Title (English)

Effect of dietary salicylic acid supplementation on performance and blood metabolites of sows and their litters

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Running Title (English)

Salicylic acids for lactating sows and litters

Abstract (English)

The core intention to undertake this experiment for a period of 21 days is to evaluate the effect of salicylic acid (SA) supplemented diet on the performance and blood metabolites of sows and their litters. Sows weighing 208.5± 18.34kg and their neonates were used. From day 114 of gestation to 21st day of lactation(weaning), ten multiparous sows (n=5/ treatment) (Landrace x Yorkshire) were assigned randomly into one of two treatments: CON (basal diet) and TRT [CON+ 0.05% salicylic acid (SA)]. There was no significant difference in the body weight, backfat thickness, backfat loss, and body condition score in SA treated sows compared to sows fed CON diet. However, the body weight of sow was dramatically reduced by average of 16kg from farrowing to weaning time. The dietary inclusion of SA in sow diet slightly improved the survival rate (p=0.065) and showed a higher body weight (p= 0.009) in piglet. However, there was no significant difference on RBC, Fe, Hematocrit, and Hb concentrations between CON and TRT sows' groups, but the total Iron binding capacity (TIBC) was significantly reduced in sows from TRT group compared with CON group from the beginning to weaning. The outcome of this trial shows that dietary addition of SA on sow diet from early lactation could increased the birth weight and TIBC of neonates at the end of the trial.

Keywords (English)

Salicylic acids; reproductive performance ; blood metabolites; piglet performance

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Abstract

The core intention to undertake this experiment for a period of 21 days is to evaluate the effect of salicylic acid (SA) supplemented diet on the performance and blood metabolites of sows and their litters. Sows weighing 208.5± 18.34kg and their neonates were used. From day 114 of gestation to 21st day of lactation(weaning), ten multiparous sows (n=5/ treatment) (Landrace x Yorkshire) were assigned randomly into one of two treatments: CON (basal diet) and TRT [CON+ 0.05% SA]. There was no significant difference in the body weight, backfat thickness, backfat loss, and body condition score in SA treated sows compared to sows fed the CON diet. However, the bodyweight of sow was dramatically reduced by an average of 16kg from farrowing to weaning time. The dietary inclusion of SA in the sow diet slightly improved the survival rate (p=0.065) and showed a higher body weight (p= 0.009) in piglets. However, there was no significant difference in RBC, Fe, Hematocrit, and Hb concentrations between CON and TRT sows' groups, but the total iron-binding capacity (TIBC) was significantly reduced in sows from the TRT group compared with the CON group from the beginning to weaning. The outcome of this trial shows that dietary addition of SA on sows diet from early lactation could increase the birth weight and TIBC of neonates at the end of the trial.

Key words: Salicylic acids, reproductive performance of sows, blood metabolites, piglet performance

Introduction

Minimizing disease prevalence has always been a big issue in pig production since it affects not only the overall health and wellbeing of animals but also causes economic losses for producers. Around the 1940s producers started to use antibiotics as growth promoters (AGP) in livestock feed and have successfully enhanced pig performance. However, in the last few decades, the use of antimicrobials in growth promotion raised safety and public health concerns which led to its ban in western as well as South Korea and thus increased pressure to do so in many other countries. The phase-out of AGP aroused the interest of nutritionists to start exploring potential alternatives in order to alleviate this problem. The nutritional strategies have been found to be the best options to ensure prudent use of antimicrobials without compromising production performance. Several alternatives have been documented so far, such as probiotics [1,2] prebiotics [3] yeast culture [4], essential oils and spices [5], and organic acids [6].

Salicylic Acid (SA) is a lipophilic monohydroxy benzoic acid, a type of phenolic acid, a beta-hydroxy acid (BHA), and an active ingredient of acetylsalicylic acid (aspirin; ASA). It is a colorless crystalline organic acid broadly used in organic synthesis and has a hormonal function in plants. Besides, it is derived from the metabolism of salicin mainly extracted from the bark of willow trees (Salix spp.), from which it gets its name [7]. Reports show that salicylic acid and its salts have been used in the human diet [8], but its bioavailability was reported to be low [9]. Moreover, it was reported that there are bacteria such as *mycobacterial, Yersinia*, and *pseudomonas* species, which are able to synthesize salicylic acid to enhance iron chelation, this, in turn, explain the ability of gut bacteria to be the source of SA although it is not readily available in absence of dietary exposure [10]. Also, Paterson et al [10] observed the effects of aspirin and its pro-drugs and suggested that SA is likely to be a biopharmaceutical with a central, broadly defensive, and plays a better role in animals compared with plants. In humans, it was reported that acetylated salicylic acid (aspirin) undergoes abrupt hydrolysis to generate salicylic acids and the generated phenolic acids lead to anti-inflammatory effects [11] and a report by Peterson at al [8] show that plant-based feedstuffs are vital sources of these phenolic acids which helps in disease resistance.

In a broad sense, functional roles of organic acids (OA) include improving nutrient digestibility, digestion, intestinal health, growth performance as well as preservative property [12]. They reduce the number of coliform bacteria in the gut, reduce scouring in piglets as well as post-weaning diarrhea control [13-15]. It is well documented that the fundamental unit for developing better antimicrobial alternatives refers to a better understanding of defense systems used to resist pathogens and their interactions. On the other hand, it has been reported that acetylsalicylic acid improved average daily gain and tends to improve the feed efficacy in weaning pigs [13]. Concerning the age of piglets, literature data indicated that various OAs act differently in accordance with their mode of action. For example, Formic acid (FA) when added on to a sow's diet showed some improvement on reproductive parameters [16] and Luise et al. [17] explained the beneficial effects of FA on intestinal microflora and carbon metabolism in sows. Contrary, the inclusion of benzoic acid in the corn-based diet of sow has shown no effect on backfat thickness and average weaning live weight [18]. However, the effect of salicylic acid supplementation in the sow diet has not been initiated so far. Thus, in this research, we intend to assess the impact of dietary supplementation of salicylic acid on performance and blood metabolites in lactating sows and suckling piglets.

2. MATERIALS AND METHODS

The present experiments were carried out at Gong-ju Swine Research unit and the husbandry practices were performed strictly in accordance with the guidelines of animal welfare, and the experimental protocol (# DK-2-1923#) was approved by IACUC of Dankook University (Cheonan, South Korea).

2.1. Experimental design, animals, housing, and diets

Ten multiparous sows (Landrace x Yorkshire) weighing 208.5± 18.34kg and their offspring were used in this experiment. On day 114 of gestation, sows split randomly into one of two treatments: CON (basal diet) and TRT [CON+ 0.05% salicylic acid (SA)]. The pig's arrangement was centered on body weight, parity, and expected farrowing date and thus five replications made of sow and its neonates were arranged from each treatment. Each sow was housed separately in farrowing crates (2.1 × 1.8 m) made of plastic floors combined with slats of iron (Fe). Pigs were kept in the house allowing the internal environment to be controlled easily and supplemental heat was provided for the piglet using heat-generating lamps. The newborns (9-11 piglets) were cross-fostered within 12 hours after farrowing. Piglets were weaned until 21days of age.

The sow's experimental diets were formulated based on maize and soybean meal (Table 1) with respect to the nutrient requirements recommended by the National Research Council (2012) [19]. From the onset of the experiment at d 114 of gestation to farrowing, sows were fed on gestational diets (2.5 kg/ d). There was no feed offered on the due date of farrowing, and the next day after farrowing day to weaning, sows were fed on experimental lactation diets. The daily feed allowance was given twice a day in meal form and increased gradually from the first day of lactation until sows had unlimited access to feed by week 2. After farrowing all neonates were taken off from mother and dried with an appropriate towel. Right after birth, neonates were weighed one by one and mummified bodies were removed. The number of alive, mummies, and dead neonates from each replicate was recorded to find out survival rates. Neonates received humane care considering routine management practices including teeth clipping, tail docking, ear notching, and males were castrated in the first week after birth and there was free access to drinking water throughout the whole experimental period.

2.2. Sampling and measurement

Sow's body weight (BW) was checked before (d 114), after farrowing, and at weaning (d 21) to determine the BW loss. The back-fat thickness (BFT) of the sows (6 cm off the midline at the 10th rib) was measured using the methods of Sampath et al. [20].

Neonates were weighed individually at the initial as well as at the weaning (21 days) stage. The average daily gain (ADG) was calculated as the difference of initial and final BW (Kg)over the length of lactation (day) ×1000. To estimate the survival rate, the number of piglets at birth (d1) and at weaning (day 21) was noted. From each sow, 5 ml of blood were collected from the jugular vein at the initial, after farrowing, and last day of the experiment. Three piglets per sow were also (5 ml) bled using an appropriate syringe and blood samples were stored in K_3EDTA tubes (Becton Dickinson Vacutainer ® Systems). With immediate effect, samples were moved in the icebox to the laboratory where they were directly centrifuged to produce serum and the latter was used to measure blood parameters. The erythrocyte counts and haematocrit were analysed using ADVIA 2120 RBC reagent. The total iron-binding capacity (TIBC) was analysed using Roche cobas® 6000 analyzer (Switzerland) whereas haemoglobin (Hb) and Fe were obtained using STAT-Site® M Hgb portable.

2.3. **Statistical analysis**

The data were statistically analyzed using the student's t-test in SAS software (SAS version 9.4, 2014, Inst. Inc., Cary, NC, USA). The individual sows were considered as the experimental unit. Variation in the data was referred to as SEM, and probability values < 0.05 denotes statistical significance

3. Results and Discussion

The body weight fluctuations during lactation are very crucial parameters to measure productivity and guarantee efficient feed utilization [21]. The currents trends in swine industries focus on measuring BW and BF of sows in order to manipulate feeding levels that could eventually stabilize the body condition and hence achieve optimum reproductive performance, litter performance, and sow longevity it should be noted that feeding during the last stage of gestation is considered as key to easing the farrowing process [22].

Table 2 shows that there was no significant difference, in body weight changes (BWC), backfat thickness (BFT), backfat loss (BFL), and body condition score (BCS) in SA treated sows compared to sows fed the CON diet. Moreover, the dietary inclusion of SA did not show any change on the total number of piglets born alive, stillborn, and mummified bodies during the 16hours for parturition. The body weight (BW) of sow was dramatically reduced by an average of 16 kg from farrowing to weaning time. Though this is a normal mechanism that after farrowing sows reduce their body weight there is a peak of energy requirement which eventually cause the body to mobilize body reserves [23]. The farrowing process may change the gut physiology and lead to limited feed consumption and this reduction of feed intake is followed by an increase in feed consumption [24,25]. Previous studies reported that sows especially the first and second parties are unable to consume enough feed that can meet the nutritional requirement which in turn may affect reproductive performance [26,27]. In this regard, during late gestation, we had only allowed less feed intake to avoid weight gain which should later cause farrowing complications [28]. Even though there was no significant difference in feed intake between TRT and CON but a comparison made introvert that feed intake increased from gestation to farrowing which can be explained by high energy demand for maternal milk production purpose. The current results exhibited that the sow's reproductive parameters such as total number of piglets born alive, stillborn, and mummified bodies showed no difference between CON and TRT1, this reveals that the current experimental diet may have no remarcable effect of reproductive outcomes of sows. Known fact shows that when the litter size is increased, the chances of increase in low-birth weight is high. Low birth weight piglets present a challenge to the swine industry because they have fewer muscle fibers, and fatten at a younger age resulting in lower meat yields than their larger littermates [29] but fortunately there was no negative influence of SA on birth weight in the present trial. Nevertheless, the current trial did not provide enough information to the reason for this outcome.

The effect of SA on litter performance is presented in Table 3. The dietary inclusion of SA in the sow diet slightly improved the survival rate (P=0.065) and piglets born from sow-fed SA supplementation had greater body weight compared to piglets born from sow-fed on the CON diet (P= 0.009). Research elicited a number of factors that may affect the survival rate such as piglets' birth weight, first suckling and colostrum intake, and late gestational sow feeding strategies. For example, the individual birth weight was shown to be a key determinant of the survivability of piglets [30]. Previously, Wientjes et al [31] supported this finding showing that birth weight has positively related to mortality during the first three days after birth in large litters. Colostrum feeding must also be considered since it enhances immunity and minimizes the emergence of diseases during the growing phase of piglets [30]. Thus, the reason for the greater survival rate may be attributed to higher birth weight, adequate and timely colostrum feeding, and good managerial aspects. In general, the gut health status of sows resulted from dietary inclusion of OAs of sows had potential effects on the gut health status of their litters, and the gut microbial population plays a crucial role in anatomical, physiological, and immunological organ development of the host animals[32]. The dietary SA inclusion might have had influence on the sow's microbial stability and subsequently to neonates.

The supplemental effect of dietary SA on the blood profiles of sows is presented in Table 4. There was no significant difference between CON and TRT sows' groups on red blood cell (RBC), Fe, Hematocrit (HCT), and hemoglobin (Hb) concentrations, however, TIBC was significantly reduced in sows (p=0.044) from the TRT group compared with the CON group from the beginning to weaning period. During parturition, sows lose a high amount of minerals through blood and thus neonates were normally born with Fe deficiency, and hence Fe can be compensated with an Fe injection during the first week of parturition. Colostrum and maternal milk consumption are considered to reduce mineral counts as well as Fe contents. It is known that the requirement of Fe grows relative to the demand to supply growing fetus during late pregnancy [33] since during this time the fetus is actively generating HCT and therefore it is clear that the negative Fe balance in sows may lead to anemia in newborn piglets [34]. Although the relationship by which sow's Fe deficiency may lead to stillbirth is still speculative, Zhao et al [35] indicated that the barrier of Fe transfer via placenta may result in an anemic high number of stillborn and anemic piglets. The TIBC is defined as the maximum level of Fe by which transferrin may bind within 100 mL of serum and acts as biological indicators of Fe transportation in pregnant sows [34]. Since this is the first study on the dietary effects of SA in the swine diet, we could not explain well the reason for the increase of TIBC in piglets as well as their mother at the weaning stage.

The supplemental effect of dietary SA on the blood profiles of neonates is presented in Table 5. There was no significant difference between piglets born from sow-fed on the CON diet and piglets born from sow-fed SA supplementation on RBC, Fe,HCT, Hb concentration. However, the TIBC of piglets born from sows fed on SA supplementation has significantly improved (p= 0.023). Fe content has increased with time from the first week until weaning day. It is well known that sows' milk Fe content is limited and cannot meet the piglet requirement for growth and expansion of blood volume, this reason Fe supplementation is imperative to adjust its adequacy in the bloodstream. The increase in Fe contents and its binding capacity may be correlated not only with its supplementation in the first week after but also may be related to the TIBC level in mother-sow since it has also shown continuous increase throughout the whole experiment. Nevertheless, the effect of other organics acids on Fe and TIBC is poorly elicited and thus we are unable to make enough comparison.

Conclusion

The outcome of this trial shows that dietary addition of SA in lactating sows diet significantly increased the birth weight and TIBC of neonates at the end of the trial. However, there was no significant difference observed in the reproductive performance of sows and we could not elucidate the exact cause for this outcome at present, thus our research team has planned to conduct further studies with different levels of SA on sows' diet to improve the productivity.

Authors' contributions

Conceptualization of this study and writing: SM and IHK; Data curation: SM and TP; Formal analysis: SM and TP; Review, editing and Supervision: IHK.

Disclosure

Authors have acknowledged that there is no potential conflict of interest.

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Table 1. Composition of experimental diets (as-fed basis).

Item	Lactating diet
Ingredients, g/kg	
Maize, ground	510.0
Soybean meal (480 g/kg CP)	267.3
Wheat bran	10.0
Rice bran	50.0
Rapeseed meal (430 g/kg CP)	35.0
Tallow	60.5
Molasses	35.0
Dicalcium phosphate	16.4
Limestone	7.6
NaCl	5.0
L-lysine-HCl (780 g/kg)	1.2
Vitamin premix[a]	1.0
Trace mineral premix[b]	1.0
Salicylic acid (%)	0.05
Nutrient content, g/kg	
Dry matter	888.7
Metabolizable energy, MJ/kg	14.47
Crude protein	183.4
Crude fat	91.6
Lysine	10.8
Calcium	10.6
Total phosphorus	7.3

[a] Provided per kilogram of complete diet: vitamin A, 12,100 IU; vitamin D3, 2000 IU; vitamin E, 48 IU; vitamin K3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; D-pantothenic, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166mg; vitamin B6, 2 mg; and vitamin B12, 28 µg. [b] Provided per kilogram of complete diet:Cu(as$CuSO_4\cdot 5H_2O$), 15 mg; Zn (as $ZnSO_4$), 50 mg; Mn (as MnO_2), 54 mg; I (as KI), 0.99 mg; and Se (as$Na_2SeO_3\cdot 5H_2O$), 0.25 mg.

Table 2. Effect of Salicylic acid (SA) on sow performance

Item	CON	SA	SEM	p-value
Number of sows	5	5		
Parity	2.4	2.4	0.4	1.00
Lactation length, d	21	21		
Sow weight, kg				
Pre-farrowing	229	228.4	7.7	0.796
Post-farrowing	211.9	210	8.4	0.748
Weaning	195.6	195.1	9.2	0.568
BW change[1]	19.5	19.4	8.4	0.692
BW change [2]	17.1	18.3	2.2	0.626
Backfat thickness, mm				
Pre-farrowing	16.8	17.6	1.1	0.202
Post-farrowing	17.3	18.2	1.5	0.070
Weaning	16.3	17.2	1.5	0.707
BF change[1]	0.5	0.6	1.3	0.406
BF change[2]	1	1	0.3	0.770
Body condition score				
Pre-farrowing	3.1	3.6	0.4	0.558
Post-farrowing	3.5	3.5	0.3	0.180
Weaning	3.1	3.3	0.3	1.000
Days to estrus, d	2.6	2.8	0.3	0.593
Litter size				
Total born, head	11.5	11.2	0.7	0.817
Total alive, head	10.5	10.2	1.7	0.851
Stillbirth, head	0.6	0.8	0.4	0.457
Mummification, head	0.3	0.4	0.3	0.644
Survival rate, %	92.3	92.3	4.4	0.788

[1]BW or BF change is the calculated difference between pre- and post-farrowing BW or BF. [2] BW or BF change is the calculated difference between post- farrowing and weaning.

Table 3. Effect of Salicylic acid supplementation in sows on litter performance.

Item	CON	TRT1	SEM	*P*-Value
Pigs/litter				
d1 (start, foster)	10.5	10.2		
d21	9.8	10	1.70	0.820
Survival rate, %	92.4	98.3	2.74	0.065
Litter weight, kg				
d1 (start, foster)	1.20	1.44	0.06	0.009
d21	6.39	6.56	0.10	0.176
Pig weight, kg				
Overall	247	244	5.27	0.579

Abbreviation: CON: corn soy bean meal based basal diet TRT1, Basal diet supplement with 0.05% SA; SUR: Survival rates; ($P < 0.05$) as measure of significant value

Table 4. Effect of dietary supplementation of 'Salicylic acid' additive on blood profile in lactating sows

Items	CON	TRT1	SEM	P-Value
Initial				
Red blood cell, 106/μℓ	6.8	6.7	0.30	0.372
Fe, ug/dL	152.2	143.8	6.00	0.298
Hb, mg/dL	12.9	12.4	0.48	0.282
Hematocrit, %	48.1	47.9	1.42	0.170
TIBC, ug/dL	566.2	550.8	9.22	0.068
After farrowing				
Red blood cell, 106/μℓ	6.6	5.9	0.45	0.186
Fe, ug/dL	150.8	164.8	21.00	0.525
Hb, mg/dL	12.8	12	0.57	0.191
Hematocrit, %	40.3	42.6	6.06	0.709
TIBC, ug/dL	521	474.2	62.29	0.078
Finish				
Red blood cell, 106/μℓ	6.8	5.9	0.80	0.538
Fe, ug/dL	114.5	141.8	18.00	0.147
Hb, mg/dL	13.3	12.2	1.40	0.675
Hematocrit, %	50.5	43.9	5.30	0.502
TIBC, ug/dL	505	443.8	34.00	0.044

Abbreviation: CON: corn soy bean meal based basal diet TRT1, Basal diet supplement with 0.05% SA; Fe, iron; Hb, hemoglobin; TIBC; total iron binding capacity.

Table 5. Effect of dietary supplementation of 'Salicylic acid' additive on blood profile in suckling piglets1

Items	CON	TRT1	SEM[2]	P Value
Initial				
Red blood cell, 106/$\mu\ell$	4.5	5.1	0.39	0.221
Fe, ug/dL	56.9	68.6	15.54	0.275
Hb, mg/dL	8.1	9.2	0.67	0.119
Hematocrit, %	35.8	38.6	2.84	0.351
TIBC, ug/dL	571.1	589.4	72.51	0.805
Finish				
Red blood cell, 106/$\mu\ell$	6.3	6.1	0.60	0.778
Fe, ug/dL	99.9	109.6	10.73	0.293
Hb, mg/dL	11.3	10.4	1.25	0.462
Hematocrit, %	40.7	37.5	3.55	0.394
TIBC, ug/dL	655.6	579.1	30.07	0.023

Abbreviation: CON: corn soybean meal based basal diet TRT1, Basal diet supplement with 0.05% SA Fe, iron; Hb, hemoglobin; TIBC; total iron binding capacity.