and growth. The perinatal cortisol concentrations represent the maximum levels observed throughout the entire life span. Glucocorticoids involved in metabolic control play an important role in the development of tissues and organs in the neonatal calf [2]. The very high cortisol levels observed up to 1 wk after birth may facilitate absorption of IgG [3]. GH receptors have been demonstrated in fetal human gut tissue from Week 20 of pregnancy [4]. The high level of growth hormone is thought to mediate its biologic effect via the specific GH receptors that stimulate the proliferation of crypt cells [5]. Thus, particularly the high concentration of GH may be important for the development and the function of the digestive tract.

Diarrhea is a major problem in the adjustment phase of the gut. In calves, mortality related to diarrhea constituted 52% of total deaths [6]. Because retrospective surveys attempting to determine the relative importance of various stressors have characteristically identified aberrations of intestinal function as a major causative factor, an understanding of factors mediating postnatal growth and development of the gastrointestinal tract is essential [1]. Further, the economic loss due to diarrhea that are cured are substantial because the disease interferes with growth. However, the effect of diarrhea on hormonal regulation of gut development and gonadal maturation has so far only received limited attention. We therefore aimed to characterize the alterations of GH, cortisol, and LH concentrations in young calves affected with clinical diarrhea. We further included insulin levels in the study as a measure of the metabolic status of the calves.

2. Materials and methods

2.1. Animals, health status, blood collection, and nutrition

Holstein Friesian ($n = 11$) and Red Holstein ($n = 9$) calves, 10 males and 10 females, were clinically observed during the first three weeks of life. Diarrhea occurred spontaneously, i.e., was not artificially inoculated and was attributable to hemolytic and non-hemolytic *E. coli* infections and one rotavirus and coronavirus infection, as determined in feces sampled at Days 3, 9, and 21 of life. Respiratory rate, heart rate, rectal temperature, and hematocrit values were always in the normal range in the affected calves and further calves were free of any sign of dehydration. Based on the clinical investigations diarrheas were judged as mild forms of diarrhea. The calves were classified in three groups according to

health status. Group 1 (controls): healthy calves ($n = 9$). Group 2: calves with diarrhea at Day 9 ($n = 7$). Group 3: calves with diarrhea at Days 3 and 9 ($n = 4$). All animals in all groups were free of illness at Day 21.

Blood samples (10 ml) were collected using indwelling catheters implanted in the jugular vein one day before sampling every 15 min for 6 hr from 9:00 a.m. to 3:00 p.m. at Days 3, 9, and 21. Plasma was stored at −20°C until assayed.

Calves stayed the first two days of life with their mothers, then they were fed 1.5 l mature cow milk at 8:00 a.m. and 6:00 p.m. From the 10th day of life calves were given 3 l standard milk replacer twice a day. During diarrhea, calves received only an electrolyte substitution therapy consisting of 100 g Floracid Comp./1.5 l water (containing 35.9 mg glucose monohydrate; A. Albrecht, Aulendorf/Germany). Calves consumed always all of the offered food. Starting at the first week of age, calves were offered hay and maize silage and some concentrates; water was always freely accessible.

2.2. Radio- and enzyme-immunoassays

Blood plasma concentrations of GH were analyzed by RIA using bGH antiserum (monkey; AFPB55), highly purified bGH (AFP-11182B) for iodination and USDA-bGH-B-1 as standard. Cortisol was quantified after sample extraction with tertiary-butylmethyl ether [7]. The cortisol antibody used (code "HCo-21-HS; S16; 9.1.84") was raised in rabbits against cortisol-21-hemisuccinat-BSA. Cross-reactivities are as follows: cortisol: 100%, pregnenolone < 1%, corticosterone 15%, DOC 19%, progesterone 7.5%. The cortisol antibody dilution was 1 : 165,000. LH was measured by an EIA according to Mutayoba et al. [8] with some modifications. The monoclonal antibody to LH generated against bovine LH (bLH, Lot $10/518b_7$) was a characterized by Matteri et al. [9]. As a tracer, 40 μg USDA-bLH-B5 (AFP5500) was biotinylated as previously described [8]. Microtiter plates (96 well Easy Wash, Corning, NY, USA) were coated with antibodies raised against mouse IgG in sheep. Antibodies were precipitated [10] and used in a concentration of 1 μg/100 μl carbonate buffer (0.05 M $NaHCO_3$, pH 9.6) per well. After 24 hr of incubation at 4°C the plates were blocked with 300 μl 2.5% casein in 0.05 M NaOH (adjusted to pH 7.2 with HCl) at room temperature for 2 hr. The plates were washed four times with 300 μl 0.05% Tween 20 in 10% PBS. The air-dried plates were stored at −20°C for up to 12 wk without appreciable loss of binding capacity. Standard (USDA-bLH-B-5) or plasma samples (50 μl) were pipetted into the wells and 100 μl monoclonal LH antibody (1 ng/ml) in assay buffer (0.1% hydrolyzed gelatin, 0.12 M NaCl, 0.02 M Na_2HPO_4, 0.01 M EDTA, 0.05% Tween 20, 0.005% chlorhexidine digluconate (20%), 0.002% phenol red and 5% bull plasma containing less than 0.1 ng LH/ml). After incubation at room temperature for 20 h plates were washed twice and 100 μl of tracer (5 ng/ml assay buffer) was added. The mixture was incubated at room temperature for 30 min. After two washing steps, 100 μl of a streptavidin-peroxidase conjugate (Sigma, Deisenhofen, Germany) solution (200 ng/ml assay buffer) were added per well. The further assay steps are comparable to Mutayoba et al. [8]. The minimum detectable level was 0.1 ng bLH/ml plasma. Cross-reactions with ovine FSH, bovine prolactin, bovine growth hormone were less than 0.1% and 0.5% with bovine TSH. Serial dilutions of bull plasma were parallel to the standard curve and the recovery of added hormone to plasma

Table 1
Basal GH concentrations, frequency, and amplitudes of GH pulses (LSM ± SE) in healthy calves (Group 1; $n = 9$) and in calves with diarrhea at Day 9 (Group 2; $n = 7$) or Days 3 and 9 (Group 3; $n = 4$)[a]

Day of life	Basal (ng GH/ml)			Frequency (episodes/6 hr)			Amplitudes (ng GH/ml)		
	Calves of group								
	1	2	3	1	2	3	1	2	3
3	44.8 ± 4.3	49.4 ± 4.9	81.2 ± 6.6***	4.6 ± 0.4	3.4 ± 0.5	3.3 ± 0.6	38.8 ± 4.8	40.5 ± 5.4	34.0 ± 7.2
9	41.1 ± 4.3	35.5 ± 4.9	30.7 ± 6.6	3.1 ± 0.4	3.8 ± 0.5	3.0 ± 0.6	34.9 ± 4.8	27.4 ± 5.4	29.8 ± 7.2
21	37.6 ± 4.3	46.6 ± 4.9	39.0 ± 6.6	4.2 ± 0.4	3.6 ± 0.5	4.3 ± 0.6	31.8 ± 4.8	30.3 ± 5.4	29.3 ± 7.2

[a] Significant differences between the values of the ill calves in Group 2 or 3 compared to those in the control Group 1 are shown by *** $P < 0.001$.

samples in the EIA was 102 ± 7%. Concentrations of insulin were determined by a commercially available RIA-kit (CIS Diagnostik GmbH, Dreieich, Germany). Coefficients of variation within and between assays were as follows: GH 10% and 12%, cortisol 6% and 10%, LH 9% and 11%, and insulin 10% and 13%.

2.3. Statistics

Plasma GH, cortisol, LH, and insulin concentrations were analyzed using procedure mixed of SAS [11]. Differences in mean hormone concentrations were analyzed by SAS using a mixed model. The statistical model included fixed effects of age, group of health status, time, sex, breed, and as a random effect the animal. Age was nested within the group of the health status. All factors were tested by *F*-test as implemented in the SAS procedure. When the *F*-test indicated significance, linear contrasts of least square means were performed. GH and LH pulses were determined by the PULSAR program by using standard deviation criteria [12]. Basal concentrations, pulse amplitude and pulse frequency were thereby determined and the GH secretion parameters were subsequently analyzed by procedure mixed of SAS. Significance of differences in calves from Group 1 versus 2 or 3 were tested by linear contrasts. Data are presented as least square means and their standard errors (LSM ± SE).

3. Results

3.1. GH—effects of age, sex, or breed

Basal GH concentrations were highest at Day 3 and decreased thereafter ($P < 0.001$; Table 1). There was no effect due to sex or breed on mean plasma concentrations of GH ($P > 0.7$).

3.2. GH—effects of diarrhea

Mean concentrations of GH in ill calves in Groups 2 and 3 were higher than in control calves at Day 3 (Fig. 1; $P < 0.01$; $P < 0.001$). By Day 9 calves in Groups 2 and 3 had lower

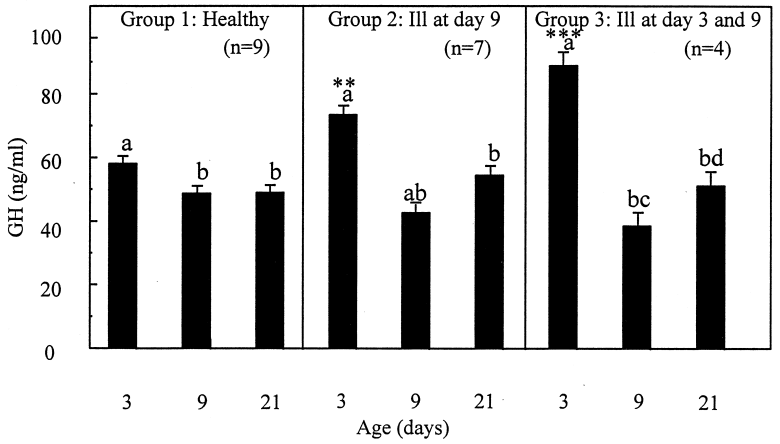

Fig. 1. GH blood plasma concentrations (LSM + SE) in healthy control calves (Group 1), calves with diarrhea (mainly hemolytic and non-hemolytic *E. coli* infections) at Day 9 (Group 2) and calves with diarrhea at Days 3 and 9 (Group 3). Values represent 25 samples per calf per day. Significant differences between calves in Groups 2 or 3 versus control Group 1 within an age group are shown by $^{**}P < 0.01$; $^{***}P < 0.001$. Means with different superscripts are significantly different within a group ($^{ab}P < 0.001$; $^{cd}P < 0.01$).

but not significantly different mean GH concentrations than control calves (P = 0.3; P = 0.14). At Day 21, these calves had similar mean concentrations of GH compared to calves in the control group (P > 0.4; P > 0.8). Basal GH concentrations were higher (P < 0.001) in calves in Group 3 than in controls at Day 3 (Table 1). At Day 9 basal GH concentrations in calves that had been ill until Day 9 had decreased slightly more compared to the control group (P = 0.2) than in those that had diarrhea at Day 3 only (Group 2; P = 0.4). No effects on the frequency and the amplitude of GH release were observed (Table 1).

3.3. Cortisol—effects of age, sex, or breed

Mean plasma concentrations of cortisol were highest at Day 3 and decreased (P < 0.001) until Day 21 (Fig. 2). When cortisol concentrations were averaged over the entire experiment, female calves tended to have higher concentrations than males (8.0 ± 0.7 versus 6.2 ± 0.6 ng/ml; P = 0.07) and Red Holstein calves had higher plasma concentrations than Holstein Friesian calves (8.6 ± 0.7 versus 5.7 ± 0.6 ng/ml; P < 0.01).

3.4. Cortisol—effects of diarrhea

At Day 3, calves in Groups 2 and 3 had lower (P < 0.001 and P < 0.05) concentrations than the healthy control calves. At Days 9 and 21, concentrations of cortisol in calves in Groups 2 and 3 were similar to controls (P > 0.1).

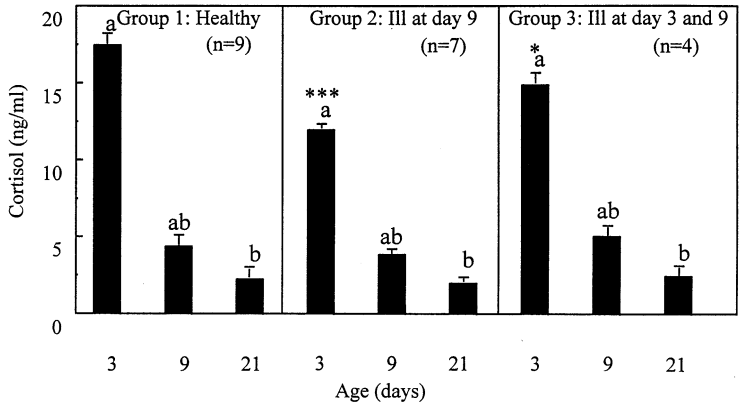

Fig. 2. Cortisol blood plasma concentrations (LSM + SE) in healthy control calves (Group 1), calves with diarrhea (mainly hemolytic and non-hemolytic *E. coli* infections) at Day 9 (Group 2) and calves with diarrhea at Days 3 and 9 (Group 3). Values represent 25 samples per calf per day. Significant differences between calves in Groups 2 or 3 versus control Group 1 within an age group are shown by $*P < 0.05$; $***P < 0.001$. Means with different superscripts are significantly different within a group ($^{ab}P < 0.001$).

3.5. LH—effects of age, sex, or breed

No secretory pulses were observed in healthy calves at Day 3. At Days 9 and 21, three and 10 pulses (0.1–0.5 ng LH/ml basal, 1.5–4.0 ng LH during pulses) were observed in healthy calves. There was no effect of sex or breed on LH concentrations ($P > 0.8$; $P > 0.2$).

3.6. LH—effects of diarrhea

Mean LH concentrations in the calves in all three groups were similar ($P > 0.4$; Fig. 3). Differences between the groups were about 0.2 ng/ml. No secretory pulses were observed in ill calves at Day 3. Only one LH pulse was observed in one ill male calf at Day 9.

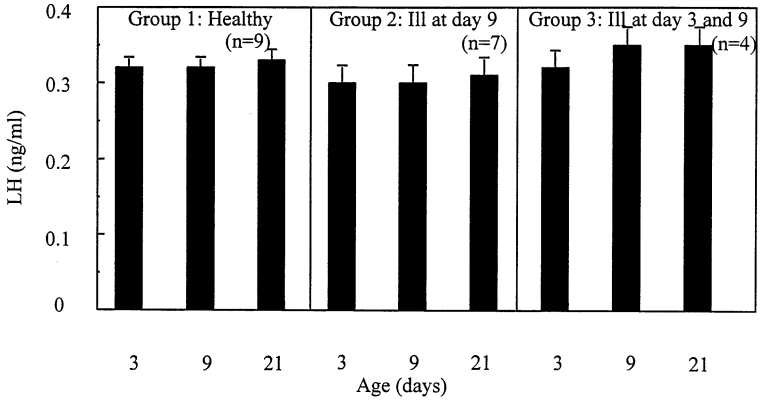

Fig. 3. LH blood plasma concentrations (LSM + SE) in healthy control calves (Group 1), calves with diarrhea (mainly hemolytic and non-hemolytic *E. coli* infections) at Day 9 (Group 2) and calves with diarrhea at Days 3 and 9 (Group 3). Values represent 25 samples per calf per day.

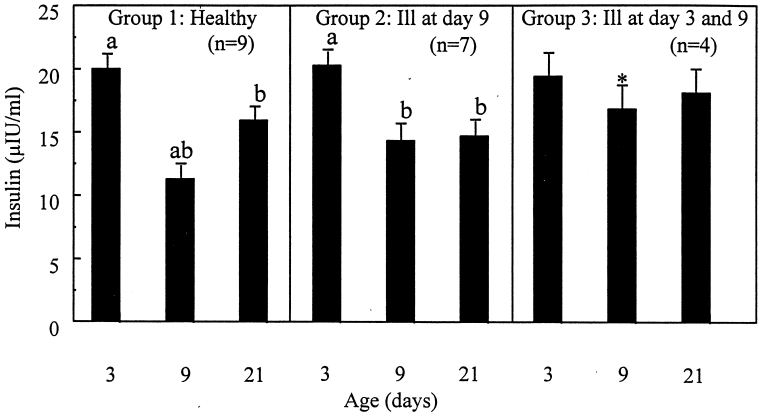

Fig. 4. Insulin blood plasma concentrations (LSM + SE) in healthy control calves (Group 1), calves with diarrhea (mainly hemolytic and non-hemolytic *E. coli* infections) at Day 9 (Group 2) and calves with diarrhea at Days 3 and 9 (Group 3). Values represent 25 samples per calf per day. Significant differences between calves in Groups 2 or 3 versus control Group 1 within an age group are shown by $^{*}P < 0.01$. Means with different superscripts are significantly different within a group ($^{ab}P < 0.001$).

3.7. Insulin—effects of age, sex, breed, or feeding

Insulin concentrations decreased after Day 3 until Day 9 ($P < 0.01$; Fig. 4) and had increased again by Day 21. There was no effect of sex or breed on insulin concentrations ($P > 0.7$). Insulin concentrations increased after feeding in the morning and decreased thereafter ($P < 0.001$).

3.8. Insulin—effects of diarrhea

At Day 9, insulin concentrations in calves that were ill during the experiment tended to be higher (Group 3: $P = 0.04$; Group 2: $P = 0.18$) than those in the healthy ones in Group 1. At Day 21 insulin concentrations in the calves in Groups 1, 2, and 3 were similar ($P > 0.4$).

4. Discussion

Within the first three weeks of life, blood plasma concentrations of GH were initially high and then decreased in healthy calves. This is comparable to the findings of Ronge and Blum [13] who described that in new-born calves GH concentrations tended to decrease during the first five weeks of life. The high concentrations of GH may have a comparable action a) on the increase in the depth of crypts and b) on the rate of proliferation of the villi of the gut [14]. In this study GH concentrations were elevated before symptoms of diarrhea could be observed. We consider it most likely that subclinical stages of infection have started to affect GH concentrations before clinical symptoms become apparent. Diarrhea was the clinical sign or symptom of the presence of the underlying infection. The host response to the infection

organism establishes the water balance perturbations that result in diarrhea. It has to be taken into account that some aspects of dehydration associated with the loss of water can compromise the interpretation of how plasma concentrations of hormones change during this process, largely because the majority of the water entering the bowl comes from the plasma compartment and the concentrations can therefore be different. Calves in Group 2 had markedly higher plasma concentrations of GH at Day 3. These calves had diarrhea six days later. Calves in Group 3 that already had diarrhea showed an even more pronounced increase in plasma concentrations of GH at Day 3. Comparable to the results of the present study is the finding that endotoxin administration increased GH concentrations in sheep [15], in finishing gilts [16], and in humans [17]. Short-term GH treatments can ameliorate some of the symptoms of the acute phase response to endotoxemia [18]. The increase in GH concentrations before any clinical signs of diarrhea were observed may be interpreted as an induction of the body's defense system. Higher concentrations of GH result in a lower permeability of the gut [19]; consequently the transportation of endotoxin from the lumen of the gut into the blood circulation may be reduced and inflammation reactions may be decreased [19]. In contrast to the typical actions of GH that are mediated by IGF, GH can also act directly on gut tissue via specific receptors even when serum IGF-1 is suppressed, as reported for infected animals [20]. The increase in GH concentration suggests that GH may play—in addition to its well-known role as a regulator of growth and immune function—an important role in intestinal healing in early postnatal calves.

During acute diarrhea the plasma concentrations of GH tended to be lower in calves that had been ill for a longer time. One major consequence of disease conditions is a reduced growth rate that may range from temporary growth restraint to permanent growth inhibition [21]. The mechanism for this reduced growth rate in cattle is thought to be due to reduced plasma concentrations of GH and IGF-1, and hence reduced anabolic stimuli [22,23]. At Day 21 of age GH concentrations were similar in the reconvalescent and the healthy calves thus, indicating that GH concentration is not stimulated beyond this time.

Plasma concentrations of cortisol in early postnatal calves seem to be of particular importance for the susceptibility to diarrhea. Cortisol may vary extensively [24,25], but there is indication that concentrations are high after birth and decrease during the first week of life in ruminants. The number of leukocytes, glucose concentrations and the weight gains increase in accordance with the concentrations of cortisol [26]. The results of the current work indicate that even before calves suffered from clinically apparent diarrhea, cortisol concentrations were decreased. Although the possibility exists that the calves of Group 2 and 3 had a low cortisol level at birth, which in turn may have induced a lower absorption of IgG [27] and a lower resistance to diarrhea, we have no data to support this. However, our results, taken together with those of other research workers [3], lead to the speculation that glucocorticoid concentrations increase to a peak around the time of onset of labor and then decline [28]. Thus, any delay in the birth process could result in lower glucocorticoid concentrations at the time of delivery, and perhaps a subsequent decrease in the ability of the calf to absorb colostral immunoglobulins. Therefore, it may be speculated that those calves that had low concentrations of cortisol had a higher susceptibility to diarrhea because their passage rate of IgG out of the colostrum into the blood circulation was diminished. However,

it is well known that cortisol may modulate susceptibility by mechanisms involving systems other than just IgG passage rate.

Increased cortisol concentrations were reported for calves during *Escherichia coli* infections [29] and endotoxin treatment [18], whereas cortisol concentrations were not changed in diarrheic calves during *Ostertagia ostertagi* infections [30]. Also, in the present study no significant increases in cortisol concentrations were observed during diarrhea. These results may be typical for mild forms of diarrhea.

The low basal concentrations of LH were unchanged by diarrhea, but the onset of pulsatile LH release was delayed. The concentrations of LH in calves of the same age or slightly older ones correspond with results of previous reports [31,32]. The pituitary is sensitive to exogenous GnRH stimulation in the infantile bull calf at 4 to 6 wk of life [33]. Stressors inducing a rise of cortisol concentration may cause a decrease in LH concentration via down-regulating the sensitivity of the adenopituitary toward GnRH in adult cattle, but in new-born cattle the pituitary is more likely to be influenced by opioids [34]. Therefore, it may be concluded that the low basal concentration of LH is not modified by early postnatal diarrhea. However, the delayed onset in LH pulses may result in delayed gonadal development, because in bull calves the early increase in pulsatile LH release is critical for initiation of reproductive performance [35].

Insulin concentrations have recently been shown to be reduced in milk-fed calves with acute, but mild diarrhea due to cryptosporidia or coronavirus infections [36]. However, after *E. coli* endotoxin treatment increased insulin concentrations were reported [29] that correspond with the results of the present study in which mainly *E. coli* and only one case with corona- and rotavirus infection was observed. This might be the reason that only slight effects of diarrhea on the concentration of insulin were observed in the present study. However, there was always a distinct stimulating effect on blood plasma concentrations of insulin after feeding the calves in the morning one hour before the start of the blood sampling intervals.

In summary, low cortisol concentrations were related to a higher susceptibility toward diarrhea in neonatal calves. Increased GH concentrations that are evident even before the clinical appearance of infections may be contribute to lower permeability of the gut for infectious agents. GH may therefore play an important role in activating the body's defenses against diarrheic infections and, additionally in intestinal healing. In diarrheic calves the onset of pulsatile LH release was delayed. In conclusion, even mild diarrhea alters the concentration of reproductive and metabolic hormones in early postnatal calves. Whether the observed disturbances might have consequences for reproductive performance and growth remains to be investigated.

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