Effects of niacin supplementation and different concentrate proportions on ruminal lipopolysaccharide concentration, immunological response and health of dairy cows

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Summary

High concentrate proportions used in diets of high-yielding cows may lead to subacute ruminal acidosis, compromise the ruminal mucosal barrier and force transfer of lipopolysaccharides (LPS) with an eventual systemic inflammatory response. Because niacin (NA) increases performance and might exert anti-inflammatory effects, the present study investigates the effects of 60 % vs. 30 % concentrate proportions with or without 24 g niacin/cow/day on ruminal LPS content and indicators of inflammatory response. The experiment was carried out with four experimental groups, 60+NA, 60-, 30+NA, 30- and lasted from calving to week of lactation (WoL) 36. Ruminal LPS concentration was generally increased after feeding 60 % concentrate diets and also modified by niacin, parity and WoL. Also total leukocytes, hematocrit, fibrinogen and aspartat-aminotransferase were influenced in an interactive manner, while glutamate dehydrogenase and gamma-glutamyl transferase activities were elevated due to the 60 % concentrate proportion. The stimulation index of peripheral blood mononuclear cells was subjected to a 2-way interactive effect of concentrate and niacin feeding, being highest in 30- groups (cows and heifers). In conclusion, long-term feeding of high-energy diets increases the LPS load of the rumen and compromises the liver.

Keywords: *concentrate-to-roughage ratio, cow, PBMC, blood count, liver enzymes*

Zusammenfassung

Effekte einer Niacinsupplementation bei verschiedenen Konzentratanteilen auf den ruminalen Lipopolysaccharidgehalt, die Immunantwort und die Gesundheit von Milchkühen

Die bei hochleistenden Milchkühen eingesetzten hohen Konzentratanteile können zur subakuten Pansenazidose und zur Pansenmukosaschädigung verbunden mit erhöhtem Lipopolysaccharid (LPS)-Transfer und einer Immunantwort führen. Da Niacin (NA) leistungssteigernde und anti-inflammatorische Effekte haben kann, wurde der Effekt von 60 % vs. 30 % Konzentratanteil mit oder ohne 24 g Niacin/Kuh/Tag auf den ruminalen LPS-Gehalt und Indikatoren einer Immunantwort untersucht. Für vier Versuchsgruppen, 60+NA, 60-, 30+NA, 30- dauerte der Versuch von der Kalbung bis zur 36. Laktationswoche (WoL). 60 % Konzentrat führte zu erhöhtem ruminalem LPS-Gehalt, der auch durch Niacin, Parität (primi-/pluripare Kuh) und WoL beeinflusst war. Gesamtleukozytenzahl, Hämatokrit, Fibrinogen und Aspartat–Aminotransferase unterlagen einem interaktiven Effekt, während Enzymaktivität von Glutamatdehydrogenase und Gamma-Glutamyltransferase auch durch den 60 % Konzentratanteil erhöht waren. Der Stimulationsindex der mononukleären Zellen des peripheren Blutes wurde durch eine 2-fach-Interaktion von Konzentrat*Niacin beeinflusst und war am höchsten in den 30-Gruppen (Kuh/Färse). Insgesamt führt die Langzeitfütterung eines hohen Konzentratanteils zu erhöhten LPS-Gehalten im Pansen, sowie zur Beeinträchtigung der Leber.

Schlüsselworte: *Konzentrat-Grundfutterverhältnis, Kuh, PBMC, Blutbild, Leberenzyme*

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1 Introduction

To meet the energy demand of high yielding cows feeding of energy-dense diets is required. Consequently, rapid fermentable high concentrate proportions are used in such rations. This strategy predisposes cows for diverse nutritional disorders such as subacute ruminal acidosis (SARA) associated with an altered rumen microbial population (Andersen et al. 1994a; Nocek, 1997; Ametaj et al., 2005; Emmanuel et al., 2008; Zebeli and Ametaj, 2009) and increases concentration of lipopolysaccharides (LPS) in the rumen fluid (Nocek, 1997; Andersen, 2003).

Under SARA conditions, Kleen et al. (2003) suggested a translocation of rumen endotoxins into the bloodstream as the rapidly fermentable carbohydrates induce hyperacidity and consecutive parakeratosis with the possible consequence of provoking a systemic inflammation. Feeding of diets with a high concentrate proportion was frequently followed by increases in the ruminal LPS concentration. Gozho et al. (2005) showed that a diet with 60 % concentrates comprising mainly of cereal grains resulted in a grain-induced SARA and increased free LPS concentration in the rumen. LPS is released by gram-negative bacteria in rumen fluid (Nagaraja et al., 1978a; Andersen et al., 1994a; Khafipour et al., 2009; Zebeli and Ametaj, 2009) or from bacterial cell lyses due to excessive activity of autolytic enzymes that facilitates growth during the rapid bacterial growth phase (Wells and Russell, 1996). Plaizier et al. (2012) reviewed several studies which showed that inducing SARA by a nutritional challenge based on feeding excessively high grain diets increases LPS in the rumen of cattle (Nagaraja et al., 1978b; Emmanuel et al., 2008; Khafipour et al., 2009).

Only few studies have detected LPS in peripheral blood during grain induced SARA (Gozho et al., 2007; Nagaraja and Lechtenberg, 2007; Plaizier et al., 2008), probably due to its rapid clearance. When hepatic and whole body clearance capacity is exceeded, LPS might induce an inflammatory response characterized by the release of multiple pro-inflammatory cytokines and acute phase proteins (APP) which finally results in an acute phase response.

Recently, niacin was shown to modulate the LPS-induced inflammatory response in mice (Zhou et al., 2014) as it was found to attenuate the production of pro-inflammatory cytokines and to exert anti-inflammatory effects mediated by its hydroxycarboxylic acid receptor HCA2.

Apart from major inflammation markers other indicators such as fibrinogen and white blood cells can be used as indicators of an acute phase response (Arthington et al., 1996; Horadagoda et al., 1999). Therefore, the objective of the present study was to investigate the effects of feeding diets with either high or low concentrate proportions (60 % vs. 30 %), both in the absence or presence of niacin (24 g/cow/day) on ruminal LPS content, hematological variables, plasma parameters and immunological functional parameters to characterize the impact of feeding energy-dense diets on health of dairy cows.

2 Material and methods

2.1 Animals, experimental design, feeding and sample collection

The experiment was conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institut, Braunschweig, Germany according to the German Animal Protection Act concerning the protection of experimental animals and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (33.9 42502-04/085/09). The current investigations complete the results of a comprehensive feeding experiment which was recently published (Rauls et al., 2015). A total of 64 German Holstein cows were assigned to four groups of different dietary treatments according to milk yield, number of lactation and body weight. A high concentrate level of 60 % (in total ratio on dry matter basis) and a low concentrate level of 30 % were tested with the presence or absence of 24g niacin/cow/d and resulted in four experimental groups: 60+NA, 60-, 30+NA and 30- The niacin used was powdered non rumen protected NA with a content of at least 99.5 % NA (Lonza Ltd., Basel, Switzerland) which was included in the pelletized concentrate. The rations were formulated according to the recommendation of nutrient and energy supply of the Society of Nutrition Physiology (GfE, 2001). The cows had free access to water. The study started individually for each cow with the day of calving and was terminated in WoL 36. The composition of concentrate and roughage is shown in Table 1. The cows were housed in a free stall barn equipped with slatted floors and cubicles covered with rubber mattresses. The roughage was offered in self- feeding stations (type MJ/ RIC; Insentec B.V., Marknesse, The Netherlands), which were re-filled daily. Concentrate was offered by computerized concentrate feeding stations (type MJ, Insentec, B.V., Marknesse, The Netherlands). The cows were equipped with ear transponders to monitor the daily feed intake. The available amount of concentrate was adjusted twice weekly according to the individual roughage intake. In this way, the concentrate-to-roughage ratios were implemented in the different feeding groups while the feed was offered for ad libitum intake. A subgroup of 36 cows from a total of 64 animals (four pluriparous and five primiparous in each group) were studied more intensively: in WoL 5 and 16, blood samples were taken by jugular venipuncture into sterile heparinized monovettes for preparation of peripheral blood mononuclear cells (PBMC) and processed as described by Renner et al. (2011). To perform subsequent cell proliferation assays, the samples were stored at -80 °C. In WoL -3, 1, 3, 16 and 36 before or after calving, blood samples from the jugular vein were taken into lithium heparinized tubes for determination of clinical-chemical traits and citrate blood was taken to determine fibrinogen content. After centrifugation (Heraeus Varifuge® 3.OR Heraeus, Osterode, Germany, 2300 g, 15 °C, 15 min), plasma was stored at -20 °C until analysis. Furthermore, blood samples from a jugular vein were taken in WoL 2, 3, 4, 5, 10, and 36 (EDTA tubes) for the determination of

Table 1
Composition, nutrient and energy content of the concentrates and roughage (cf. Lohölter et al., 2013)

	Concentrate [1]				Roughage [2]
	Con 60+NA	Con 60 -	Con 30+NA	Con 30 -	
Components (%)					
Soybean meal	26.8	26.8	26	26	-
Wheat grain	50	50	50	50	-
Maize grain	20.8	20.8	20	20	-
Mineral premix [3] including supplemental niacin	2.4	-	4	-	-
Mineral premix [3]	-	2.4	-	4	-
Chemical composition (g/kg DM)					
Ash	49	51	62	63	58
Crude protein	224	219	219	217	106
Ether extract	30	30	30	30	34
Crude fibre	34	34	34	33	220
NDFom	156	185	130	128	440
ADFom	47	47	47	47	241
Niacin (g/kg DM)	1.76	-	3.52	-	-
Energy [4] (MJ NEL/kg DM)	8.3	8.3	8.2	8.2	6.4

[1] Con 60+NA, Con 60 -, Con 30+NA, Con 30 – used in groups 60+NA, 60 -, 30+NA, 30 -
[2] 60 % maize silage and 40 % grass silage on DM basis
[3] Per kg mineral feed: 140 g Ca; 120 g Na; 70 g P; 40 g Mg; 6 g Zn; 5.4 g Mn; 1 g Cu; 100mg I; 40 mg Se; 5 mg Co; 1 000 000 IU vitamin A; 100 000 IU vitamin D3; 1500mg vitamin E
[4] Calculation or roughage based on nutrient digestibilities measured with wethers (GfE, 1991). For the concentrates, tabulated values were used (DLG, 1997)

hematocrit and total and differential leukocyte counts which were evaluated immediately afterwards. Samples of rumen fluid were taken with an oral rumen tube and a hand vacuum pump from the ventral sac of the rumen in Wol -3, 1, 3, 16 and 36. After centrifugation (Beckmann J2-HS, Beckman Coulter Inc., Brea, CA, USA, 2400 g, 5 min), 2 ml of the supernatant were poured into Eppendorf-tubes and again separated by centrifugation (Hettich, Tuttlingen, Germany, 10 000 g, 15 min). The supernatant was percolated through a 0.22 µmol filter into Eppendorf-tubes and after heating for 30 minutes at 100 °C stored at -20 °C for subsequent LPS measurement.

3 Analyses

3.1 Feedstuffs

The composition of the feedstuffs (ash (ash), crude protein (CP), crude fibre (CF), ether extract (EE), neutral detergent fibre (NDFom) and acid detergent fibre (ADFom)) was determined according to the methods of the Association of German Agricultural Analysis and Research Centres (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) VDLUFA (2012).

The niacin content in feedstuffs was determined microbiologically using *Lactobacillus plantarum* as an indicator strain (VDLUFA method Vol. III 13.9.1., HPLC-Method).

3.2 Ruminal lipopolysaccharides

The ruminal LPS-content was determined using the *Limulus amoebocyte* lysate assay (LAL QCL-1000, Lonza Group Ltd., Basel, Switzerland). Pretreated rumen samples were diluted using pyrogen-free water until their LPS concentrations were in a range of 0.1 to 1 endotoxin units (EU)/mL relative to the reference endotoxin (*Escherichia coli* O111:B4) and assayed as described by Gozho et al. (2005).

3.3 Hematological variables

Total leukocytes were counted using an improved Neubauer cell counting chamber and the evaluation of the differential leukocyte count after panoptic staining of the smear according to Pappenheim (Kraft and Dürr, 2005) was performed counting 200 cells. Hematocrit was determined after centrifugation with a hematocrit centrifuge. A 1 mL plasma sample of each cow was sent to the laboratory of the Clinic for Cattle of the University of Veterinary Medicine Hannover, Foundation, Hannover, Germany. It was analyzed for activity of glutamate dehydrogenase (GLDH, EC 1.4.1.3), gamma-glutamyl transferase (GGT, EC 2.3.2.2) and aspartate aminotransferase (ASAT, EC 2.6.1.1) using a fully automatic apparatus (Cobas-Mira, Hoffmann-La Roche and Co. AG Diagnostika, Basel) and employing photometric standard procedures. Plasma fibrinogen was determined with gravimetric methods (Clauss method) directly after sampling.

PBMC viability and concanavalin A (ConA, Sigma- Aldrich, Steinheim, Germany, C 5275) stimulated proliferation were analyzed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay. PBMC were isolated from heparinized blood, diluted with phosphate buffered saline (PBS) at a ratio of 1:1, by density gradient centrifugation using Biocoll separation solution (Biochrome AG, Berlin, Germany). Isolation and subsequent proliferation assay were carried out as described by Renner et al. (2011). The results of the ex vivo tests were expressed as stimulation index (SI), defined as the ratio between the density of ConA-stimulated and ConA-non-stimulated cells.

3.4 Calculations

The energy balance was calculated as follows:

- The daily feed intake and live weight were condensed to weekly means before data analysis.
- The net energy requirements for maintenance (NEM) and lactation (NEL) were based on the equations published by the Society of Nutrition Physiology (GfE, 2001):
- Net energy maintenance (NEM) (MJ NEL/d) = 0.293 × $BW^{0.75}$
- Energy concentration of milk (MJ/kg) = 0.38 × milk fat (%) + 0.21 × milk protein (%) + 0.95
- NEL (MJ/d) = [Energy concentration of milk (MJ/kg) + 0.086] × milk yield (kg/d)

The energy balance was calculated as follows:

- Net energy balance (MJ NEL/d) = energy intake (MJ NEL/d) – [NEM (MJ NEL/d) + NEL (MJ NEL/d)]

3.5 Statistical analysis

Statistical analyses were performed using the software package SAS version 9.1 (SAS Institute Inc., 2004). All parameters were analyzed as repeated measures using the MIXED procedure. Dietary concentrate proportion, niacin supplementation, parity and WoL and the interactions between these factors were considered as fixed effects. Cows were treated as random effect. All results were presented as least squares means ± standard errors (SE). For all analyses, significance was declared when p-values were ≤ 0.05 and a tendency was noted when 0.05 < P < 0.10.

4 Results

4.1 Feed intake and energy balance

The results of the aimed concentrate-to-roughage proportions and performance parameters of the different feeding groups were already published in Rauls et al. (2015). The present results refer to the more intensively investigated subgroup of 36 animals out of the total of 64 cows. The concentrate-to-roughage proportions were nearly achieved: group 60+NA: 60 % to 40 %; group 60-: 57 % to 43 %; group 30+NA: 28 % to 72 %; group 30-: 30 % to 70 %. The consumed daily amounts of NA supplementation were slightly lower than the targeted amounts: group 60+NA obtained 21.1g ± 0.1 g/cow/day, group 30+NA 18.4 g ± 0.1 g/cow/day. The native amount of niacin in the unsupplemented groups was 2.0 g ± 1.1 g/cow/day in group 60- and 0.2 g ± 0.0 g/cow/day in group 30-. A 4-way interaction (C*NA*P*WoL = <0.001) was

Table 2

Effects of different concentrate proportion of the diet (30 vs. 60 % on a dry matter basis, **C)**, niacin supplementation (24 and 0 g/cow/day, **NA)**, parity (primi- vs. pluriparous cows, **P)** and week of lactation **(WoL)** on feed and energy intake, energy balance and ruminal lipopolysaccharide concentration (LS MEANS and standard error)

	group				p-value								
	60+NA[2]	60 -[3]	30+NA[4]	30 -[5]	C	NA	C*NA	P	WoL	C+P	NA*P	C*NA*P	C*NA* P*WoL
Intake	**(n = 9)**	**(n = 9)**	**(n = 9)**	**(n = 9)**									
DMI[1] (kg/d)	20.0 ± 0.2	18.3 ± 0.3	18.3 ± 0.3	19.8 ± 0.2	0.770	0.595	<0.001	<0.001	<0.001	0.427	0.001	0.002	<0.001
Energy intake (MJ NEL/d)	150.4 ± 1.8	136.3 ± 1.8	126.3 ± 1.8	137.3 ± 1.8	<0.001	0.334	<0.001	<0.001	<0.001	0.692	0.003	0.003	<0.001
Energy balance (MJ NEL/d)	15.7 ± 2.1	11.8 ± 2.0	14.7 ± 2.2	18.9 ± 2.0	0.148	0.943	0.054	<0.001	<0.001	0.002	0.111	0.072	<0.001
Ruminal Lipopolysaccharides													
LPS (EU/ml)	41457 ±3825	35486 ±4213	14975 ±4047	16570 ±3793	<0.001	0.584	0.345	0.959	<0.001	0.658	0.173	0.550	0.004

[1] Dry matter intake
[2] 60 % concentrate with 24g niacin/cow/day
[3] 60 % concentrate without niacin
[4] 30 % concentrate with 24g niacin/cow/day
[5] 30 % concentrate without niacin

detected for DMI, energy intake and energy balance (Table 2). In the beginning, the DMI of both groups receiving 60 % concentrate increased more rapidly than in the 30 % concentrate counterparts. Group 60+NA (cows and heifers) showed over the whole experiment a higher DMI than group 60-. This does not apply to the combination of low concentrate and supplemental niacin. Here, only the heifers developed temporarily a higher DMI while the cows from group 30+NA showed a lower DMI than group 30-. As energy intake is closely related to the DMI, it developed similarly. The energy intake increased in cows from both groups receiving 60 % concentrate proportion. The cows from group 60+NA had higher energy intake than group 60- over the entire trial. The energy intake of the 30 % concentrate groups was differently affected by parity, niacin, concentrate and WoL over the course of the trial.

Different energy intake and milk performance resulted in different energy balances. While the cows from group 60+NA almost reached a positive energy balance from WoL 5 on, the cows from group 60- remained in a nearly continuously negative energy balance until WoL 27. Heifers from all groups were in a positive energy balance.

4.2 Rumen fluid analysis/LPS

Ruminal LPS concentration was differently influenced by concentrate proportion, niacin supplementation, WoL and parity as indicated by the significant 4-way interaction (C*NA*P*WoL = 0.004, Table 2). LPS concentration was mainly higher when diets containing 60 % concentrate were fed compared to groups that received 30 % concentrate, except of heifers which developed different results in the beginning (WoL1 and 3, Figure 1). Niacin supplementation rather seemed to result in increased LPS and the heifers of 60+NA group appeared to respond more sensitively in early lactation (WoL 3), while heifers that received 30% concentrate showed the opposite with consistent lower LPS.

4.3 Hematological variables

A high variation between and within groups was visible for total leukocyte count (C*NA*P*WoL = 0.001, Table 3) during frequent sampling in the first lactation weeks. Afterwards, leukocyte number was similar between WoL10 and 35 being higher in cows and heifers receiving 60 % concentrate. The percentage of lymphocytes was influenced by concentrate proportion (p = 0.001) and niacin (p = 0.006) as it was lower in the groups receiving 60 % concentrate and higher in niacin supplemented groups. The percentage of banded neutrophil granulocytes was influenced by a C*NA interaction (p = 0.043) as it was higher in 60+ than in 30+ and 30- group (both p < 0.001).

The hematocrit (C*NA*P*WoL = 0.004) was until WoL 10 differently affected in cows and heifers, while 60 %

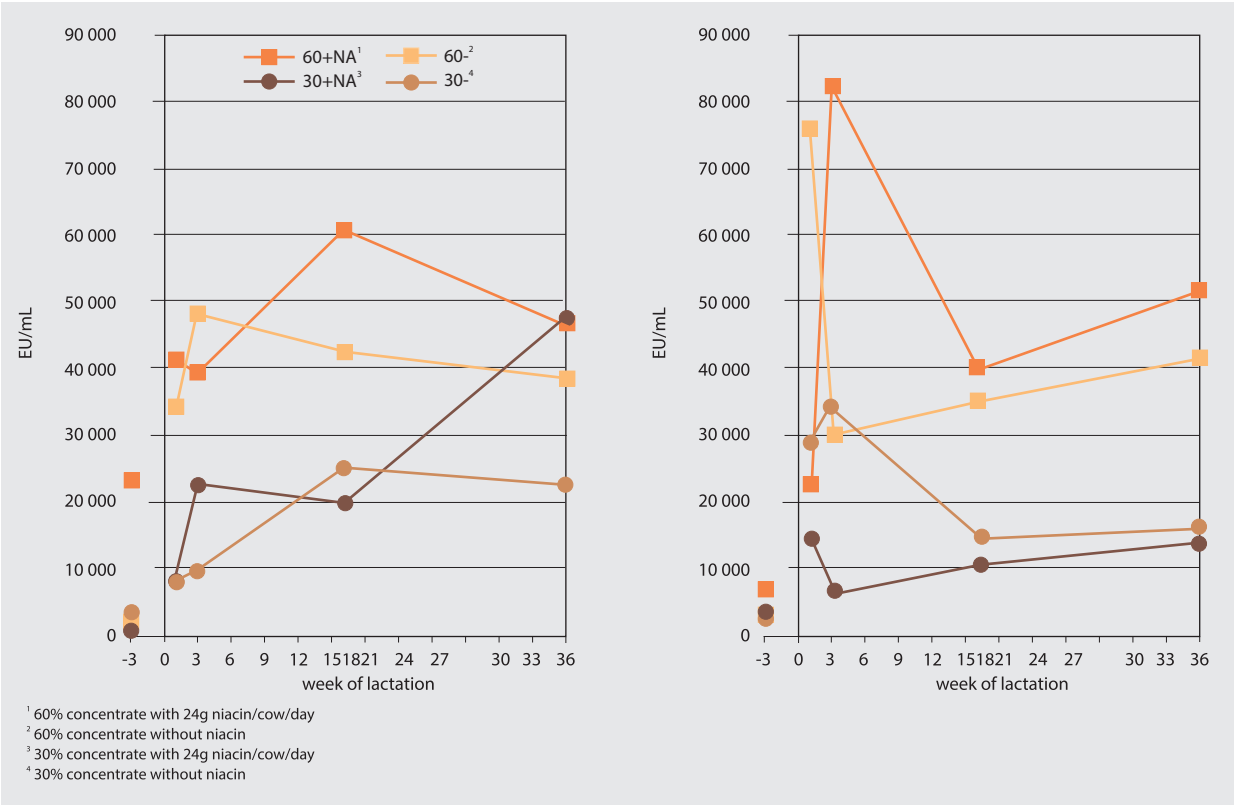

Figure 1
LPS concentration in rumen fluid of cows (left) and heifers (right) in week of lactation 1, 3, 16 and 36 in the different feeding groups.
The findings of the 3rd week a. p. were without niacin supplementation; Data points show LS means of n = 9.

Table 3

Effects of different concentrate proportion of the diet (30 vs. 60 % on a dry matter basis, **C**), niacin supplementation (24 and 0 g/cow/day, **NA**) parity (primi- vs. pluriparous cows, **P**) and week of lactation **(WoL)** on hematological variables and the SI for PBMC (LS MEANS and standard errors)

	group				p-value						
	60+NA[5]	60 -[6]	30+NA[7]	30 -[8]	C	NA	C*NA	P	WoL	C*P	NA*P	C*NA *P	C*NA* P*WoL
	(n = 9)	(n = 9)	(n = 9)	(n = 9)									
Hematolgical variables													
Total leukocytes (G/l)	7.14 ± 0.27	7.63 ± 0.24	6.78 ± 0.24	7.19 ± 0.22	0.104	0.067	0.868	0.079	0.018	0.001	0.077	0.235	0.001
Lymphocytes (%)	61.7 ± 2.1	53.8 ± 1.9	65.5 ± 1.8	62.7 ± 1.6	0.001	0.006	0.185	0.938	0.176	0.185	0.170	0.161	0.055
Monocytes (%)	0.7 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.136	0.054	0.226	0.968	<0.001	0.496	0.499	0.973	0.338
Segmented neutrophil granulocytes(%)	27.3 ± 1.8	31.8 ± 1.6	25.9 ± 1.5	28.0 ± 1.4	0.099	0.038	0.453	0.235	0.192	0.581	0.700	0.712	0.547
Banded neutrophil granulocytes (%)	9.4 ± 0.7	7.2 ± 0.6	5.5 ± 0.6	5.8 ± 0.5	<0.001	0.122	0.043	0.299	<0.001	0.274	0.691	0.623	0.240
Hematocrit (%)	30	31	28	29	0.001	0.151	0.690	0.050	0.134	0.676	0.091	0.282	0.004
Plasma Parameters													
GLDH[1] (U/l)	30.7 ± 4.6	21.4 ± 4.7	18.3 ± 4.8	14.5 ± 4.6	0.046	0.169	0.557	0.442	0.003	0.550	0.347	0.318	0.313
ASAT[2] (U/l)	92.1 ± 4.9	71.9 ± 4.9	71.7 ± 4.9	80.6 ± 5.0	0.239	0.260	0.005	0.473	0.004	0.669	0.581	0.668	0.022
GGT[3] (U/l)	31.4 ± 2.3	34.4 ± 2.3	27.0 ± 2.3	28.4 ± 2.3	0.027	0.347	0.728	0.228	<0.001	0.843	0.215	0.816	0.339
Fibrinogen (g/l)	5.7 ± 0.3	5.6 ± 0.3	5.2 ± 0.3	4.8 ± 0.3	0.031	0.381	0.668	0.188	0.001	0.482	0.417	0.563	0.037
Lymphocyte proliferation													
SI[4] for PBMC MTT assay	11.1 ± 1.5	7.8 ± 1.4	6.8 ± 1.2	12.8 ± 1.2	0.786	0.310	0.002	0.955	<0.001	0.224	0.237	0.402	0.265

[1] Glutamatdehydrogenase
[2] Aspartat-aminotransferase
[3] Gamma-glutamyltransferase
[4] Stimulation index
[5] 60 % concentrate with 24g niacin/cow/day
[6] 60 % concentrate without niacin
[7] 30 % concentrate with 24g niacin/cow/day
[8] 30 % concentrate without niacin

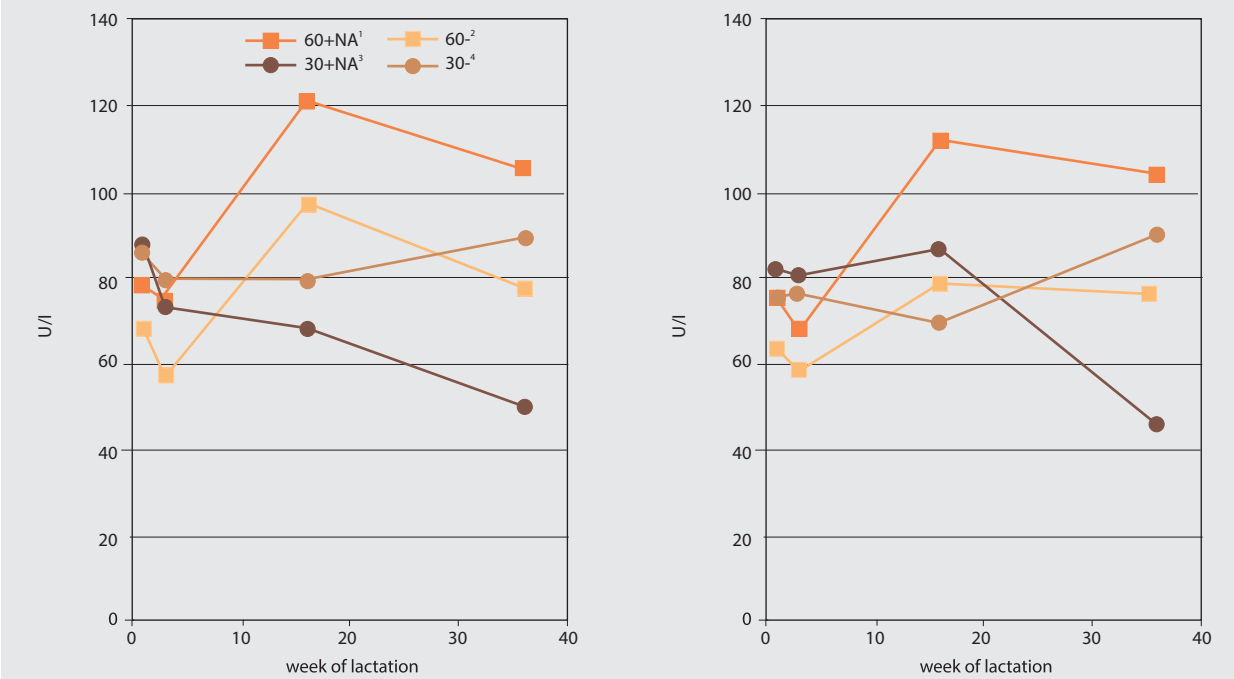

Figure 2
Plasma ASAT concentration of cows (left) and heifers (right) in WoL 1, 3, 16 and 36 in the different feeding groups.
Data points show LS means of n = 9.

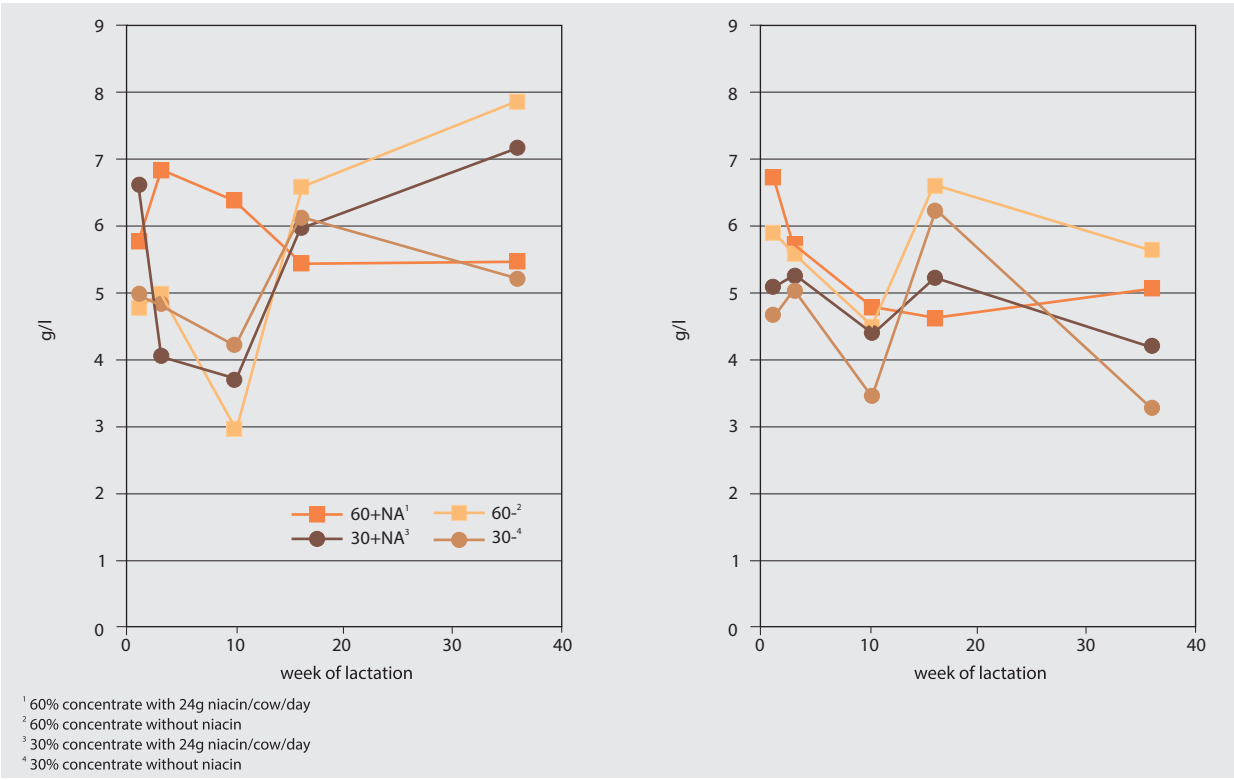

Figure 3
Plasma fibrinogen concentration of cows (left) and heifers (right) in week of lactation 1, 3, 10, 16 and 36 in the different feeding groups.
Data points show LS means of n = 9.

concentrate groups showed higher values over the course of the trial.

GLDH and GGT activity were higher due to high concentrate proportion, whereby serum ASAT activity (C*NA*P*WoL = 0.022, Table 3, Figure 2) was highest in group 60+NA (cows and heifers reacted similarly) while all other groups were over the course of time differently influenced by concentrate, niacin and parity.

Fibrinogen reacted similarly in all groups developing rather lower values in the first weeks with exception of cows from group 60+NA, which showed higher values. Between WoL 10 and 16, fibrinogen increased in all groups again with exception of group 60+NA (cows and heifers). To the end of the trial cows had rather increased, while heifers decreased fibrinogen (Figure 3).

A C*NA interaction influenced the SI ($p = 0.002$, Table 3) for the PBMC as stimulation ability was higher in 30- than in 30+ and 60- group ($p = 0.008$ and 0.048, respectively).

5 Discussion

In this study, the effects of different concentrate proportions on ruminal LPS concentrations were investigated. Zebeli et al. (2015) showed that rumen LPS mediates inflammatory responses while Zhou et al. (2014) found that niacin attenuates LPS-induced inflammatory responses in mouse alveolar macrophages in vitro. Therefore, the present study additionally investigated the impact of high dose niacin supplementation.

Feeding of the high concentrate diets (60 % of the total DMI) increased ruminal LPS concentrations which agrees with results reported by Gozho et al. (2006) who showed that a 60 % concentrate diet with gradual adaption was followed by a grain-induced SARA and an increase in free ruminal LPS. Also, Motoi et al. (1993); Andersen et al. (1994a); Gozho et al. (2007); Emmanuel et al. (2008); Khafipour et al. (2009) and Li et al. (2012) found an increased ruminal LPS concentration due to experimentally induced SARA. Additionally, Plaizier et al. (2012) found even greater increases in rumen LPS due to SARA challenges in studies that used diets consisting of a mixture of forages and grain just like the present study did. The authors explained these findings by the effects of concentrate feeding on microbial population in the rumen as it shifts towards gram-negative bacteria. As LPS is part of the outer membrane, it is released at bacterial cell death and lysis which might be increased in times of low ruminal pH and also during rapid growth phase of bacteria (Hurley, 1995; Wells and Russell, 1996). Also several other investigators showed that feeding cattle with high concentrate/low roughage diets is associated with major changes in the gastrointestinal microbiota in favor of gram-negative bacteria (Krause et al., 2003) resulting in a notably higher concentration of LPS in the rumen fluid (Gozho et al. 2007; Emmanuel et al., 2008; Zebeli and Ametaj, 2009). However, the mechanisms of release and removal or neutralization of endotoxins in the rumen fluid of dairy cows fed high concentrate proportion is still unclear. In the present study high concentrate/low roughage proportion in the diet might have modified rumen microflora also towards a shift to gram-negative bacteria and to an accumulation of short chain fatty acids (SCFA). The lack of fibre in the diet reduced time of chewing which is important for neutralizing the short chain fatty acids. Therefore, the rumen pH was probably lowered promoting bacterial cell death and lysis and therefore the release of LPS. Additionally, the rumen epithelium might have been damaged due to the depressed pH and therefore more LPS could have reached the blood circulation. In the present investigation the pH was only determined once (WoL -3, 1, 3, 16 and 36) via oral rumen tube as published by Rauls et al. (2015). Due to the way of sampling, the enhanced saliva production might have influenced the measured rumen pH. Therefore, the rumen pH values measured in the present trial may not be suitable to make a statement about the potential occurrence of SARA. Nevertheless, a SARA was reported to be induced by feeding 60 % concentrate in the diet (Gozho et al. 2005) as previously described. However, in the present study a negative correlation between pH and SCFA concentration (Rauls et al., 2015) was found ($r = -0.41$, $p = <0.0001$), caused by a negative correlation between pH and valeric acid ($r = -0.27$, $p = 0.02$). Nevertheless, since valeric acid is mostly in a very low concentration, the predictive value is rather low. Similarly, Allen (1997) postulated that rumen pH is mainly dependent on the concentration of total volatile fatty acids (VFA = SCFA) and on rumen buffering. A depressed rumen pH can essentially influence the release of LPS as mentioned above. But in contrast to that, Haubro and Jarlov (1990) and Andersen et al. (1994b) did not find increases in ruminal LPS concentrations due to induced SARA. Gozho et al. (2007) showed in three studies that feeding concentrate increases ruminal LPS and that the magnitude of the response depends on the level of concentrate in the diet and probably on how long such diets are fed before inducing SARA. Rapid growth of bacteria is associated with bacterial lysis due to excessive activity or autolytic enzymes during cell growth and division in the rapid growth phase (Wells and Russell, 1996). Andersen (2000) suggested that as much as 60 % of ruminal LPS is produced by rapidly growing gram-negative bacteria. During a SARA, the damaged rumen epithelium might lead to pathogen and/or LPS infiltration (Nordlund et al., 1995). When hepatic or body clearance is exceeded, LPS might induce inflammatory responses (Andersen, 2000). LPS content was not analyzed in peripheral blood samples. It is believed that free ruminal LPS that translocate into the portal vein can be cleared by the liver before reaching the peripheral blood circulation (Andersen, 2000). Also, Gozho et al. (2007) did not find detectable LPS in the peripheral circulation during a grain induced SARA. However, others detected very low LPS concentrations in the systemic circulation when compared to the ruminal ones. Zhang et al. (2013) detected free plasma LPS levels in a study investigating the influence of DMI and the effect of a *Saccharomyces cerevisiae* fermentation product on LPS-content. Therefore, it cannot be excluded that small amounts of LPS were present in systemic blood of the cows in the present experiment, especially of those fed the high-concentrate diets.

As we found no correlation between LPS and the SI for PBMC, fibrinogen or other hematological parameters and liver enzyme activity, results give no clear evidence for a connection between ruminal LPS and immune parameters of the present trial. However, fibrinogen which is considered only as weak APP in the bovine was elevated in cows fed the high-concentrate diets which contrasts to the findings of Pyörälä (2000).

Moreover, Gozho et al. (2007) attribute only an ancillary role to fibrinogen in SARA diagnostics under field conditions. Besides fibrinogen, which is synthesized by the liver, the high-concentrate feeding was associated with increases in GLDH and GGT activity in peripheral blood which might further hint at a LPS-associated involvement of the liver. Regarding the cellular components, the differential blood count was influenced by niacin supplementation. Niacin supplementation increased the proportions of lymphocytes and decreased that of the segmented neutrophil granulocytes. From a functional viewpoint it is interesting to note that stimulation ability of PBMC was differently influenced by niacin. In interpretation of these interactions between concentrate proportion in the diet and niacin supplementation it has to be considered that niacin acts both at the ruminal and the systemic level whereby direct effects from systemically absorbed niacin (Rauls et al., 2015), or indirect effects from an altered ruminal fermentative pattern and microbial protein synthesis (Niehoff et al., 2009) might affect the proliferative PBMC response. However, the nature of these interactions cannot be explained yet and requires further elucidation.

6 Conclusion

The aim of the present study was to investigate the influence of 60 % vs. 30 % concentrate in the diet of dairy cows with the presence or absence of 24 g/cow/day niacin supplementation. It was shown that ruminal LPS concentration, total leukocytes, hematocrit, ASAT and fibrinogen were subject to varying degrees of a 4-way interaction (C*NA*P*WoL). The SI of the PBMC was influenced by a C*NA interaction. Niacin supplementation increased lymphocyte percentages, while segmented neutrophil granulocytes decreased and there was also found a decreasing tendency for the total leukocytes and the monocytes. High concentrate proportion in the diet increased the GLDH and GGT.

As the mode of action of niacin is influenced by many different criteria like volume of niacin, parity, concentrate proportion, specific period, a large variety of diverse results has been generated. Therefore, further investigations should be conducted (only cows or heifers, shorter period) under more restricted experimental conditions to achieve more precise results.

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