Late gestation diet supplementation of resin acid-enriched composition increases sow colostrum immunoglobulin G content, piglet colostrum intake and improve sow gut microbiota

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Abstract:	A study was made of the effects of dietary supplementation of tall oil fatty acid (TOFA) and resin acid (RA) on sow colostrum yield, colostrum composition and gut microbiota. TOFA and RA are commonly termed resin acid-enriched composition (RAC) and conjugated linolenic, pinolenic, abietic, dehydrobiotic acids are characteristic components of RAC. The experiment was conducted in three trials in three respective herds. Sows were allocated either a control diet or a control diet supplemented with 5g RAC/day/sow during the last week of pregnancy. In one of the herds faecal microbiota populations of sows at farrowing were assessed using 16S rRNA gene sequencing. Colostrum samples were examined for nutritional composition, acute phase proteins (APP) and immunoglobulin (Ig) content. All piglets were individually weighed at birth and 24 hours later in order to calculate colostrum yield (CY), and later at three to four weeks to calculate average daily gain (ADG). The RAC-fed sows had significantly higher IgG levels ($P < 0.05$) in all three herds but treatment did not influence colostrum IgA and IgM concentration. Protein, lactose and fat content of colostrum did not significantly differ between sows of the two diet groups ($P > 0.05$), but those fed RAC had higher levels of colostrum serum amyloid A (SAA). CY was significantly higher in RAC-fed sows in herds 2 and 3 with heavier piglets between 3 and 4 weeks of age ($P < 0.05$), but not in herd 1 ($P > 0.05$). RAC supplementation significantly increased some beneficial and fermentative bacteria (Romboutsia and Clostridium sensu stricto) than the control diet group ($P < 0.01$) while some opportunistic pathogens (Barnesiella, Sporobacter, Intestinimonas and Campylobacter), including Proteobacteria, were suppressed. Therefore, RAC added to the sow diet at late pregnancy increases colostrum IgG, colostrum availability for neonate piglets, and seems to promote better maternal intestinal microbial sources.
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Late gestation diet supplementation of tall oil fatty acid and resin acid increases sow colostrum IgG content, piglet colostrum intake and modulates sow gut microbiota

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Short title: Tall oil fatty acid, resin acid and sow performance

Abstract

A study was made of the effects of dietary supplementation of tall oil fatty acid (TOFA) and resin acid (RA) on sow colostrum yield, colostrum composition and gut microbiota. TOFA and RA are commonly termed resin acid-enriched composition (RAC) and conjugated linolenic, pinolenic, abietic, dehydrobiotic acids are characteristic components of RAC. The experiment was conducted in three trials in three respective herds. Sows were allocated either a control diet or a control diet supplemented with 5g RAC/day/sow during the last week of pregnancy. In one of the herds faecal microbiota populations of sows at farrowing were assessed using 16S rRNA gene sequencing. Colostrum samples were examined for nutritional

composition, acute phase proteins (APP) and immunoglobulin (Ig) content. All piglets were individually weighed at birth and 24 hours later in order to calculate colostrum yield (CY), and later at three to four weeks to calculate average daily gain (ADG). The RAC-fed sows had significantly higher IgG levels ($P < 0.05$) in all three herds but treatment did not influence colostrum IgA and IgM concentration. Protein, lactose and fat content of colostrum did not significantly differ between sows of the two diet groups ($P > 0.05$), but those fed RAC had higher levels of colostrum serum amyloid A (SAA). CY was significantly higher in RAC-fed sows in herds 2 and 3 with heavier piglets between 3 and 4 weeks of age ($P < 0.05$), but not in herd 1 ($P > 0.05$). RAC supplementation significantly increased some beneficial and fermentative bacteria (*Romboutsia* and *Clostridium sensu stricto*) than the control diet group ($P < 0.01$) while some opportunistic pathogens (*Barnesiella*, *Sporobacter*, *Intestinimonas* and *Campylobacter)*, including Proteobacteria, were suppressed. Therefore, RAC added to the sow diet at late pregnancy increases colostrum IgG, colostrum availability for neonate piglets, and seems to promote better maternal intestinal microbial sources.

Keywords:

Tall oil fatty acid, resin acid, colostrum, Immunoglobulin G, Sow

Implications

Colostrum plays an essential role in piglet survival and growth, providing the piglets with a vital source of both immunoglobulins and energy. Despite this, both colostrum yield and quality vary considerably among sows. Therefore, feeding sows with alternative additives or compounds is common practice to improve colostrum quality and production. RAC contains free fatty acid, resin acids and improves performance in species other than the pig. This study demonstrated that RAC supplementation in

the sow diet increases colostrum yield, colostrum IgG, APP and abundance of beneficial gut microbiota and subsequent litter performance.

Introduction

Colostrum plays an essential role in piglet survival and growth, providing a source of both immunoglobulin (mainly IgG) and energy (Rooke and Bland, 2002). Piglets are born with a limited energy reserve and are devoid of immune protection due to the epitheliochorial structure of the placenta (Rooke and Bland, 2002; Salmon *et al.*, 2009). Therefore, colostrum is the sole external source of a piglet's nutrients and maternal immunity. Inadequate colostrum intake by the piglet is a major direct and subjacent cause of mortality during the initial days after birth (Decaluwé *et al.*, 2014). In addition, insufficient intake of maternally-derived immunoglobulins has a negative effect on piglet health, and thus also influences weight gain and survival at later stages in life (Rooke and Bland, 2002). Both the colostrum yield and colostrum quality vary considerably among sows (Devillers *et al.*, 2007). This variation can be attributed to sow, piglet and environmental traits (Devillers *et al.*, 2007; Quesnel, 2011). Therefore, an improvement in CY and its composition, especially IgG, IgA and IgM, benefits piglets. Thus, feeding sows with alternative additives to improve colostrum is common practice in modern pig production. RAC has been used in feed as a novel additives to improve performance in broiler (Vienola *et al.*, 2017). RAC modulates the microbial population in the digestive system of broilers and changes the microbial metabolism and improves the feed conversion ratio and gut microbiota (Kettunen *et al.*, 2017; Vienola *et al.*, 2017). RAC, a novel dietary product, typically comprises resin acids (~8%) and free fatty acids (~90%), and 2 to 3% neutral components. RA of RAC has been used to enhance immunity and regulate inflammation and wound healing (Kang *et al.*, 2008; Park *et al*., 2017). However,

Conjugated linolenic, pinolenic and oleic acids are characteristic fatty acid components of RAC and the effects of their supplementation in gestating and lactating diets have been well studied (Bontempo *et al.*, 2004; Corino *et al.*, 2009; Yao *et al.*, 2012; Yin *et al.*, 2017). Studies indicated that dietary supplementation of essential fatty acids improves sow colostrum immunoglobulins, and significantly increases average daily gain of piglets and subsequent weaning weight. However, the peculiar fatty acid composition of RAC, and its content of resin acids, may suppress the pathogenic bacteria and influence the growth of beneficial microbiota (Doorman and Deans, 2000).

The aim of this study was to explore the role of RAC on sow colostrum yield, colostrum quality, gut microbiota populations and subsequent piglet performance. Our hypothesis was that using RAC in the sow gestating diet could induce stimulation of the mucosal immune system, modulate beneficial microbiota and result in potentially higher levels of CY and colostrum immunoglobulin, thereby contributing to improved colostrum quality and immune protection of sucking piglets.

Materials and Methods

The experiment was carried out in three trials (herds 1-3) in commercial pig farms in Finland (two herds) and in the Netherlands (one herd) during January 2016 to April 2016, December 2016 to March 2017 and June 2017 to August 2017 respectively. The experiment was repeated in different batches of sows that farrowed during that time.

Animals and experimental design

A total of 44 multiparous sows (Yorkshire × Landrace) of mixed parities (from 1 to 7, 3.8 ± 0.2, mean ± SE) in herd 1, 47 multiparous sows (Yorkshire × Landrace) of mixed parities (from 1 to 7, 3.6 ± 0.2) in herd 2, and 30 multiparous sows (Topigs 20)

of mixed parities (from 1 to 6, 3.1 ± 0.2) in herd 3, were allocated to two dietary treatment groups. During gestation, sows were housed in groups of 15 to 20. The group housing rooms were equipped with individual feeding stalls. Sows were shifted to a farrowing house ~1 week before the expected date of farrowing. Sows were kept in individual farrowing crates (200 cm × 80 cm). Upon arrival at the farrowing rooms, sows were fed a standard diet, according to the national standards for lactating sows, (CON; n = 21, n = 23, n = 15 for herd 1, 2, 3 respectively) or the same standard diet supplemented with 5g RAC/day/sow in feed (Progres®, Hankkija Oy/Suomen Rehu, Hyvinkää, Finland, patent no FI124918) (RAC; n = 23, n = 24, n = 15 for herds 1, 2, and 3 respectively). The supplementation was continued until the start of farrowing. Parturition was observed, with as little interference as possible in the farrowing process. The birth of the first piglet was considered to represent the beginning of parturition. During the first 24 h piglets were allowed to consume only maternal colostrum and after 24 h were also allowed to drink a milk supplement. Six piglets from each litter were selected and ear-tagged based on body weight at birth (BW_B) in a block of three categories, 2 piglets <1 kg, 2 piglets 1.4 - 1.8 kg and 2 piglets >1.8 kg, respectively representing small, normal and large piglets. Altogether 126, 138, 90 piglets were included in the CON diet and 138, 144, 90 piglets in the RAC diet in herds 1, 2 and 3 respectively. Piglets were allowed to eat creep dry feed after one week in addition to maternal milk. All the selected piglets were monitored until 3 - 4 weeks of age. The researchers and farm workers gave no additional help or care to the piglets, unless there was a clear risk of them becoming crushed by the sow.

Parameters and measurements

All the piglets were weighed at birth, 24 h after birth and at weaning (3 weeks in herds 1 and 2, 4 weeks in herd 3). The CY was calculated as the sum of the

individual piglets' colostrum intake (CI) within a litter, as described by Devillers *et al.* (2004), using the following variables: BW_B (kg), weight at 17 to 24 h of age (BW_{24}, kg), duration of CI (t in min and 17 h ≤ t ≤ 25 h), and time between birth and first suckling (t_{FS}, min). The regression equation was: $CI = -217.4 + 0.217 \times t + 1861019 \times BW_{24}/t + BW_B \times (54.80\text{-}1861019/t) \times (0.9985 - 3.7 \times 10^{-4} \times t_{FS} + 6.1 \times 10^{-7} \times t^2_{FS})$. The tFS was estimated to be 35 min, which was based on our observations from a previous study (Hasan *et al.*, 2016). An error of 15 min in t_{FS} generates a 6 g/kg BW_B miscalculation of CI for piglets or less than 2% error (Devillers *et al.*, 2004). Observed sow parameters were parity, gestation length, farrowing duration, and numbers of live born and stillborn piglets. Observed piglet parameters were birth interval, pre-weaning mortality, BW_B, BW_{24}, body weight at weaning, and average daily gain (ADG). If needed, piglets were allowed to be cross-fostered after BW_{24} was taken, among the litters of the same treatment group, except for the six ear-tagged piglets that stayed with their original mothers till weaning.

Sampling colostrum, blood and faeces

Twenty millilitres of colostrum were collected from each sow within the first two hours after birth of the first piglet. Colostrum samples were collected from the first three teats of same side of the anterior udder. Samples were subdivided and stored at -20°C until further analysis. Fresh faecal samples were individually collected from the rectum of sows using sterile 50 ml tubes (n = 21 CON; n = 23, RAC) from herd 1. After collection, samples were kept in an icebox and transported immediately to the laboratory and stored at –80°C before total genomic DNA extraction. Sow blood samples were collected from the *vena saphena* at the beginning of farrowing using lithium heparin tubes, and centrifuged at 2000 rpm/min for 10 minutes, the plasma

being separated and stored at -20°C for further analysis. Blood samples were collected only from herds 1 and 2.

Colostrum and blood sample analysis

The standardized and complete methods for colostrum composition are described in Hasan *et al.* (2016). Concentration of Ig was quantified using swine IgG, IgM and IgA ELISA quantification Kits (Bethyl Laboratories, Montgomery, Texas, USA). The intra- and inter-assay coefficients of variation were 4.8% and 6.7% respectively. The colostrum total solid (TS), fat, protein and lactose contents were analysed using MolkoScanTM FT+ (Foss, Hillerød, Denmark), according to a validated method described in previous study Hasan *et al.* (2016). Colostrum and plasma serum amyloid A (SAA) were analysed with commercial multispecies indirect ELISA (Phase^{TM} SAA Assay, Tridelta Development Ltd., Kildare, Ireland) according to the manufacturer's instructions for swine. Colostrum and plasma haptoglobin (HP) concentrations were analysed with a haemoglobin-binding assay developed for cows (Makimura and Suzuki, 1982) with modifications, in which tetramethylbenzidine was used as a substrate and 5 µl of sample volume. Pooled and lyophilized aliquots of porcine acute phase serum were used as standards. The assay was calibrated using a porcine serum sample of known HP concentration provided by the European Commission Concerted Action Project (number QLK5-CT-1999-0153).

Microbial characterization

Microbial genomic DNA was extracted from 250 mg of each faecal sample using a QIAamp DNA Stool DNA kit (Quagen, ct. no. 51504) according to the manufacturer protocol. The yield and purity of DNA extracts were quantified using a Nanodrop 2000 (Thermo Fisher Scientific). The 16S PCR amplification and sequencing was done as described in Pereira *et al.* (2017) with modifications in primers. The 16s

region amplified was V3-V4 and mixed primers 341F_1-4 (CCTACGGGNGGCWGCAG) and 785R_1-4 (GACTACHVGGGTATCTAATCC), with partial Illumina TruSeq adapter sequences added to the 5’ ends (F_1; ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT, F_2; ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTgt, F_3; ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTagag, F_4; ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTtagtgt, R_1; GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACT, R_2; GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTa, R_3; GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTtct, R_4; GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTctgagtg). The PCR amplification steps and MiSeq sequencing was done at the DNA Sequencing and Genomics Laboratory, Institute of Biotechnology, University of Helsinki, Finland, similarly as described by Pereira *et al.* (2017). Sequenced 16S rRNA gene amplicons were processed using the MOTHUR software package (v 1.39.5) (Schloss *et al.*, 2009). The two paired-end reads were joined and the sequences were demultiplexed and quality filtered, with the removal of sequences containing bases <200 bp. Sequences were assigned to operational taxonomic units (OTUs) at ≥97% similarity with chimera filtering using the USEARCH algorithm (Edgar, 2013). The Ribosomal Database Project (RDP) classifier (Cole *et al.*, 2013) was used to annotate the representative OTU sequences, and taxonomic information was obtained for each OTU. The OTUs table-based data were further visualized using Calypso (Zakrzewski *et al.*, 2016).

Statistical analysis

All statistical analyses were performed using SPSS 24.0 (IBM Company Headquarters, Chicago, IL), considering statistical significance at $P < 0.05$ (2-sided tests) and tendency to significance when $P > 0.05$ but < 0.1. Normally distributed variables are reported as LSmean ± SEM and all the variables presented were normally distributed. Normality of the data was analysed with the Kolmogorov-Smirnov test and the Levene's test was used to verify homogeneity of variances. In order to analyse which variables were associated with CY and IgG, we performed univariate analysis before modelling. Interaction terms were tested and variables were included in the final model if $P < 0.25$. Backward stepwise elimination was used for final models. Sow data were subjected to linear regression with treatment (RAC and CON) and herd as fixed factors, and parity and farrowing duration as covariates. For piglet data, all the colostrum variables were tested with univariate analysis with ADG as the dependent variable: only lactose and CI were found significant. We analysed ADG with a mixed model where herd and feed were fixed factors, lactose and CI covariates, and sows were a random factor nested within herd. Microbiota statistical analysis was done with Calypso for Shannon Index, Simpson's Index, ANOVA and correlations.

Results

Sow reproductive performances

The effects of the dietary RAC supplementation on the performance of sows are presented in the Table 1. Sow dietary RAC had no significant effect on the gestation length, farrowing duration, litter size and birth interval ($P > 0.05$). However, there was a tendency for fewer stillborn piglets in herds 1 and 2 between RAC and control treatments.

Piglet growth performance

223 Piglet growth performance is shown in Table 2. Dietary supplementation of RAC had
224 no effect on BW_B. Piglets from RAC-fed sows were significantly heavier ($P < 0.05$) at
225 3-4 weeks of age in herd 2, and there was tendency towards this in herd 3. We did
226 not establish a similar trend in herd 1, but the overall differences among herds were
227 significant. After univariate analysis, in a linear mixed model, herd and CI had a
228 significant influence on ADG ($P < 0.01$) whereas feed and lactose did not ($P > 0.05$).
229 *Colostrum yield, colostrum quality and composition*
230 There was a higher CY in herd 2 ($P < 0.05$) and tendency towards this in herd 3 ($P =$
231 0.09) for RAC-fed sows (Table 2). The RAC-fed sows had significantly higher IgG
232 levels ($P < 0.05$) in all three herds, although the treatment did not influence colostrum
233 IgA and IgM concentration. However, the RAC supplementation of sow diets had no
234 effect on the CY in herd 1 ($P > 0.05$). Colostrum protein, lactose and dry matter
235 percentages were unaffected by the dietary treatment (Table 3). In addition, feeding
236 sows with RAC significantly reduced colostrum fat content only in herd 1. When all
237 three herds were analysed together in a regression model, neither treatment nor herd
238 had significant effects on CY. In a regression model, colostrum IgG was significantly
239 influenced by the treatment and herd ($P < 0.01$), but farrowing duration and parity did
240 not have any effect on colostrum IgG ($P > 0.05$).
241 *Colostrum and plasma concentration of APP*
242 The effect of the sow dietary RAC supplementation on colostrum and plasma
243 concentration of SAA and HP are presented in Table 3. The RAC-supplemented
244 sows tended to have increased colostrum SAA ($P = 0.09$, P = 0.07 and $P = 0.09$ for
245 herd 1, 2, and 3 respectively) and a similar tendency was also observed for plasma
246 SAA. On the other hand, RAC-supplemented sows had significantly lower HP in
247 colostrum in herd 2, but the changes were not similar in other herds or for plasma.

Microbial composition after RAC supplementation

After quality filtering as described above, a total of 90434 DNA sequence reads were generated from sow faecal samples, and reads were analysed for assignment of OTUs (≥97% identity level). The diversity of the microbial communities in the different treatment groups was gauged using Shannon and Simpson's indices. The diversity indices showed the number of different taxa present in each sample, a higher number indicating greater diversity. The Shannon indices were 3.3 ± 0.07 and 3.5 ± 0.07 for sows fed RAC and CON respectively. The Simpson's indices were 0.09 vs. 0.07 for sows fed RAC and CON respectively. The differences between Shannon and Simpson's indices were not significant ($P > 0.05$).

Collectively, 11 bacterial phyla, 141 genera, and 430 species (OUT) were identified in the faecal samples of sows. At phylum level, Firmicutes, Bacteroidetes and Proteobacteria were the three predominant phyla, representing 91.90% vs. 86.97%, 4.67% vs. 7.45% and 2.19% vs. 4.16% of all the sequences in RAC and CON-fed sows, respectively (Figure 1). There was a significant difference in the relative proportions between the treatment and control groups. The abundance of Firmicutes significantly increased ($P < 0.05$) in RAC-fed sows. A contrary trend was observed for Bacteroidetes and Proteobacteria, where feeding sows with RAC significantly reduced their presence ($P < 0.05$).

The distribution of the 16S sequences that were assigned to the genus level was analysed and is represented in Table S1 (only genera with ≥0.1% of the total sequences are displayed). The following 10 genera were defined as the most abundant, with more than 1% of total DNA sequences: *Romboutsia*, *Clostridium sensu stricto*, *Oscillibacter*, *Acidaminobacter*, *Christensenella*, *Sporobacter*, *Intestinimonas*, *Flavonifractor*, *Thermotalea*, *Barnesiella* and *Ruminococcus*. A total

of 19 differentially abundant genera were identified among the dietary treatments at the genus level, which included the five most abundant and fourteen less abundant genera (Table S1). RAC treatment resulted in significant increases in the abundance of *Romboutsia* and *Clostridium sensu stricto* ($P < 0.05$) and significant decreases in the abundance of *Barnesiella*, *Sporobacter*, *Intestinimonas*, *Campylobacter* and some other genera presented in the Table S1 ($P < 0.05$).

Discussion

This experiment showed that dietary supplementation of RAC increased CY of gestating sows (in two herds) and colostrum IgG content. In herds 2 and 3, a sow diet supplemented with RAC influenced the weight of piglets at 3-4 weeks of age. The experiments were conducted consecutively in three different trials in commercial settings with the agreement with farmer not to use any medication without researchers concern. However, due to own farm policy, piglets of herd 1 received all intramuscular antibiotic treatment at day 1. In the sows, RAC supplementation in a late gestation diet increased abundance of beneficial bacteria and decreased numbers of opportunistic pathogens. The supplemented RAC consisted of resin acids (mostly abietic, dehydroabietic and pimaric acids) and free fatty acids (mostly linoleic, oleic and pinolenic acids and CLA). Fractional distillation of crude tall oil, obtained as a by-product of the Kraft process in wood pulp manufacture, produces distilled tall oil, and further refinement of distilled tall oil produces RAC. Previous studies reported that supplementing a gestating sow diet with a fatty acid such as CLA affects piglet performance. If the sow diet contains 0.5% CLA in the last week of gestation and first week of lactation, piglets are significantly heavier at weaning (Bontempo *et al.*, 2004; Corino *et al.*, 2009). Although the results reported here are in line with others on the effects of feeding sows free fatty acid it is difficult to compare

298 the various studies on CLA because of differences in methodologies and the specific
299 composition of this novel RAC.
300 Colostrum is characterized by the high concentration of IgG and relatively low
301 concentration of IgA and IgM (Klobasa and Butler, 1987). Passive immunity or
302 sufficient intake of colostral immunoglobulins by piglets is important during the
303 piglet's early life (Rooke and Bland, 2002). In the present study, IgG concentration
304 was significantly higher in the sow fed with RAC in all three herds. Rooke and Bland,
305 (2002) reported that IgG synthesis by piglets is positively correlated with the amount
306 of maternal IgG absorbed, and early life maternal IgG has a significant influence on
307 later immune development, thus reinforcing the need for an adequate IgG intake from
308 colostrum.
309 The colostrum concentration of IgG is highly variable and can be affected by many
310 factors, such as parity, season, genotype, farrowing duration and teat location
311 (Klobasa and Butler, 1987; Rooke and Bland, 2002; Quesnel, 2011). In our study, the
312 concentration of IgG was not influenced by sow parity or farrowing duration, and a
313 similar result was obtained in our previous study (Hasan *et al.*, 2016) and in those of
314 Quesnel (2011). To minimize the effect of teat location in the concentration of IgG,
315 the colostrum samples were collected only from the three anterior teats from the
316 same side of the udder.
317 All IgG in colostrum is derived directly from the plasma of the sow. Variation in IgG
318 concentration in colostrum is very high among sows (Klobasa and Butler, 1987) and
319 so is individual variation of IgG concentration in the serum of a sow (Quesnel, 2011).
320 Supplementing a diet of a gestating sow with immunomodulating and bioactive
321 compounds increases colostrum IgG concentration. For example, conjugated
322 linolenic acid (CLA), non-specific immunostimulating products, shark liver oil, fish oil,

323 sources of essential oils, fermented liquid feed, polyunsaturated fatty acid (PUFA),
324 seaweed extract and mannan oligosaccharides have all been tested and found to
325 have effect (Bontempo *et al.*, 2004; Yao *et al.*, 2012).
326 Literature on the use of RAC in animal feed is scarce. To date, no studies have been
327 done related to RAC supplementation of gestating sow diets and the effect on sow
328 performance. The mechanism of performance improvement may also lie in systemic
329 effects of the RAC, but investigation of such effects was beyond the scope of this
330 paper, although the mechanisms of some RAC components, e.g. CLA, pinolenic and
331 abietic acids, have shown anti-inflammatory and immunomodulatory effects in
332 humans and animals (Takahashi *et al.*, 2003; Bontempo *et al.*, 2004; Kang *et al.*,
333 2008; Corino *et al.*, 2009; Yao *et al.*, 2012). The novel feed additives of resin acids
334 (abietic and dehydrobietic acids) found to inhibit prostaglandin E2 (PGE_2) production
335 by activation of peroxisome proliferator-activated receptors (PPARs), especially
336 PPARγ (Takahashi *et al.*, 2003; Kang *et al.*, 2008). Decrease PGE_2 have direct effect
337 on production of IgG (Calder, 1996). Studies also indicated that dietary CLA
338 increases plasma IgG and colostrum IgG in pigs (Bontempo *et al.*, 2004; Corino *et*
339 *al.*, 2009; Yao *et al.*, 2012), this also be possible by the pathway of cyclooxygenase
340 and lipoxygenase by decreasing the PGE_2 production (Calder, 1996) . In addition,
341 dietary PUFA are involved in interleukin (IL) production, which in addition to isotype-
342 specific lymphokines play an important role in regulating immunoglobulin synthesis
343 (Coffman *et al.*, 1986). Although dietary PUFA especially CLA has an effect in the
344 IgG increase, the doses were very low in our study compare to the others. In our
345 opinion, the novel RA has a potential role in the increase of IgG based on the current
346 research of anti-inflammatory, immunomodulatory and inhibitory effect on pathogenic

bacteria (Takahashi *et al.*, 2003; Kang *et al.*, 2008; Park *et al*., 2017; Vienola *et al.*, 2017).

Colostrum SAA tended to be higher in all three trials in RAC-fed sows. Age-dependent studies showed that SAA concentration is highest in neonate piglets (Moya *et al.*, 2007) and calves (Orro *et al.*, 2008). However, Larson *et al.* (2003) suggested that SAA in colostrum have local beneficial effects on the neonatal gut. Research has also found that colostrum-associated SAA peptide enhanced innate protection by stimulating intestinal epithelial cells and mucous production and thereby preventing binding of enteropathogenic bacteria (Larson *et al.*, 2003). Therefore, the tendency to have higher colostrum SAA content, for the sow whose diet has been supplemented with RAC, might positively affect piglet growth.

The beneficial effects of certain gut microorganisms are well known: boosting the production performance of the animal being one. Therefore, we investigated the RAC effect on faecal microbiota of sows and showed that it did not induce changes in microbiota diversity. Firmicutes was the most prevalent phylum, particularly in the RAC fed sows, compared with the control group. Bacteroidetes represented another dominant phylum and their numbers were significantly lower in RAC-fed sows. In the present study, a lower relative abundance of Proteobacteria was recorded for the RAC group, which can be considered beneficial. The increased prevalence of Proteobacteria represents a marker for an unstable microbial community (dysbiosis), a potential diagnostic criterion for diseases (Shin *et al.*, 2015). However, Proteobacteria are also linked with intestinal inflammation (Mukhopadhya *et al.*, 2012). This phylum includes bacteria known to cause intestinal disease in humans and animals (Salyers and Whitt, 2002). Such a result indicated that the RAC might contribute to the balance of the intestinal microbiota at phylum level. Other studies

conducted in the chicken showed that most of the components of RAC, specially the resin acids, inhibited the growth of pathogenic bacteria (Kettunen *et al.*, 2017; Vienola *et al.*, 2017). We found the RAC used in this study stabilized gut microbiota and reduced the risk of pathogen colonization. *Barnesiella*, *Sporobacter*, *Intestinimonas* and *Campylobacter* in the RAC group decreased, while *Romboutsia* and *Clostridium sensu stricto* significantly increased. *Barnesiella*, *Sporobacter*, *Intestinimonas* and *Campylobacter* are well known initiators of inflammatory diseases and gastrointestinal disorders in humans and animals (Weijtens *et al.*, 1999; Zhang *et al.*, 2017; *Romboutsia* and *Clostridium sensu stricto* produce short-chain fatty acids (SCFAs) by anaerobic fermentation of dietary components, the main energy source for the colonocytes, and protect from inflammation (Lopetuso *et al.*, 2013). *Clostridium sensu stricto* is reported to promote the intestinal mucus barrier and thus be able to inhibit adherence of pathogenic microbes (Wlodarska *et al.*, 2015). Overall, this study demonstrated that addition of RAC to the late gestation diet of sows enhanced colostrum production (in two farms out of three), colostrum SAA, and especially colostrum IgG concentration, ensuring availability of more energy and sustained piglet immunity. Supplementation of the gestation diet may change the gut microbiota, alleviate farrowing stress, improve sow physiology and produce more viable piglets. Further studies should be directed at unveiling the exact cellular mechanism of RAC in farrowing sows and the anti-inflammatory and possible immunomodulatory effects via colostrum in weaned piglets.

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Declaration of interest

The authors declare that they have no conflict of interest.

Ethics statement

The experimental protocol was approved by the Animal Experiment Board in Finland (permission ESAVI/333/04.10.03/2011) and by the Dutch authority CCD (The study does not require a special license under the Dutch Animal Procedures Act, decision 26.04.2017).

Software and data repository resources

All the relevant data included in the manuscript and /or in supplementary table.

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532 **Table 1** *Effect of dietary supplementation of RAC on sow farrowing characteristics.*

variable	Herd 1		Herd 2		Herd 3	
	RAC	CON	RAC	CON	RAC	CON
Gestation length, day	115	115	115.6±0.1	115.6±0.1	114.3±0.2	114.3±2
Farrowing duration, min	215.3±15.6	208.2±14.8	195.4±17.3	206.1±19.5	379.1±57.2	352.3±72.2
Litter size	16.5±0.7	15.6±0.8	16.1±0.8	17.3±0.9	17.4±0.9	16.2±1.0
Live born piglets	15.9±0.6	14.6±0.8	15.3±0.8	15.8±0.7	14.6±1.2	14.6±0.8
Still born piglets	0.6±0.1	1.0±0.2	0.7±0.3	1.5±0.4	2.8±1.1	1.6±0.5
Birth interval, min	14.5±1.3	14.4±1.3	13.8±1.1	13.6±1.1	21.7±2.7	21.1±2.9

533 LS mean ± SE

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Table 2 *Effect of dietary supplementation of RAC on sow CY, piglet CI and piglet growth performance.*

variable	Herd 1		Herd 2		Herd 3	
	RAC	CON	RAC	CON	RAC	CON
BW_B, g	1449.8±18.3	1440.8±21.8	1256.7±16.8	1293.5±18.2	1290.3±21.0	1242.2±20.5
Weight at 3-4 weeks, g	6770.2±139.6	7079.7±159.7	6939.1±130.7[a]	6562.8±168.8[b]	7872.1±86.2	7785.5±108.1
ADG, g	250.4±5.7	265.9±6.4	256.6±5.7[a]	235±7.4[b]	223.2±2.8	226.6±3.3
Age at 3-4 weeks, days	21.0	21.0	21.5±0.0	21.8±0.0	29.1±0.0	28.8±0.0
CY, g	4571.0±344.5	4754.3±277.6	4203.5±205.2[a]	3803.96±203.6[b]	4552.3 ±237.3	4371.6 ±129.4
CI, g	318.6±9.1	347.87±9.5	291.97±8.26[a]	258.48±8.2[b]	313.2±8.7	323. 8± 10.6

LS mean ± SE

[ab] Means in the same row with different superscript differ ($P < 0.05$) between the same dietary treatment in same herd.

565 **Table 3** *Effect of dietary supplementation of RAC on colostrum composition,*

566 *colostrum quality, colostrum and blood APPs.*

variable	Herd 1		Herd 2		Herd 3	
	RAC	CON	RAC	CON	RAC	CON
Fat %	4.2±0.3	4.2±0.3	4.1±0.26[a]	5.2±0.0[b]	5.0 ± 0.3	4.5 ± 0.2
Protein %	17.1±0.63	16.5±0.5	16.8±0.3	17.1±0.5	16.9 ± 0.7	15.9 ± 0.5
Lactose %	4.3±0.0	4.4±0.0	4.2±0.0	4.15±0.0	4.2 ± 0.09	4.4 ± 0.08
Dry matter %	27.5±0.7	27.0±0.7	27.06±0.4	28.35±0.7	28.0 ± 0.8	26.6 ± 0.5
IgG, mg/mL	86.3±5.27[a]	70.9±5.4[b]	76.5±3.2[a]	65.5±4.2[b]	108.9 ± 7.3[a]	92.1 ± 6.6[b]
IgA, mg/mL	9.8±0.7	9.7±0.5	11.0±0.6	12.3±0.8	-	-
IgM, mg/mL	4.5±0.3	3.9±0.3	4.9±0.3	4.7±0.4	-	-
Colostrum SAA, mg/L	361.0±61.4	296.41±41.0	423.3±63.7	314.2±38.2	563.7±66.32	479.9±37.2
Colostrum HP, mg/L	1234.5±95.2	1250.5±91.4	1212.4±66.4[a]	1473.0±104.9[b]	1318.7±125.6	1316.3±87.0
Plasma SAA, mg/L	22.4±8.6	18.5±7.7	16.5±2.9	13.4±1.3	-	-
Plasma HP, mg/L	1696.0±112.3	1839.9±124.1	1893.3±81.4	1897.3±108.3	-	-

567 LS mean ± SE

568 [ab] Means in the same row with different superscript differ ($P < 0.05$) between the

569 same dietary treatment in same herd.

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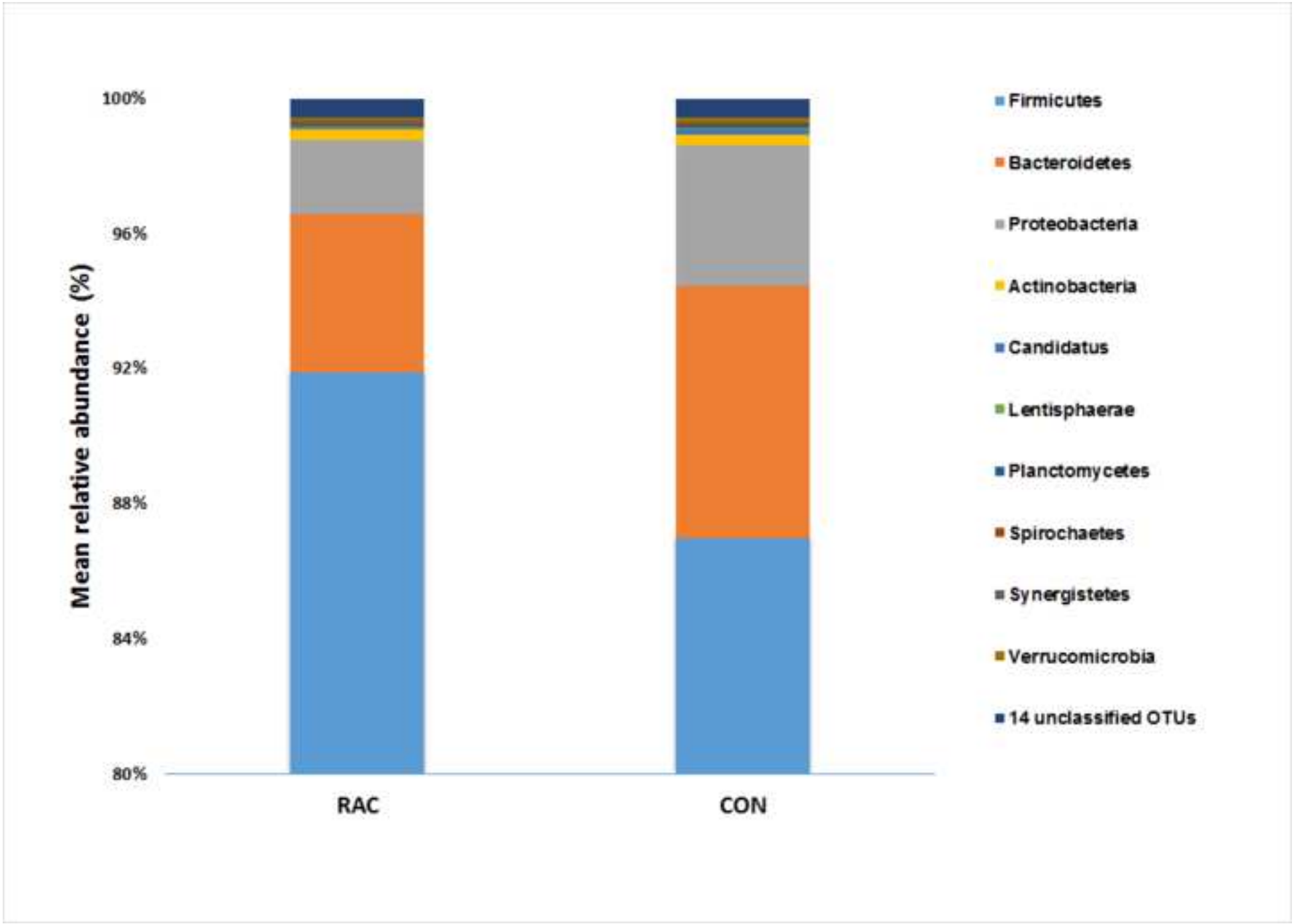
Figure captions

Figure 1 The distribution of bacterial phyla in faecal samples under conditions of dietary treatment during late gestation. Values represent the mean proportions of the phylum.

Figure 1

Click here to download Figure Figure 1.tif



Late gestation diet supplementation of tall oil fatty acid and resin acid increases sow colostrum IgG content, piglet colostrum intake and modulates sow gut microbiota

Shah Hasan, Sani Saha, Sami Junnikkala, Toomas Orro, Olli Peltoniemi and Claudio Oliviero

Supplementary Table S1 Abundant taxa (genus) in the faecal microbiota of sows fed the RAC and control diet.

Taxa.genus	**RAC**	**CON**	***P***
Romboutsia	4.47	3.95	0.045
Clostridium_sensu_stricto	4.13	3.52	0.011
Oscillibacter	2.65	2.64	0.72
Acidaminobacter	2.41	2.59	0.4
Christensenella	2.35	2.38	0.8
Sporobacter	2.22	2.5	0.048
Intestinimonas	1.9	2.2	0.0094
Flavonifractor	1.56	1.67	0.31
Thermotalea	1.46	1.26	0.34
Barnesiella	1.34	1.75	0.016
Ruminococcus	1.32	1.18	0.74
Lachnospiracea_incertae_sedis	1.23	1.28	0.56
Terrisporobacter	1.18	1.01	0.036
Gracilibacter	1.03	0.99	0.5
Anaerovorax	1.01	0.92	0.53
Turicibacter	1.01	0.98	0.66
Alkalibacter	0.98	0.99	0.78
Desulfovibrio	0.97	1.03	0.85
EscherichiaShigella	0.89	1.31	0.2
Clostridium_IV	0.89	1.01	0.4
Clostridium_XlVa	0.86	0.94	0.34
Prevotella	0.75	0.96	0.11
Acetanaerobacterium	0.72	0.71	0.75
Bacteroides	0.7	0.88	0.52
Dethiosulfatibacter	0.7	0.74	0.95
Alkalibaculum	0.69	0.72	0.68
Cellulosibacter	0.63	0.61	0.63
Falsiporphyromonas	0.62	0.56	0.38
Anaerobacterium	0.62	0.72	0.51
Pseudoflavonifractor	0.62	0.64	0.71

Fastidiosipila	0.59	0.64	0.66
Anaerotruncus	0.58	0.65	0.17
Lactobacillus	0.54	0.56	0.95
Blautia	0.52	0.57	0.15
Phascolarctobacterium	0.5	0.69	0.0015
Faecalibacterium	0.48	0.6	0.093
Mogibacterium	0.47	0.44	0.22
Sporacetigenium	0.45	0.44	0.54
Ruminococcus2	0.43	0.48	0.034
Subdoligranulum	0.43	0.54	0.12
Saccharofermentans	0.41	0.42	0.68
Murimonas	0.4	0.41	0.91
Prolixibacter	0.38	0.48	0.18
Anaerocella	0.36	0.51	0.015
Coprococcus	0.36	0.48	0.02
Collinsella	0.36	0.35	0.69
Pyramidobacter	0.36	0.36	0.88
Anaerovibrio	0.34	0.4	0.12
Acetatifactor	0.33	0.34	0.94
Sporobacterium	0.32	0.36	0.27
Parabacteroides	0.32	0.35	0.42
Paraeggerthella	0.32	0.31	0.47
Peptococcus	0.32	0.32	0.5
Cruoricaptor	0.32	0.34	0.8
Hydrogenoanaerobacterium	0.31	0.27	0.23
Anaerofilum	0.28	0.33	0.011
Subdivision5_genera_incertae_sedis	0.28	0.35	0.084
Solobacterium	0.27	0.29	0.66
Defluviitalea	0.27	0.34	0.86
Holdemanella	0.26	0.3	0.17
Oligosphaera	0.26	0.3	0.24
Caminicella	0.26	0.29	0.53
Streptococcus	0.25	0.26	0.8
Alkalitalea	0.24	0.22	0.59
Alloprevotella	0.23	0.26	0.22
Robinsoniella	0.23	0.22	0.52
Lactivibrio	0.23	0.22	0.96
Vampirovibrio	0.22	0.24	0.31
Aquisphaera	0.22	0.23	0.93
Treponema	0.2	0.25	0.055
Hydrogenibacillus	0.19	0.23	0.22
Mitsuokella	0.19	0.26	0.25
Papillibacter	0.19	0.21	0.35

Tannerella	0.18	0.25	0.14
Eubacterium	0.17	0.24	0.18
Faecalicoccus	0.17	0.19	0.65
Anaerosporobacter	0.16	0.21	0.045
Enterococcus	0.16	0.3	0.047
Ornithobacterium	0.16	0.13	0.56
Anaerosinus	0.16	0.16	0.88
Catabacter	0.15	0.18	0.055
Ethanoligenens	0.15	0.2	0.1
Peptostreptococcus	0.15	0.12	0.6
Paraprevotella	0.14	0.23	0.054
Anaerophaga	0.14	0.21	0.1
Cloacibacillus	0.13	0.095	0.19
Bilophila	0.13	0.14	0.61
Dorea	0.12	0.14	0.25
Dendrosporobacter	0.12	0.15	0.4
Roseburia	0.11	0.16	0.11
Helicobacter	0.1	0.13	0.16
Paludibacter	0.1	0.1	0.25
Psychrosinus	0.097	0.11	0.52
Campylobacter	0.094	0.21	0.0078
Eisenbergiella	0.09	0.11	0.23
Labilithrix	0.083	0.13	0.065
Megasphaera	0.074	0.071	0.2
Macellibacteroides	0.063	0.099	0.075
Pseudobacteroides	0.0052	0.051	0.3