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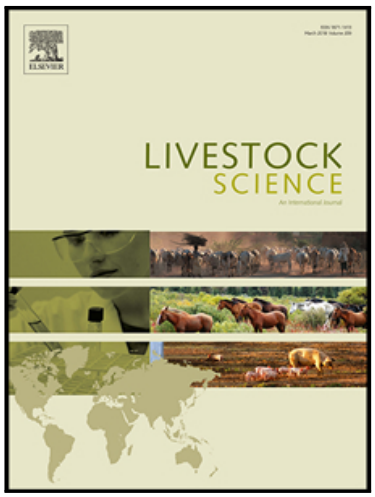

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The effects of dietary resin acid inclusion on productive, physiological and rumen microbiome responses of dairy cows during early lactation

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Highlights

- Two novel dietary supplements with resin acids were compared in transition cows
- Rumen microbiota was analysed by 16S rRNA sequencing 2 and 10 weeks after calving
- Time but not the diets affected rumen microbiome and lactation parameters
- Plasma cytokine levels responded to time and to one resin acid treatment
- Milk fatty acid composition reflected the changes in cow energy status

ABSTRACT

Dairy cows have intense fluctuations in digestive, metabolic and hormonal systems around calving which predispose them to various disorders and health problems. The aim of the current experiment was to investigate feed and nutrient intake, rumen fermentation, rumen bacterial communities, milk production, milk fatty acid composition and plasma biomarker profiles of dairy cows to assess the modulation of these functions by in-feed resin acid inclusion. Thirty-six Nordic Red cows were used in a continuous feeding trial starting 3 weeks prepartum and lasting for 10 weeks into the lactation. The cows were fed grass silage *ad libitum* and the dietary treatments were 1) control with basal concentrate (CON), 2) CON supplemented with tall oil fatty acids (TOFA; 90 % fatty acids and 9% resin acids) at 7.0 g/cow/day and 3) CON supplemented with resin acid concentrate (RAC; 37.5% resin acids) at 1.7 g/cow/day. The mixture of resin

acids in TOFA and RAC, consisting mostly of abietic and dehydroabietic acids, originated from coniferous tree species *Pinus sylvestris L.* and *Picea abies L*.

Feed intake and milk production were measured throughout the experimental period. Milk and blood samples were collected at weeks 2, 3, 6 and 10, and rumen fluid was sampled at weeks 2 and 10 of lactation to analyse rumen fermentation and rumen bacterial communities. The dynamics in feed intake and milk production with progressing lactation showed typical curvilinear trends (*P* for time <0.001). The time effect was also significant for most other measured parameters including plasma metabolites, immunological biomarkers, rumen pH and a number of ruminal bacterial species. Diet organic matter and neutral detergent fibre digestibility of TOFA was higher ($P < 0.05$) than those of the other diets. TOFA also resulted in mild immunomodulatory effects but in general there were no major effects of in-feed resin acid supplementation or diet × time interactions on the measured parameters. This indicates that under the conditions of the current experiment, the dietary supplements were not able to modulate the performance of dairy cows.

Key words: bacterial community, immunomodulation, milk fatty acid composition, rumen fermentation, prepartum, transition period

1. Introduction

Around calving, dairy cows undergo a few metabolically challenging weeks which are referred to as the transition or periparturient period. The time is characterized by hormonal and immunological fluctuations associated with calving and the physiological adaptations due to onset of milk production (e.g. Drackley, 1999; De Koster and Opsomer, 2013; Mordak and Stewart, 2015; Sundrum, 2015; Abuelo et al., 2019). These adaptations pose a significant metabolic stress to high-yielding dairy cows (Sundrum, 2015), creating a temporary negative energy balance (Drackley et al., 2005), and inducing insulin resistance with the potential to cause metabolic disorders (De Koster and Opsomer, 2013). An undisturbed transition from the late pregnancy to the peak lactation is of economic interest for dairy farmers, because a rapid onset of lactation improves possibilities for a high milk production over the production cycle, and because the transition period is critical for the health of the cow (Drackley et al., 2005; LeBlanc et al., 2006). It has been estimated that 75% of cases of illness in lactating dairy cows take place during the first month after calving (LeBlanc et al., 2006).

The negative energy balance of high-yielding dairy cows during the postpartum period makes them susceptible to performance-reducing infections (Wankhade et al., 2017). The postpartal systemic

inflammation is partly caused by gut leakage. Dairy cows are susceptible to increased gut epithelium permeability while shifting from a forage-based diet before calving to a rapidly fermentable diet after calving (Khiaosa-ard and Zhebeli, 2014). As a consequence, an increasing amount of bacterial endotoxins such as lipopolysaccharide (LPS) translocate across ruminal and intestinal epitheliums. The translocated endotoxins enter the blood circulation and activate the innate immune response through toll-like receptor 4, inducing a low-grade systemic inflammation (Eckel and Ametaj, 2016). The circulating endotoxins may be further distributed into organs and tissues and induce local inflammations (Eckel and Ametaj, 2016). The immune activation and inflammation are energy-consuming processes, which direct the energy partitioning away from milk production (Bradford et al., 2015). As a result, a decreased milk yield is often observed with increasing gut LPS leakage, for instance, in cows with subacute ruminal acidosis (Khafipour et al., 2009). Moreover, LPS leakage from the gastrointestinal tract has been suggested as a trigger of various metabolic diseases during the periparturient period (Eckel and Ametaj, 2016).

To improve the production capacity and well-being of dairy cows, anti-inflammatory drugs can be applied to reduce the postpartal systemic inflammation (Bradford et al., 2017). - However, the feed industry has shown increasing interest in supporting the development of the gastrointestinal microbiota of dairy cows by alternative feed ingredients with capacity to improve the productivity and to reduce the need for anti-inflammatory or antimicrobial medication. Recent research efforts towards this direction include dietary interventions such as nicotinic acid (Tienken et al., 2015; Kinoshita et al., 2016), conjugated linoleic acid and polyunsaturated fatty acids (Qin et al., 2018), over-supplementation with metabolizable protein (Hare et al., 2019), methionine (Liang et al., 2019), and a *Saccharomyces cerevisiae* fermentation product (Knoblock et al., 2019).

Tall-oil fatty acid (TOFA) mixture which contains natural fatty acids and resin acids from coniferous trees is a novel feed material that has not been studied in periparturient cows. The material has an antimicrobial activity against Gram-positive pathogens such as *Clostridium perfringens* and *Staphylococcus aureus in vitro* (Roy et al., 2018), while the Gram-positive commensals such as *Lactobacillus* spp. seem to be relatively tolerant to it (Vienola et al., 2018). In sows (Hasan et al., 2019) and broiler chickens (Vienola et al., 2018), dietary TOFA has improved the composition of intestinal microbiota. Moreover, TOFA inclusion has improved the production performance of broiler chickens (Kettunen et al., 2017; Vienola et al., 2018) and sows (Hasan et al., 2019). In monogastric animals, dietary resin acids have been suggested to reduce inflammation-associated upregulation of intestinal matrix metalloproteinases and therefore to enhance intestinal integrity and homeostasis (Aguirre et al., 2019). Although coniferous resin acids have not previously been used in ruminant diets, they are ubiquitous in the winter forage of moose (*Alces alces*, L.) which is an important game species in North America and Eurasia (e.g. Bergqvist et al. 2001). It should be

noted that the lipid-soluble resin acids in TOFA are biodegradable, and do not harm aquatic environments in the ways that saponified resin acids of the wastewaters of the wood industry used to do (e.g. Luchnikova et al., 2019).

The objective of the present study was to evaluate the effects of two novel dietary supplements with coniferous resin acids on the production parameters, rumen microbiota and plasma biomarkers of metabolic and immunological status on lactating dairy cows during the transition period and early lactation. The hypothesis was that the dietary resin acids would have beneficial effects on the microbial community of rumen, and also - either directly or indirectly via microbes – reduce the metabolic or immunological stress of the transition period, thus allowing higher milk production. The two test products provided a similar quantity of resin acids per cow per day, but one was in oil form and the other was a dry product in a wheat flour carrier. The study thus also aimed to compare the effects of these two forms of providing coniferous resin acids to lactating dairy cows. e.

2. Materials and methods

2.1. Animals and treatments

Thirty-six Nordic Red cows were used in a continuous feeding trial that started 3 weeks prepartum and lasted for 10 weeks into the lactation. The cows entered the experiment gradually and the time between the calving of the first and the last cow was 117 days. No primiparous cows were included in the experiment and the average parity of cows was 3.3 (s.d. 1.07). All cows completed the whole experimental period.

The diet consisted of grass silage *ad libitum* and pelleted concentrate fed separately from automated feeders. Throughout the experiment, two separate grass silage batches were used as a mixture. Both silages were made from primary growth of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward at Jokioinen, Finland (60°48'N, 23°29'E). Grass was slightly wilted, precision chopped and ensiled into horizontal silos using a formic acid based additive (AIV2 Plus Na, Eastman, Oulu, Finland) at a target rate of 5 L/ton fresh matter.

The cows were divided into blocks of three animals according to the expected date of calving. The cows within the block were then assigned to the experimental treatments in a random order. The dietary treatments were: 1) Control with basal concentrate (CON); 2) Basal concentrate including a subset of 1.8 kg/cow/day of TOFA-supplemented concentrate, providing 7.0 g TOFA/cow/day; and 3) Basal concentrate including a subset of 1.8 kg/cow/day of RAC-supplemented concentrate providing 1.7 g of RAC/cow/day. Treatments 2 and 3 both resulted in an intake of 0.63 g resin acids/cow/day. The dosing of the TOFA was

based on the previous usage of TOFA in sows: 5 g of TOFA/sow/day for one week pre-parturition (Hasan et al. 2019) and also on unpublished pilot trial with TOFA to test the palatability of TOFA. The dosing of RAC was selected to provide the same amount of resin acids/cow/day (0.63 g/cow/day) as the 7.0 g TOFA/cow/day -dosing. The daily dose of TOFA and RACsupplemented concentrate pellets for each cow was mixed within the basal concentrate pellets and the mixture was given every time the cow ate at the feeding stations. The main components of the concentrates were rapeseed meal or expeller (37%), wheat (18%), barley (17%), sugar beet pulp (8%) and molasses (5%). All experimental concentrates included minerals and vitamins and were pelleted and produced by Hankkija Ltd. (Hyvinkää, Finland).

The two dietary treatments contained coniferous resin acids from Scots pine (*Pinus sylvestris L.*) and Norway spruce (*Picea abies L.*), mainly including abietic, dehydroabietic, palustric and pimaric acids. The two resin acid-containing supplements TOFA and RAC were produced by thermal distillation from crude tall oil, a by-product of cellulose industry by Forchem Ltd. (Rauma, Finland). While TOFA is already commercially available (Progres®, Hankkija Ltd., Hyvinkää, Finland), RAC is at present a product candidate for livestock feeds. The product form differs in these two substances: TOFA is a liquid containing 9% resin acids, 90% fatty acids (mainly linoleic and oleic acids) and 1% unsaponifiables, and RAC is a powder in which 37.5% resin acids have been dried onto food grade whole grain wheat flour.

2.2. Experimental procedures

National Ethics Committee (Regional State Administrative Agency, Finland; license number ESAVI/14482/2018) approved the experimental procedures in accordance with the guidelines established by the European Community Council Directive 2010/63/EU. The cows started to receive the experimental concentrates three weeks before the expected calving date. After calving, the amount of concentrate was increased so that all cows received 12 kg concentrate per day from the automatic feeders and 300 g of the basal concentrate without TOFA or RAC from the milking parlour during every milking (twice a day) by day 14.

Cows were housed in a free-stall barn and fitted with transponder collars that allowed identification in the milking parlour, scale and feeding area, where each cow had an individual automated weighing feeding trough (Insentec Ltd., Marknesse, the Netherlands). Cows were fed silage four times daily at 0700, 1300, 1600, and 1800 h by an automatic feeding wagon (TR Feeding Robot, Pellon Group Ltd., Ylihärmä, Finland). Uneaten feed was removed and weighed daily at 1200 h before fresh feed was offered. At least 5% refusal was targeted daily to ensure *ad libitum* silage intake. Concentrate feeds were delivered from automatic feeders and milking parlour. Water was accessible freely throughout the experiment. Silage was sampled twice per week and frozen to produce a composite sample of four weeks. The three experimental

concentrates and the milking parlour concentrate were sampled once per week to produce a composite sample of four weeks.

Colostrum from the first milking after calving was weighed and sampled for chemical composition and immunoglobulin analyses. Cows were milked in a 2×6 auto tandem milking parlour at 0700 and 1700 h and the amount of milk was recorded every day. Milk samples were taken at 2 consecutive milkings (morning and evening) at weeks 2, 3, 6, and 10 after calving. Milk samples for resin acid analysis were taken at morning and evening milkings from the same individual eight cows per treatment at weeks 2, 3, and 6 after calving and stored frozen (-20 °C). Once during the experiment, pooled milk of 6 cows (ranging from 4 to 10 weeks into lactation) per treatment was collected and submitted to the evaluation of sensory properties. Milk taste and odour were estimated using a scale of 1 to 5, where 1 was the lowest and 5 was the highest score (5 = normal quality of raw milk), according to standard procedures (ISO, 2009). The results were not analysed statistically (n = 1 per treatment) but interpreted as an indication of the sensory quality of milk in response to resin acid intake of cows.

All cows were weighed every time when they passed the milking parlour. The body condition scores (BCS) of cows were evaluated 3 weeks before expected calving date, and at weeks 2 and 10 after calving using a scale from 1 to 5 (Edmonson et al., 1989). The animals were monitored daily for health problems, and any abnormalities and infections were recorded, and treated according to the general barn guidelines.

Blood samples were taken from tail vein 3 weeks before expected calving, and at week 2, 3, 6, and 10 after calving. The samples were taken in the morning after milking but before cows entered the feeding area thus being close to a fasting value, as 300 g of concentrate was given at the milking parlour. Plasma samples were frozen and stored at -20 °C for later analysis.

For rumen fermentation and rumen microbial community analysis, 0.5-1 litre of rumen fluid was collected using oesophageal stomach tube (Ruminator, profs-products.com, Wittibreut, Germany) ca. 3 h after morning feeding. All cows were sampled at 2 and 10 weeks after calving. The pH of rumen fluid was measured immediately after sampling using a portable pH meter. Rumen liquid samples for ammonia and VFA analyses were stored at -20 ˚C prior to analyses, while those for bacterial community determination were aliquoted and stored at -80 ˚C until DNA extraction.

Diet digestibility was analysed using acid insoluble ash (AIA) as an internal marker. Spot samples of faeces were taken after morning and evening milkings over a 4-day period from 21 cows (7 per treatment) which

at the time of sampling were 4 to 10 weeks into lactation. The samples were frozen immediately at -20 °C, and later pooled into one sample per cow per period.

2.3. Analytical methods

Feed, milk, rumen fluid and faecal analyses were conducted as described by Savonen et al. (2020). The mid-infrared spectral readings of the milk and colostrum samples were acquired with a MilkoScan FT600 spectrometer (Foss, Hillerød, Denmark) in the laboratory of Valio Ltd. (Seinäjoki, Finland). The selected resin acids in milk were determined according to Apajalahti et al. (2020). The limit of quantitation was 0.02 mg/kg for abietic and dehydroabietic acids.

Blood plasma was analysed for BHBA (automated enzymatic method (3-hydroxybutyrate)) and non-esterified fatty acids (NEFA; automated enzymatic method), while Na-fluoride plasma was analysed for glucose (automated IFCC-standard method (hexokinase), all performed on Konelab Prime 60 (Thermo Fisher Scientific Ltd., Vantaa, Finland). Blood serum was analysed for insulin concentration (solid-phase, enzyme-labeled chemiluminescent immunometric assay performed on Immulite 2000 XPi, Siemens Healthineers, Erlangen, Germany). The analyses were conducted at Movet Ltd. (Kuopio, Finland).

Colostrum immunoglobulin G (IgG) concentration and the plasma concentrations of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-α), intestinal fatty acid-binding protein (I-FABP), LBP and serum amyloid A (SAA) were analysed using commercial ELISA kits following the protocols from the manufacturers (detailed information in Supplemental Table S1). Reading of the optical density during the ELISA analyses was performed with a Multiskan™ FC microplate photometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The precision of ELISA analyses was assessed by calculating the intra- and inter-assay coefficients of variation (Supplemental Table S1). The coefficients of variation of all biomarkers were below the up-limits suggested by the manufacturers. Colostrum immunoglobulin content was also estimated by the Brix value measured using a refractometer (Lappo Ltd., Piikkiö, Finland), according to Hasan et al. (2016).

Total DNA was extracted from 0.5 ml of rumen liquid for rumen bacterial community analysis as described by Rius et al. (2012). Universal primers targeting 16S rRNA gene V4 region were used for bacterial amplicon sequencing (Caporaso et al., 2011). Libraries were prepared and sequenced on Illumina MiSeq platform using 2 × 300 bp chemistry in Finnish Functional Genomics Centre (Turku, Finland). Demultiplexing of sequences, adapter removal and sorting sequences by barcode were performed by the sequencing centre. Sequencing data was further processed using Qiime v 1.9.1 (Caporaso et al., 2010) as described in Tapio et

al. (2016). Singleton operational taxonomic units (OTU) were removed and the data from each sample were rarefied to the similar sequencing depth prior to further analyses.

2.4. Calculations and statistical analyses

The feed values metabolizable energy (ME), metabolizable protein and protein balance in the rumen were calculated according to Luke (2021). Milk fatty acid (FA) concentrations were predicted from milk mid-infrared spectral readings using calibration equations described by Soyeurt et al. (2011), where performances of the individual prediction equations used were reported. Only FAs and FA groups with fair ($R^2 \geq 0.89$), good ($R^2 \geq 0.97$), or excellent ($R^2 \geq 0.99$) prediction accuracy (Grelet et al., 2014) were considered.

The statistical analyses were performed using SAS (release 9.4, SAS Institute Inc., Cary, NC, USA). Analyses of variance (procedure MIXED) were conducted with Tukey's test in a mixed linear model including block and diet as fixed effects and cow as a random effect (individual cows as experimental units). The repeated-measures ANOVA was used for parameters measured at different time points. Pearson's correlation analysis was performed between colostrum IgG concentration and Brix value using PROC CORR. The normality of variables was inspected with PROC UNIVARIATE.. For immunological variables, whose residuals were not normally distributed, the observations with a studentized residual larger than 3 or smaller than -3 were removed as outliers. After the removal of outliers, all the variables achieved a normal distribution of the residuals. The autoregressive covariance structure was used except for immunological data where it was selected among compound symmetry, unstructured, spatial power, and autoregressive, as the structure producing the smallest Bayesian information criterion

For rumen bacterial community analysis, OTUs that had less than 0.01% relative abundance across all samples were filtered out. Data was normalized using cumulative-sum scaling and $\log_2$-transformation to account for the non-normal distribution of taxonomic count data, as implemented in Calypso (Zakrzewski et al., 2017). The alpha diversity of rumen bacterial communities was calculated using Shannon index, Simpson index and Simpson evenness measure E as implemented in Qiime (Caporaso et al., 2010). Significance was calculated using non-parametric Kruskal-Wallis test for multiple group comparisons as implemented in *ggpubr* R package (Kassambara, 2020). Beta diversity or grouping of samples based on treatment and sampling week was evaluated by distance-based permutational multivariate analysis of variance (adonis), calculated using Bray-Curtis dissimilarities in *vegan* R package (Oksanen et al., 2019). Significance was defined at $P < 0.05$ level after 999 permutations.

3. Results

3.1. Feed intake, production responses and diet digestibility

The composition of grass silage and concentrate feeds used in the current experiment is presented in Table 1. The fermentation quality of the silage was good (separately for prepartum and postpartum periods: pH 4.30 and 4.33, proportion of ammonia N in total N 34.3 and 34.5 g/kg, lactic acid 27.6 and 29.0 g/kg dry matter (DM), acetic acid 14.3 and 15.6 g/kg DM, and butyric acid 1.08 and 0.81 g/kg DM). The feed intakes prepartum and during the first 10 weeks of lactation are presented in Table 2 and 3, respectively. The good nutritional and fermentation quality of grass silage elicited high voluntary silage intake and the proportion of separately fed concentrates in the total diet DM postpartum remained moderate (40 % on DM basis). Dietary treatments did not significantly influence feed or nutrient intake except for the slightly higher intake of the control concentrate when compared with those containing resin acids, but the difference was numerically very small (150 g/day or 1.5 % of the total concentrate intake). Feed intake increased rapidly after calving until plateauing at week 6, but there was no significant diet × time interaction (Figure 1A).

The rumen fermentation parameters at week 2 and 10 of lactation are presented in Table 4. Rumen pH was somewhat lower at week 2 when compared with week 10 (6.61 vs 6.70, respectively; P for time < 0.05) as well as proportion of acetate in rumen VFA (642 vs 654 mmol/mol, respectively; P for time < 0.001), but otherwise differences in rumen fermentation between weeks 2 and 10 were minimal. At week 10, the proportion of propionate was higher in CON than TOFA (P < 0.05) with RAC being intermediate, while in case of butyrate, the concentration was higher in TOFA than RAC. These minor effects on rumen VFA proportions resulted in a slightly more lipogenic profile of TOFA than CON, while RAC did not differ from either of them.

Digestibilities of DM, organic matter (OM) and neutral detergent fibre (NDF) were higher (P < 0.05) in TOFA than RAC and CON (Table 5). The resin acids were not totally digestible as both abietic and dehydroabietic acid were detected in faeces at levels of 23.6 and 6.53 mg/kg DM for TOFA, and 28.2 and 9.12 mg/kg DM for RAC, respectively. The daily outputs of the resin acids estimated based on DM intake and DMD could be calculated and a total tract digestibility of 58% was achieved averaged over both diets assuming an intake of them to be 0.63 g/day as planned.

The yields of milk, ECM, milk components and milk composition did not differ between treatments over the 10-week lactation period (Table 6). The effect of time was statistically significant for all reported performance parameters (P < 0.001), while no diet × time interaction was observed. However, both resin acid-supplemented groups showed a numerical increase in milk production during the first weeks of lactation (Figure 1B), and during weeks 2 and 3, the milk production on RAC was on average 2.1 kg/day higher than that of the control diet (P < 0.075). The significant time effect showed the typical quadratic

trend for milk, ECM (Figure 1B) and milk component yields. The colostrum composition showed typical clearly elevated concentrations of fat and particularly protein compared to normal milk, but no differences in colostrum composition were observed in relation to dietary treatments (Table 2).

No diet or diet × time interactions were detected for milk FA composition. However, the proportions of medium chain and saturated fatty acids increased while those of C18:1cis9, mono- and polyunsaturated as well as long chain fatty acids decreased with progressing lactation (Supplementary Table S2). Abietic and dehydroabietic acids were not transferred into milk as their concentrations in milk of 8 cows at weeks 2, 3 and 6 were in all cases below detection limit (0.02 mg/kg milk). This result was in line with the sensory analysis of milk which also indicated no difference between the dietary treatments. The milk smell scores were 4.88, 4.67 and 4.75 and taste scores 4.83, 4.88 and 4.75 for diets CON, TOFA and RAC, respectively.

Cow body condition score was similar in all diets 3 weeks prepartum (Table 2) and during lactation (Table 6). Most of the cows remained healthy through the trial period, but a few of them were diagnosed with udder or respiratory infections and were treated accordingly. The veterinary records revealed that the number of cows diagnosed with any type of infection was 8, 6 or 3 for CON, TOFA, and RAC, and the number of the total number of days when a cow was diagnosed with infection was 11, 11, and 4 for CON, TOFA, and RAC, respectively.

3.2. Plasma biomarkers

The effects of sampling time and dietary treatments on plasma insulin, glucose, NEFA and BHBA concentrations are presented in Figures 2 A-D. The effect of sampling time was significant ($P < 0.001$) for all parameters while a diet × time interaction was only significant ($P < 0.05$) for insulin. Blood glucose concentration showed an increase with progressing lactation (from week 2 to week 10), while blood NEFA concentration decreased. The NEFA concentration was highest for RAC (0.435 mmol/l) and lowest for CON (0.328 mmol/l) ($P < 0.05$), while intermediate for TOFA (0.394 mmol/l).

Irrespective of the diets, the concentrations of all immunological biomarkers showed variation between lactation weeks (time effect $P < 0.05$; Figure 3 A-H). Some biomarkers shared a similar pattern of changing over time. For instance, the plasma concentrations of IL-8 ($P < 0.001$), IL-10 ($P < 0.001$), and TNF-α ($P < 0.001$) decreased gradually over time in all groups. The concentrations of IL-1β, SAA, and LBP showed an increase at week 6 relative to week 3. A significant diet effect was found for IL-6, IL-8, and IL-10, as values for TOFA were higher ($P < 0.05$) than those for CON and RAC. The only significant diet × time interaction was found for TNF-α ($P < 0.05$), reflecting the slight increase in differences between treatments on weeks 6 and 10, compared with weeks 2 and 3.

3.3. Rumen bacterial community analyses

In total 3866135 16S rRNA gene amplicon sequences were retained after quality control and singleton removal. Sequencing depth was rarefied to 20000 reads for all samples. The time effects on rumen bacterial diversity, Shannon index, Simpson index and Simpson evenness measure E are presented in Supplementary Figure S1. Alpha diversity in CON and RAC groups was significantly ($P < 0.05$) higher at week 10 when compared with week 2, but changes did not reach statistical significance in TOFA group. No significant difference in alpha diversity was observed between CON, RAC and TOFA calculated at week 2 or at week 10 separately (Supplementary Figure S1).

Adonis analysis was performed to explore if differences in bacterial community composition can be attributed to the diet or time effect. Results indicated significant differences in distances between the groups based on sampling week ($P < 0.01$) but not based on dietary treatment ($P = 0.055$). To evaluate treatment effects on individual taxa, 85 genera were tested. In comparison to CON, on TOFA the abundances of *Sutterella* (Proteobacteria), SHD-231 (Chloroflexi), *Acholeplasmatales* (Tenericutes), and RFP12 (Verrucomicrobia) were higher but those of *Ruminococcus* and *Streptococcus* (Firmicutes) lower. RAC treatment had higher abundancies of *Alphaproteobacteria* and *Acholeplasmatales* when compared with CON. However, none of these changes remained significant after the false discovery rate control (FDR > 0.2, Supplementary Table S3).

Differences in bacterial community structure between weeks 2 and 10 after calving were more pronounced. Out of 25 genera level taxa that showed significant abundance changes ($P < 0.05$), 15 remained significant (FDR < 0.05) also after FDR correction (Figure 4). At week 2 after calving, rumen had significantly higher abundances of *Prevotella, Coprococcus*, *Rubrivivax* and undefined genera from families *Paraprevotellaceae*, *Veillonellaceae* and *Mogibacteriaceae* when compared with week 10. In contrast, at week 10, rumen bacterial community had significantly higher abundances of representatives from Bacteroidetes, Proteobacteria, Spirochaetes, SR1, Cyanobacteria, WPS-2 and TM7 phyla when compared with week 2 (Figure 4).

4. Discussion

4.1. Production responses

In the present study the cows reached the peak milk production at 3-4 weeks after calving while feed intake peaked clearly later at weeks 7-8 as typically observed (e.g. Salin et al., 2018). When calculated for the entire 10-week lactation period, the feed intake or production parameters were not significantly affected by the dietary treatments, nor did they affect milk composition. Therefore, the hypothesis that resin acids

would improve lactation parameters was not supported by the present study. A key question regarding the effect of resin acids on production parameters is the quantity of resin acids in the diet, because no dose-response studies on the effects of resin acids on lactating ruminants have been conducted. The amount of resin acids supplemented per cow per day was set to 0.63 g based on an approximation from earlier experiments by Hasan et al. (2019) on sows, given a daily dose of 0.45 g of resin acids (in 5.0 g of TOFA with 9% resin acids). It may be speculated that a higher dose would be needed. However, another experiment where resin acids were fed to lactating dairy cows as 15 g/cow/day TOFA in combination with a yeast hydrolysate product also failed to show effects on feed intake or milk production (Bayat et al., 2021).

Kairenius et al. (2020) fed pine bark meal containing resin acids to lactating dairy cows, which provided 3.5 or 7.0 g/day of combined intake of abietic acid and dehydroabietic acid, and observed slightly decreased milk production while feed intake was unaffected. However, the intake of resin acids in Kairenius et al. (2020) was confounded with other possible bioactive compounds in tree bark, which are not present in TOFA or RAC. Moreover, the bark meal inclusion reduced the energy content of the diets as an explanation of the decreased milk production. Milk production was not improved in the present study or in Kairenius et al. (2020) or Bayat et al. (2021) with the daily doses ranging from 0.63 to 7.0 g of resin acids per cow per day. Whether resin acids may help dairy cows through the sensitive first weeks after lactation should be studied in adequate dose-response experiments.

In the present study and in Kairenius et al. (2020), no changes in milk sensory quality were detected in response to resin acid supplementation. In line with this, Apajalahti et al. (2020) reported that resin acids were not transferred into meat or adipose tissue of broiler chicken.

The significantly higher OM and NDF digestibility of TOFA compared to the other diets is difficult to explain, and such effect could not be confirmed by Bayat et al. (2021). If ME intake would have been calculated from digestible organic matter intake assuming a yield of 16 MJ ME per kg OM digested, the ME intake from RAC would have been 9 MJ per day greater than from CON and TOFA (256 and 247 MJ ME per day, respectively). However, there were not even numerical differences in milk ME output, although according to the feed evaluation system (Luke, 2021), this amount of additional ME would be sufficient for 1.7 kg ECM.

4.2. Metabolic and immunological parameters

The lower blood glucose concentration and higher NEFA concentration at week 2 reflect increased mammary uptake of glucose and adipose tissue mobilization due to the onset of lactation (Drackley et al., 2005). The abrupt increase in energy and nutrient demands for the lactation cannot be immediately

compensated for by a higher feed intake, and the cow needs to mobilize its own energy reserves (Wankhade et al., 2017). This increased mobilization of body reserves leads to elevated concentrations of NEFA and BHB in blood plasma (Ospine et al., 2010; Mäntysaari et al., 2019) and fatty acid C18:1 cis9 in milk fatty acids (Gross et al., 2011; Mäntysaari et al., 2019) as seen also in the present study during weeks 2 – 3. It is worth noting that both TOFA and RAC increased plasma NEFA concentration, suggesting a negative effect on the metabolic balance of the cows.

Dietary resin acids have been suggested to protect the intestinal barrier functions of broiler chickens (Aguirre et al., 2019). To evaluate the effects of resin acids on the gastrointestinal barrier of dairy cows, we measured the plasma concentrations of two biomarkers, I-FABP and LBP. These proteins enter blood circulation when the intestinal epithelium is damaged and therefore have been used as a biomarker of the intestinal barrier in pigs (Pietro et al., 2019). However, the use of I-FABP as a biomarker in ruminants has not been reported before. In the present experiment, the dietary treatments did not affect the I-FABP or LPB values.

The cows fed TOFA had higher concentrations on inflammation biomarkers than cows consuming RAC. TOFA increased concentrations of various pro-inflammatory cytokines in blood circulation during the first 10 weeks of lactation including IL-6, IL-8 and TNF-α. Plasma concentration of the anti-inflammatory cytokine IL-10 was higher in TOFA group at the same time. Although pro- and anti-inflammatory cytokines mediate inflammation in opposite ways, simultaneous stimulations of them in response to the manipulation of inflammation have been observed in previous studies. Martel et al. (2014) gave continuous TNF-α infusion to the adipose tissue of late-lactating cows and observed increased IL-10 concentrations in the liver and adipose tissue, suggesting a stimulated anti-inflammatory response to counteract local inflammation. Furthermore, Takiya et al. (2019) fed cows with an anti-inflammatory agent and observed simultaneous up-regulations of IL-10 signaling pro-inflammatory pathways.

The different performance of the two resin acids-supplemented diets in regulating inflammation likely reflects their different composition. While RAC contains coniferous resin acids attached to a wheat flour carrier, TOFA contains fatty acids from crude tall oil in addition to resin acids. In the present experiment, both resin acid diets provided each cow a daily dose of 0.63 g of resin acids, but it is possible that the bioavailability of resin acids differed between the two supplements. Overall, the effect of the two resin acid -containing supplements on the metabolic or inflammatory status of lactating dairy cows remained inconsistent and do not support the hypothesis that resin acids would reduce the metabolic or immunological stress of the transition period.

The immunity of neonatal calves largely relies on the intake of immune components from colostrum. A previous study suggested a potential positive effect of dietary TOFA supplement on the immunity of piglets as it increased colostrum yield and colostrum IgG concentration of the sows (Hasan et al. 2019). However, colostrum IgG concentration was not affected by the diets in the present study, suggesting that ruminants may be less sensitive to TOFA than monogastric animals. The correlation of the colostrum IgG concentration and the Brix value used as a quick test at farms had a reasonable correlation (R^2 = 0.63), in line with Hasan et al. (2016) using sow milk.

4.3. Rumen fermentation and ruminal bacterial communities

Although diet composition remained the same, and feed intake increased from week 2 to week 10 resulting in a greater amount of fermented OM in the rumen, rumen pH was lower at week 2 when compared with week 10. This suggests that with progressing lactation, cows were able to stabilize rumen pH which may be due to increased absorption of VFA through rumen wall or increased buffering through saliva. This phenomenon took place simultaneously with the increase in average rumen acetate proportion out of total VFA from week 2 to 10 as well as changes in rumen microbial communities.

The transition from low energy high fibre prepartum diet to high energy postpartum diet is associated with a rearrangement of rumen microbial communities to accommodate digestion of changing dietary compounds. Bainbridge et al. (2016) demonstrated that microbial shifts continue throughout lactation and these results are in line with our observations. In our study, rumen bacterial community at week 2 after calving was significantly enriched with *Prevotella*, *Paraprevotellaceae* sp., *Veillonellaceae* sp., *Mogibacteriaceae* sp., *Coprococcus* and *Rubrivivax*, while at week 10 we observed increases in alpha diversity and in abundances of *Desulfovibrio* and several poorly known and largely unexplored taxa such as orders GMD14H09 (Proteobacteria), PL-11B10 (Spirochaetes), YS2 (Cyanobacteria), families BS11 (Bacteroidetes), F16 (TM7) or phyla WPS-2 and SR1.

Higher abundances of *Rubrivivax* and *Prevotella* have been associated with high-concentrate diets and ruminal acidosis (Fernando, 2008), conditions that cows are often exposed to postpartum. Increase in TM7 at week 10 in our study corresponds to the finding of Bainbridge et al. (2016), who observed increased TM7 abundance on 93 days in milk. However, despite observed similarities, comparing taxa affected by the stage of lactation in different studies is difficult mainly due to the inconsistency between diets. Nevertheless, changes in microbiota composition reflect the flexibility of the rumen microbial community to adjust to dietary and host physiological requirements.

The dietary treatments had minor effects on rumen bacterial community composition. Although our hypothesis of positive changes of resin acids on rumen microbiota was based on the results from earlier studies, indicating positive effects of dietary resin acid supplementation on intestinal microbiota of pigs (Hasan et al., 2019) and poultry (Vienola et al., 2018), digestive system of ruminants appeared to be less sensitive to resin acids. The observed absence of differences in rumen bacteria between the dietary treatments was in line with the lack of significant difference in rumen VFA concentration between the treatments. It is, however, possible that differences in response to resin acids between ruminants and monogastric animals is related to different gastrointestinal tract sites studied. Our results indicated that ca. 40% of resin acids supplemented bypassed the gastrointestinal tract without being digested, suggesting that the microbiome of the lower digestive tract may have been exposed to resin acids from TOFA and RAC. . In broiler chicken, depending on the dietary dose, 45-70% of resin acids were found in excreta (Apajalahti et al., 2020) suggesting that the bioactivity of the resin acids may be presented in the lower tract in broilers as well. However, the effect of TOFA and RAC on intestinal microbes was not investigated in the present study, and revealing the potential effects of resin acids on the entire gastrointestinal tract of ruminants remains a subject for further studies.

5. Conclusions

The present study investigated the effects of two feed supplements containing coniferous resin acids on the production parameters, rumen microbiota, and physiological and immunological responses of dairy cows during transition and early lactation periods. . There were no major effects of in-feed resin acid supplementation or diet × time interactions indicating that under the conditions of the current experiment, the dietary supplements were not able to modulate the 10-week performance of dairy cows. However, the resin acids of TOFA may have altered the immunological status during the periparturient period, reflected by the increased concentrations of both pro- and anti-inflammatory cytokines in blood circulation. This alteration might reflect an activation of the immune functions. Responses to dietary supplements may depend on the overall situation of the animals because it is typically easier to detect differences if the animals are challenged by various stressors. Lack of such stressors may be one reason why only minor responses to resin acid supplementation were observed in the current experiment.

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Conflict of interest

One of the authors (H. Kettunen) is employed by Hankkija Ltd., but authors declare no other conflicts of interest.

CRediT authorship contribution statement

P. Kairenius: Methodology; Formal analysis; Investigation; Data Curation; Writing - Review & Editing; N. Qin: Methodology; Formal analysis; Investigation; Data Curation; Writing - Original Draft; I. Tapio: Methodology; Formal analysis; Investigation; Data Curation; Writing - Original Draft; P. Mäntysaari: Investigation; Resources; Data Curation; Writing - Review & Editing; M. Franco: Formal analysis; Investigation; Writing - Review & Editing; P. Lidauer: Investigation; Writing - Review & Editing; T. Stefanski: Investigation; Writing - Review & Editing; M. H. Lidauer: Data Curation; Writing - Review & Editing; S. Junnikkala: Methodology; Investigation; Writing - Review & Editing; Supervision; M. Niku: Methodology; Investigation; Writing - Review & Editing; Supervision; H. Kettunen: Conceptualization; Methodology; Writing - Original Draft, Review & Editing; Funding acquisition; M. Rinne: Resources; Writing - Original Draft; Supervision; Project administration;

Data Availability

The rumen bacterial DNA sequence raw read data has been uploaded to NCBI Sequence Read Archive under submission number SUB9008163. For other raw data, see Supplemental materials or contact the authors.

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conflict 0f interest

This letter is to inform about conflicts of interest regarding our manuscript submission titled "The effects of dietary resin acid inclusion on productive, physiological and rumen microbiome responses of dairy cows during early lactation" and authored by P. Kairenius, N. Qin, I. Tapio, P. Mäntysaari, M. Franco, P. Lidauer, T. Stefanski, M. H. Lidauer, S. Junnikkala, M. Niku, H. Kettunen, and myself and submitted to Livestock Science on 22 March 2021.
One of the authors (H. Kettunen) is employed by Hankkija Ltd., which markets the feed additive used in the current study. This information is also clearly presented in the manuscript (address of H. Kettunen and producer of the additive mentioned) as well as included in the end of the manuscript in "Conflict of interest section". Hankkija Ltd. has also provided funding for this experiments, which information is also included in the manuscript.
Otherwise the authors declare no conflicts of interest.

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Table 1
Composition of the experimental feeds (mean + standard error)

	Grass silage		Concentrate feeds						
			Control		TOFA[1]		RAC[2]		MP[3]
	Prepartum	Postpartum	Prepartum	Postpartum	Prepartum	Postpartum	Prepartum	Postpartum	
Number of samples	8	12	5	8	5	8	5	8	8
Dry matter (DM), g/kg	298 ± 2.0	299 ± 1.4	867 ± 0.5	863 ± 0.5	871 ± 0.1	872 ± 0.2	868 ± 0.1	870 ± 0.2	876 ± 0.3
In DM, g/kg									
Organic matter	925 ± 12.0	920 ± 12.0	913 ± 5.3	916 ± 5.7	921 ± 0.5	921 ± 0.4	919 ± 0.3	919 ± 0.4	927 ± 1.6
Ash	74.6 ± 12.0	79.8 ± 13.4	86.7 ± 5.3	83.8 ± 5.7	79.1 ± 0.5	79.1 ± 0.4	80.8 ± 0.3	81.1 ± 0.4	73.4 ± 1.6
Crude protein	146 ± 5.2	145 ± 5.1	215 ± 4.8	217 ± 4.7	217 ± 2.4	219 ± 2.2	219 ± 1.3	219 ± 1.2	219 ± 7.0
Neutral detergent fibre	494 ± 28.9	487 ± 22.9	216 ± 1.7	215 ± 3.8	214 ± 3.9	218 ± 7.0	216 ± 3.7	216 ± 3.8	222 ± 7.6
In vitro OM digestibility, g/kg	791 ± 17	794 ± 14.0							
D-value[4], g/kg DM	701 ± 10.1	699± 10.1							
Feed values[5]									
ME[6], MJ/kg DM	11.2 ± 0.16	11.2 ± 0.16	11.9 ± 0.09	12.0 ± 0.1	12.2 ± 0.03	12.2 ± 0.0	12.0 ± 0.04	12.0 ± 0.0	12.0 ± 0.1
MP[7], g/kg DM	83.8 ± 1.09	83.8 ± 1.12	119 ± 2.4	118 ± 2.4	123 ± 2.1	121 ± 2.8	120 ± 2.3	119 ± 3.1	125 ± 3.2
PBV[8], g/kg DM	21.2 ± 4.91	20.7 ± 4.75	46 ± 4.0	49 ± 5.6	43 ± 4.5	46 ± 5.0	47 ± 3.2	50 ± 4.1	42 ± 5.4

[1]TOFA = Concentrate containing tall oil fatty acids
[2]RAC = Concentrate containing resin acid concentrate
[3]MP = Concentrate given at the milking parlour
[4]D-value = Digestible OM in DM
[5]According to Luke (2021)
[6]ME = Metabolizable energy
[7]MP = Metabolizable protein
[8]PBV = Protein balance in the rumen

Table 2

Body weight (BW), body condition score (BCS), and feed intake of dairy cows prepartum, and colostrum production and composition (n = 12 per treatment)

	Treatment			SEM	*P*-value
	Control	TOFA[1]	RAC[2]		
Prepartum period, days	18.5[b]	20.3[ab]	21.8[a]	1.10	0.1285
BW, kg	738	775	730	18.4	0.1796
BCS	3.44	3.48	3.48	0.096	0.9277
Intake, kg dry matter/day					
Total	15.7	16.0	15.8	0.432	0.9120
Silage	13.4	13.7	13.5	0.424	0.9011
Total concentrate	2.32	2.31	2.32	0.045	0.9749
Colostrum, kg at first milking	7.8	7.0	7.4	0.80	0.775
Colostrum composition					
Fat, g/kg	66.7	63.9	56.1	7.43	0.585
Protein, g/kg	144	135	147	6.4	0.421
Lactose, g/kg	33.0	33.6	30.2	1.11	0.077
Urea, mg/100 ml	62	63	86	6.2	0.016
Somatic cells, 1000/ml	878	1 816	934	567	0.433
Immunoglobulin G, mg/ml	56.1	54.2	57.6	4.81	0.874
Brix, %	23.6	22.6	23.2	0.82	0.669

[1]TOFA = Concentrate containing tall oil fatty acids

[2]RAC = Concentrate containing resin acid concentrate

Values with a different letter in a row are significantly different at 5% Tukey test.

Table 3
Feed and nutrient intake of dairy cows averaged over lactation weeks 1-10 (n = 12 per treatment)

	Treatment			SEM	*P*-value		
	Control	TOFA[1]	RAC[2]		Diet	Time	Diet × Time
Intake, kg dry matter/day (unless otherwise stated)							
Total	24.4	24.8	24.5	0.42	0.752	<0.001	0.245
Silage	14.5	15.1	14.8	0.42	0.676	<0.001	0.494
Total concentrate	9.87[a]	9.74[ab]	9.70[b]	0.056	0.076	<0.001	0.969
Organic matter	22.3	22.7	22.5	0.38	0.748	<0.001	0.162
Crude protein	4.23	4.28	4.24	0.058	0.772	<0.001	0.223
Crude fat	0.944	0.961	0.952	0.0160	0.728	<0.001	0.324
Neutral detergent fibre	9.06	9.26	9.14	0.202	0.762	<0.001	0.105
Metabolizable energy, MJ/day	261	265	262	4.0	0.713	<0.001	0.224
Metabolizable protein	2.42	2.46	2.43	0.034	0.680	<0.001	0.269
Protein balance in the rumen	0.708	0.703	0.707	0.0135	0.963	<0.001	0.143

[1]TOFA = Concentrate containing tall oil fatty acids
[2]RAC = Concentrate containing resin acid concentrate
Values with a different letter in a row are significantly different at 5% Tukey test.

Table 4
Rumen fermentation at weeks 2 and 10 (n = 12 per treatment)

	Week 2					Week 10				
	Treatment			SEM	*P*-value	Treatment			SEM	*P*-value
	Control	TOFA[1]	RAC[2]			Control	TOFA[1]	RAC[2]		
pH	6.53	6.64	6.67	0.062	0.2300	6.67	6.73	6.71	0.045	0.5875
NH_3, mmol/l	6.89	6.92	6.12	0.603	0.4123	7.01	6.40	6.15	0.572	0.5544
Total VFA[3] (mmol/l)	102	104	101	3.7	0.8570	102	104	98	3.3	0.5236
Molar proportions, mmol/mol total VFA										
Acetate (A)	638	649	640	5.7	0.3987	648	655	658	4.7	0.3126
Propionate (P)	191	178	190	4.6	0.0914	190^{a}	176^{b}	184^{ab}	3.4	0.0269
Butyrate (B)	134	138	134	3.5	0.7022	130^{ab}	136^{a}	126^{b}	3.4	0.0659
Isobutyrate	5.6	6.1	6.2	0.33	0.3963	5.7	5.8	5.5	0.26	0.6812
Valerate	15.8	15.2	15.4	0.51	0.6588	14.1	14.0	13.7	0.50	0.7448
Isovalerate	8.5	7.9	8.0	0.62	0.7130	7.8	7.4	7.5	0.52	0.7692
Caproate	6.9	6.5	5.6	0.51	0.2537	5.3^{ab}	6.0^{a}	5.1^{b}	0.36	0.0377
Molar rations										
A+B/P	4.06^{b}	4.48^{a}	4.09^{b}	0.13	0.0519	4.12^{b}	4.50^{a}	4.27^{ab}	0.093	0.0260
A/P	3.36^{b}	3.70^{a}	3.38^{ab}	0.115	0.0779	3.44^{b}	3.73^{a}	3.58^{ab}	0.083	0.0607

[1]TOFA = Concentrate containing tall oil fatty acids
[2]RAC = Concentrate containing resin acid concentrate
[3]VFA = Volatile fatty acids
Values with a different letter in a row (separately for week 2 and week 10) are significantly different at 5% Tukey test.

Table 5

Apparent total tract diet digestibility of dairy cows (n = 7 per treatment)

	Treatment			SEM	*P*-value
	Control	TOFA[1]	RAC[2]		
Dry matter	0.674[b]	0.690[a]	0.673[b]	0.0047	0.0378
Organic matter	0.688[b]	0.705[a]	0.687[b]	0.0047	0.0259
Crude protein	0.637	0.651	0.640	0.0082	0.4538
Neutral detergent fibre	0.555[b]	0.584[a]	0.552[b]	0.0077	0.0177

[1]TOFA = Concentrate containing tall oil fatty acids

[2]RAC = Concentrate containing resin acid concentrate

Values with a different letter in a row are significantly different at 5% Tukey test.

Table 6
Milk production over lactation weeks 1-10 (n = 12 per treatment)

	Treatment			SEM	*P*-value		
	Control	TOFA[1]	RAC[2]		Diet	Time	Diet × Time
Production per day							
Milk	39.8	39.3	40.9	0.94	0.358	<0.001	0.988
Energy corrected milk (ECM)	42.2	42.8	42.9	1.05	0.893	<0.001	0.855
Fat	1798	1851	1806	55.5	0.728	<0.001	0.886
Protein	1371	1358	1405	32.9	0.445	<0.001	0.519
Lactose	1810	1794	1861	45.5	0.452	<0.001	0.987
Milk composition, g/kg							
Fat	45.5	47.2	44.3	1.17	0.130	<0.001	0.806
Protein	34.9	34.7	34.6	0.48	0.950	<0.001	0.386
Lactose	45.4	45.5	45.4	0.33	0.970	<0.001	0.956
Total solids	131	134	133	2.6	0.705	<0.001	0.787
Urea, mg/100 ml	25.5	26.7	26.0	0.99	0.320	<0.001	0.001
Somatic cell count, 1000/ml	115	160	60	55.2	0.445	0.320	0.995
Body weight							
Mean, kg	665	701	656	18.5	0.213	<0.001	0.973
Change, kg/week	-3.61	-3.52	-2.82	0.868	0.781	<0.001	0.989
Body condition score, scale 1-5							
Mean	3.31	3.36	3.30	0.081	0.822	<0.001	0.931
Change, unit/month	-0.18	-0.17	-0.20	0.039	0.819	<0.001	0.921
Efficiency of milk production							
Nitrogen use efficiency[3]	0.326	0.319	0.333	0.007	0.342	<0.001	0.709
kg ECM/kg dry matter intake	1.74	1.73	1.75	0.046	0.918	<0.001	0.675
kg ECM/MJ ME[4] intake	0.162	0.162	0.164	0.004	0.920	<0.001	0.767
Energy balance[5]	-24.5	-25.4	-25.9	4.91	0.979	<0.001	0.775

[1]TOFA = Concentrate containing tall oil fatty acids
[2]RAC = Concentrate containing resin acid concentrate
[3]Nitrogen excreted in milk / Nitrogen intake
[4]ME = Metabolizable energy
[5]Energy balance was calculated for each cow by subtracting the energy required for milk production and maintenance from the total energy intake. The energy value used for ECM production (5.15 MJ per kg ECM) and ME Requirements for maintenance (0.515 MJ × kg Body weight$^{0.75}$) were based on Luke (2021).

Table 7

Milk fatty acids (FA; g/100 g FA) over lactation weeks 2, 3, 6 and 10 (n = 12 per treatment)

	Treatment			SEM	*P*-value		
	Control	TOFA[1]	RAC[2]		Diet	Time	Diet × Time
10:0	2.63	2.62	2.61	0.134	0.997	0.014	0.846
12:0	3.09	3.08	3.07	0.173	0.995	<0.001	0.783
14:0	11.1	11.0	11.0	0.35	0.997	<0.001	0.468
16:0	30.4	30.7	29.6	0.53	0.313	<0.001	0.689
18:1 cis-9	20.2	20.0	20.3	0.86	0.973	0.013	0.661
∑18:1 cis	21.7	21.6	21.9	0.91	0.971	0.014	0.664
∑18:1	24.5	24.4	24.9	0.95	0.914	0.002	0.679
Saturated FA	69.4	69.6	68.9	0.98	0.870	0.011	0.673
Monounsaturated FA	27.4	27.3	27.9	0.96	0.929	0.014	0.653
Unsaturated FA	30.6	30.4	31.1	0.10	0.873	0.016	0.689
Medium chain FA	49.2	49.3	48.4	1.00	0.745	<0.001	0.536
Long chain FA	42.3	42.0	42.8	1.16	0.865	<0.001	0.750

[1]TOFA = Concentrate containing tall oil fatty acids

[2]RAC = Concentrate containing resin acid concentrate

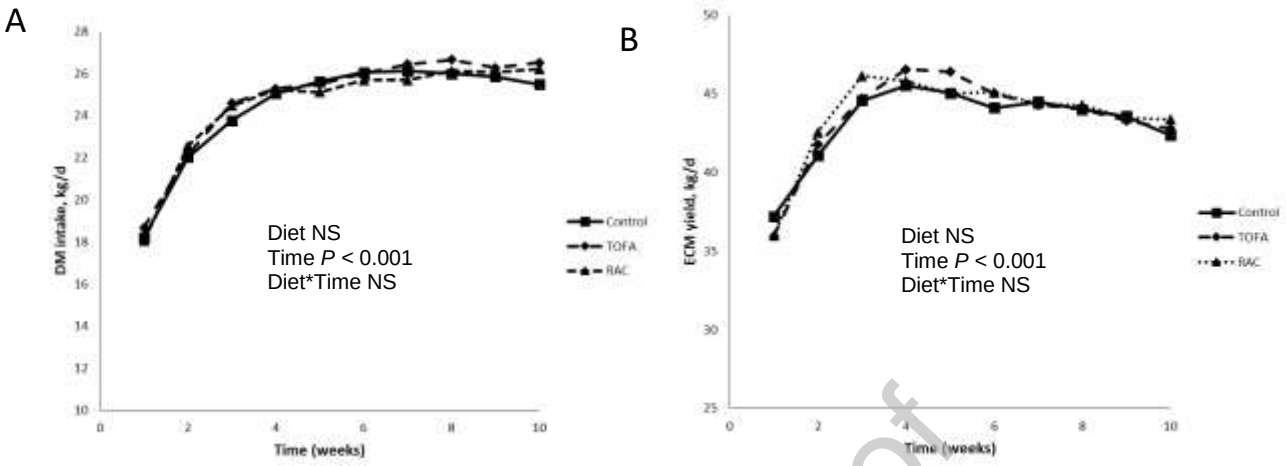


Fig. 1. Total dry matter (DM) intake (A), and energy corrected milk yield (ECM; B) of cows fed a control diet, tall oil fatty acids (TOFA) or resin acid concentrate (RAC) over first 10 weeks of lactation (n = 12 per treatment).

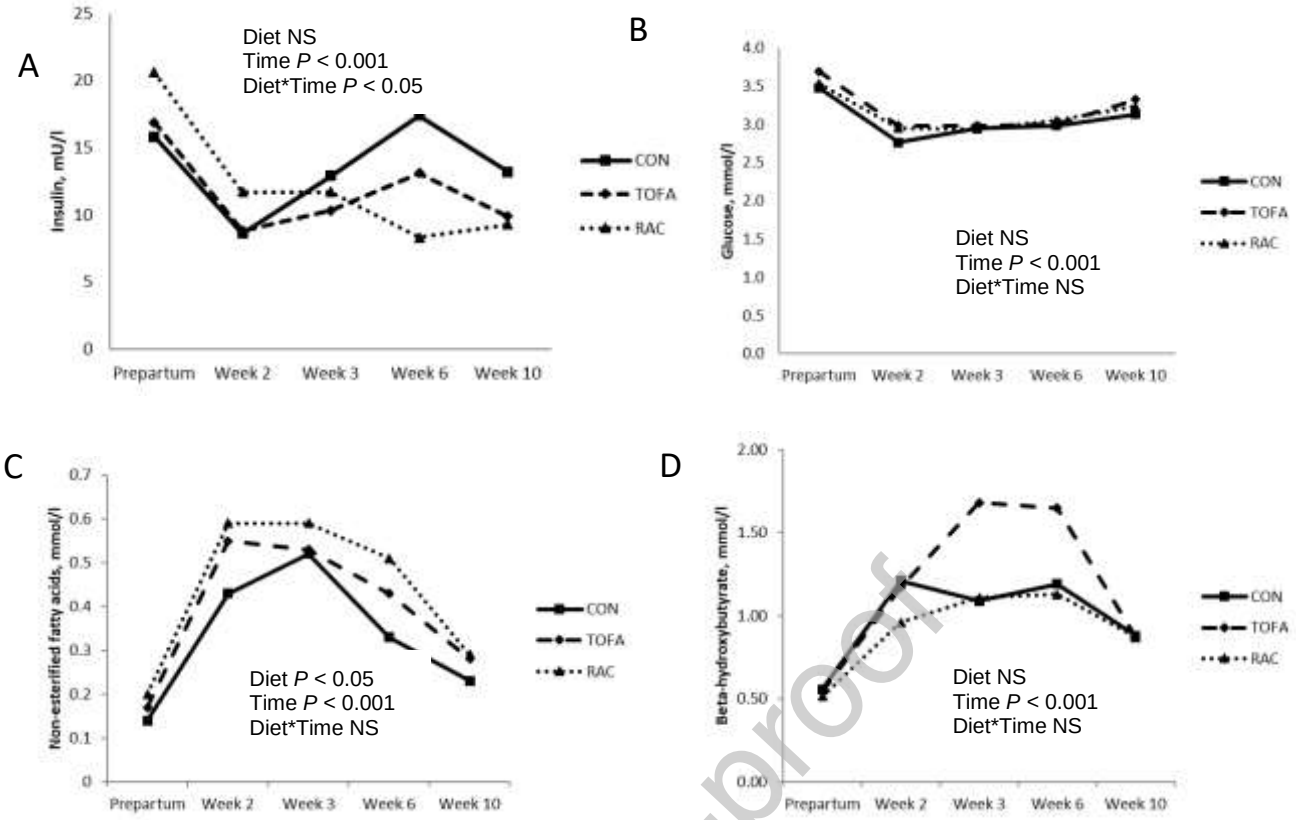


Fig. 2. Blood plasma parameters insulin (A), glucose (B), non-esterified fatty acids (C) and beta-hydroxybutyrate (D) prepartum and at lactation weeks 2, 3, 6 and 10 (n = 12 per treatment) of cows fed a control diet, tall oil fatty acids (TOFA) or resin acid concentrate (RAC) (n = 12 per treatment).

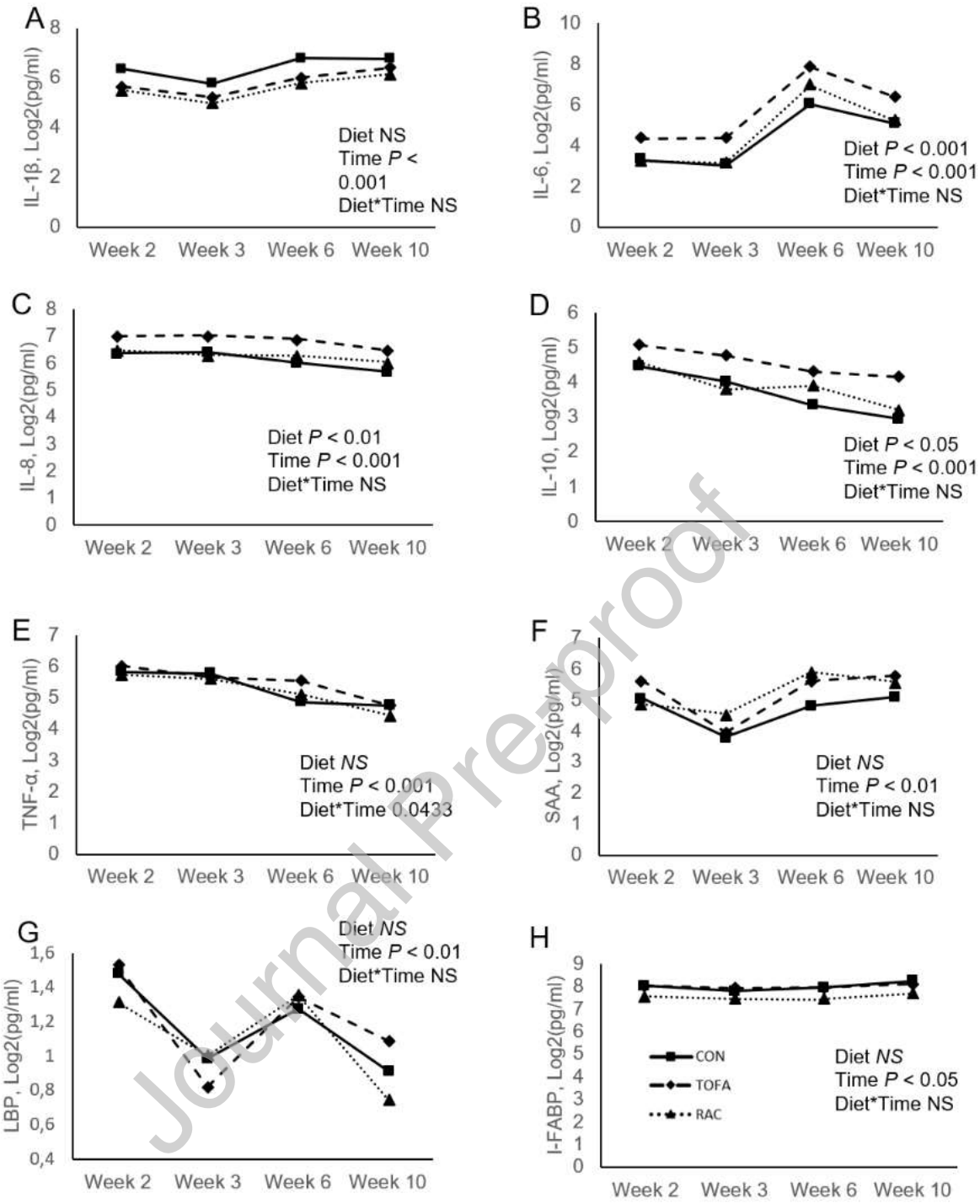


Fig. 3. Blood plasma parameters interleukin-1 beta (IL-1β; A), interleukin-6 (IL-6; B), interleukin-8 (IL-8; C), interleukin-10 (IL-10; D), tumor necrosis factor alpha (TNF-α; E), serum amyloid A (SAA; F), lipopolysaccharide-binding protein (LBP; G), and intestinal fatty acid-binding protein (I-FABP; H) at lactation weeks 2, 3, 6 and 10 (n = 12 per treatment) of cows fed a control diet, tall oil fatty acids (TOFA) or resin acid concentrate (RAC) (n = 12 per treatment).

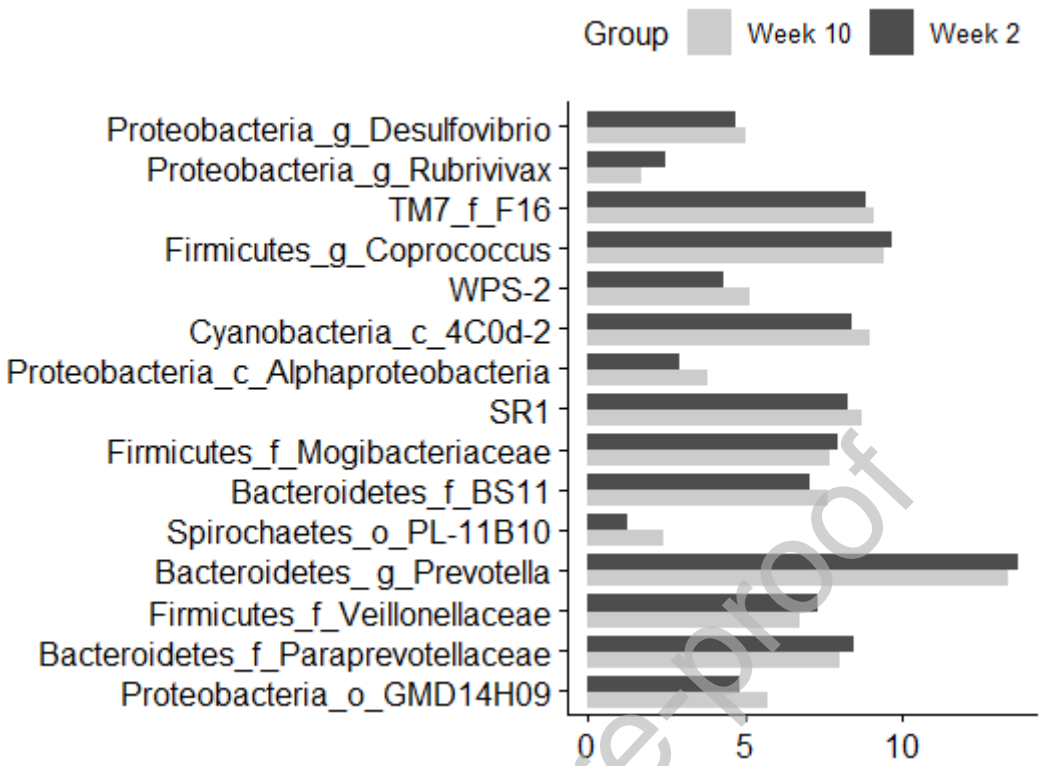
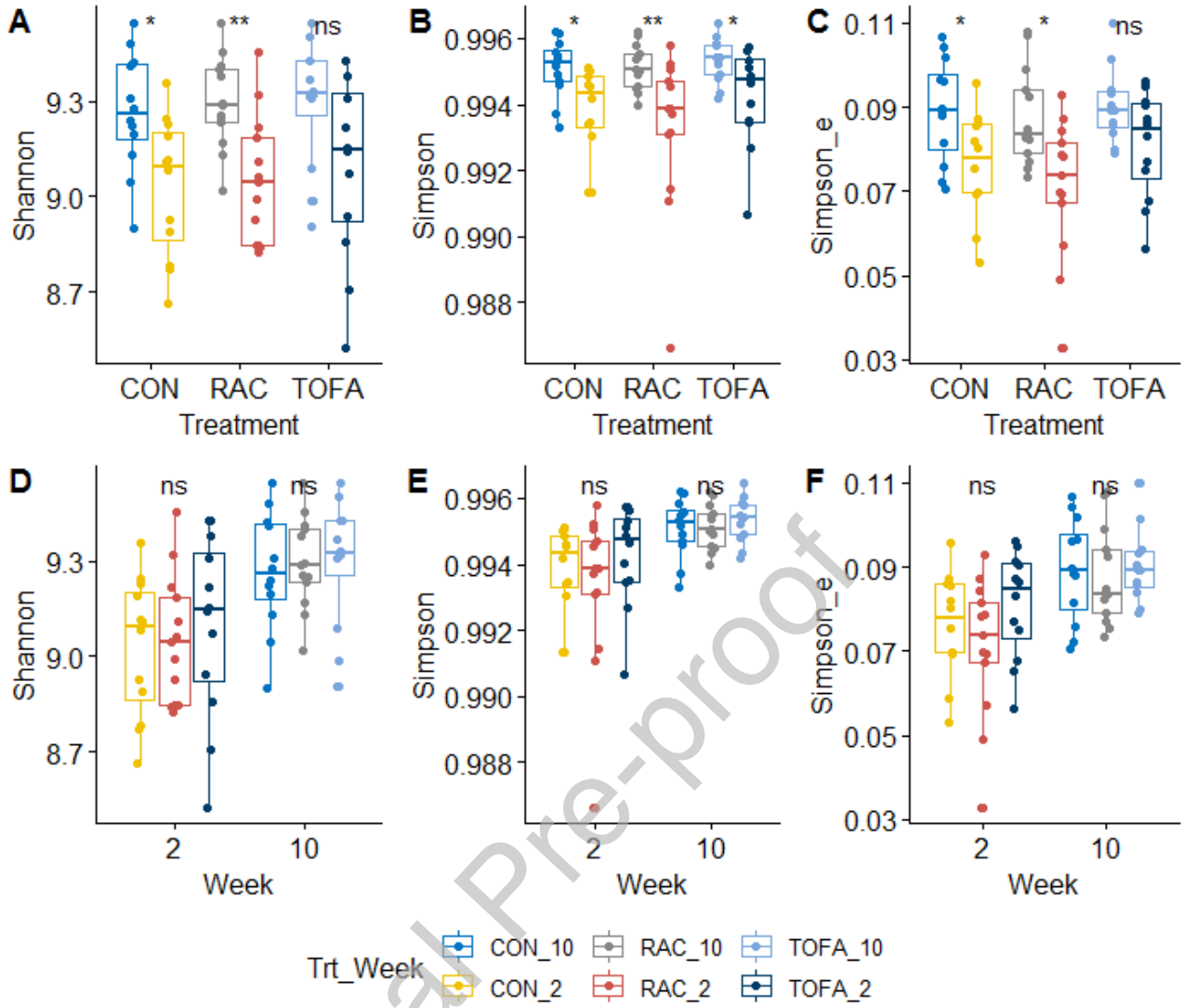


Fig. 4. Bacterial genera significantly different between week 2 and 10 after calving. Y axis represents taxa, while X axis represents cumulative sum scaled and log transformed bacterial abundances. Only statistically significant (FDR < 0.05) differences are presented (n = 36 at both time points).



Supplementary Figure S1. Alpha diversity estimates Shannon index, Simpson index and Simpson evenness measure E. Significance between weeks for each treatment (A-C) and between dietary treatments Control (CON), resin acid concentrate (RAC) of tall-oil fatty acids (TOFA) at weeks 2 and 10 separately (D-F) were estimated using Kruskal-Wallis test. Significance values are presented as: * < 0.05; ** < 0.01; ns > 0.05 (n = 12 per treatment).

Supplementary Table S1. Information of ELISA kits and the precision of analyses.

Supplementary Table S2. Milk fatty acid composition separately for weeks 2, 3, 6 and 10.

Supplementary Table S3. Microbial taxa at genus level, relative abundance of taxa in each of treatment-week groups and significance of treatment in pairwise comparisons (CON-TOFA, CON-RAC and TOFA-RAC) at week 2, week 10 and combined week 2/10 data.