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Dietary ractopamine supplementation of pregnant sows: what are the impacts on the neonate?

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*The use of additives such as ractopamine (**Rac**) in pregnant sows during early-mid pregnancy is an alternative to increase foetal and progeny growth and development. However, Rac supplementation in finishing pigs can lead to behavioural and physiological changes similar to the typical stress responses. The objective of this study was to evaluate the effects of dietary supplementation with Rac in pregnant sows from day 25 to 50 of gestation (pre-hyperplastic stage) on piglet's vitality, blood parameters, number, diameter and perimeter of muscle fibres in semitendinosus muscle and developmental characteristics of piglets at birth to weaning. Forty-one hybrid sows were divided into three dietary treatments: (1) control diet without Rac (control), (2) addition of 10 mg/kg of Rac (Rac10) and (3) addition of 20 mg/kg of Rac (Rac20). Higher numbers of low-vitality piglets (P<0.05) were observed in Rac-fed sows, regardless of dose, compared with the control group. Very low-density lipoprotein levels were lower in the Rac10 group when compared with the Rac20 group at day 21. Haematocrit was greater, and the mean corpuscular haemoglobin concentration was lower in piglets from Rac-fed sows. No significant statistical differences were detected regarding piglets body weight, average daily gain, blood gasometry, complete blood count and muscle fibre measurements in semitendinosus muscle. The use of Rac in pregnant sows reduced the vitality parameters of piglets but did not improve the performance from birth until weaning and did not negatively influence the haematological parameter and lipid metabolism.*

Implications

Neonatal piglets' vitality is described as the capacity to develop and survival prognosis. Piglets' vitality is directly related to intrapartum asphyxia and can also be influenced by several factors including birth weight, prenatal stress, foetal maturation, glucose, fat and protein catabolism. A higher vitality piglet has been positively correlated with piglets' growth and survival at weaning, representing an economic impact for the producer. The present sow feeding programs with ractopamine during early-mid pregnancy have reduced the vitality parameters of piglets, but this had no consequences on growth performance during early life of piglets, from birth until weaning.

Introduction

The genetic selection for hyperprolific sows has led to an increased litter birth weight variation, as well as to an overall

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decrease in birth weight (Foxcroft, 2007). The greater number of fetuses increases the competition for nutrients and oxygen, resulting in lighter fetuses at birth (Alvarenga *et al.*, 2013). Furthermore, low birth weight is the most common cause of mortality in the pre-weaning period and negatively influences the development and feed efficiency of the affected offspring (Alvarenga *et al.*, 2013).

Early-mid pregnancy (pre-hyperplastic stage) is a critical period for foetal growth and development. Dwyer *et al.* (1993) showed that the manipulation of maternal nutrition in the pre-hyperplastic stage from 25 to 50 days of pregnancy increases foetal growth and development, leading to improved postnatal growth and muscle gain. The supplementation with β2-adrenergic agonists is an alternative to increase foetal and progeny growth and development. Hoshi *et al.* (2005b) reported greater progeny growth rates and positive correlation between the number of muscle fibres and slaughter weight, carcass weight and *Longissimus dorsi* depth (*r* = 0.80, *r* = 0.86 and *r* = 0.67, respectively), in piglets from sows fed with 20 mg/kg of ractopamine (Rac)

from day 25 to 50 of pregnancy. Similar trials by Gatford *et al.* (2009) and Garbossa *et al.* (2015) evaluated the effects of feeding with 20 mg/kg of Rac to sows in the same stage of gestation and reported an increase in foetal weight and birth weight, respectively. In general, studies with pregnant sows showed the effects of Rac at a dose of 20 mg/kg; however, there is no information on the use of lower doses, although the effects at low doses (5 to 10 mg/kg) are known in finishing pigs (Ritter *et al.*, 2017).

Rac is a β-adrenergic agonist and its structure is similar to epinephrine and norepinephrine (Moody *et al.*, 2000). This compound binds to β-adrenergic receptors stimulating lipolysis and redirect nutrients towards muscle growth (Moody *et al.*, 2000; Cantarelli *et al.*, 2009; Araújo *et al.*, 2014; Ritter *et al.*, 2017). However, feeding pigs with Rac also increased catecholamine levels and the capacity to produce and release epinephrine and norepinephrine, leading to behavioural and physiological changes, including blood parameters and responses typical stress (Marchant-Forde *et al.*, 2003; Ritter *et al.*, 2017).

In mammals, various physiological conditions, including pregnancy, parturition, foetal systems and organs maturation, can be adapted as responses to stress-related hormones (Farrand *et al.*, 2006; Alonso-Spilsbury *et al.*, 2007). Increased stress hormones during mid and late gestation in pigs cause foetal alterations of the hypothalamic–pituitary–adrenal axis and central neurotransmitter systems leading to multiple metabolic systems changes as the use of glucose, fat and protein catabolism, changes in heart rate and blood pressure (Kanitz *et al.*, 2006). On the other hand, piglets' vitality is described as the capacity to develop and short-term survival prognosis and is an indicator of proper maturation and adaptation of the transition from intrauterine to the extrauterine environment during the birth process. Piglets' vitality may be influenced by various factors including birth weight, hormonal concentration, intrauterine environment of sow, prenatal stress, perinatal asphyxia and the environment (Alonso-Spilsbury *et al.*, 2007; Trujillo-Ortega *et al.*, 2011).

Neonatal vitality can be assessed using the Apgar scoring system, which is based on non-invasive physiological traits assessment of the newborn within 1 min after birth (Mota-Rojas *et al.*, 2006). Furthermore, piglets' blood gas measurement at birth provides crucial information on oxygen delivery, what is extremely important for subsequent survival (Trujillo-Ortega *et al.*, 2011). However, there is no scientific evidence of Rac effects in vitality or blood parameters in piglets from sows fed with β2-adrenergic agonists during pregnancy.

Our main hypothesis was that newborn piglets from Rac-fed sows show changes in vitality score and changes in blood parameters from birth until weaning. The aim of this study was to evaluate the effects of dietary supplementation with Rac in pregnant sows during 25 to 50 days of pregnancy on piglet vitality, blood parameters, muscle fibres and developmental characteristics from birth to weaning.

Material and methods

The experimental protocol was approved by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal of Science University of São Paulo (CEUA/FMVZ, protocol number 4632010617). The experiment was conducted from July/2017 to February/2018, at the Swine Research Center of University of São Paulo.

Animals
Forty-one hybrid sows (DB-90, Patos de Minas, Brazil) from first to seventh parity were used in the trial. During pregnancy, the sows were housed individually until 107 days of gestation and then transferred to farrowing crates. After farrowing, the piglets were identified according to their mothers' treatments and maintained with the sows until weaning, at 21 days of age.

Experimental design and feeding
The oestrus was checked twice daily (8:00 am and 4:00 pm), and the sows were inseminated with refrigerated semen with 24-h interval until the end of oestrus. The day of ovulation was considered as day 0 of pregnancy. The ovulation was checked every 8 h by ultrasound scanning, from the first oestrus behaviour, and the ovulation moment was considered when no follicles were found or when the number of follicles was lower than that in the previous scan (Viana *et al.*, 1999). The semen used for insemination was obtained from hybrid boars with proved fertility and same genetic line (DB LM6200, Patos de Minas, Brazil).

At day 23, pregnancy was confirmed by ultrasound scanning 100® (Pie Medical, Philipsweg, Belgium) equipped with a 3.5-mHz convex array probe. After this, sows were allocated within three dietary treatments: (1) control diet without ractopamine (control; $n = 13$ sows), (2) control diet with 10 mg/kg of ractopamine (Ourofino Saúde Animal, Cravinhos, Brazil) (Rac10; $n = 14$ sows) and (3) control diet with 20 mg/kg of ractopamine (Rac20; $n = 14$ sows). The ractopamine from Ourofino Saúde Animal was approved by the Brazilian Department of Agriculture (MAPA) under the number SP-00768 30023. Sows were blocked according to their parity and start date of the Rac supplementation period. The treatments were administered top-dressed on the sows fed from day 25 to 50 of gestation, as recommended by Hoshi *et al.* (2005a and 2005b).

During gestation, the routine management was maintained, and sows were fed a gestation diet, formulated according to Rostagno *et al.* (2017) (Table 1). From day 1 to 5 of gestation, sows were fed 1.8 kg/day; from day 5 to 50, 2.5 kg/day; from day 50 to 90, 2.3 to 2.8 kg/day according to the corporal score; and from day 90 to the day before farrowing, 2.8 kg/day. From farrowing to weaning, sows were given *ad libitum* access to feed with a lactation diet, formulated according to Rostagno *et al.* (2017) (Table 1).

Table 1 *Dietary composition, as formulated, of gestation and lactation diets to sows used during the experiment*

Ingredient (%)	Gestation	Lactation
Corn	65.10	49.56
Soybean meal	16.83	36.12
Wheat bran	15.00	-
Energy farm reproduction concentrate[1]	-	10.93
Smartsui reproduction nucleus 3%[2]	3.00	3.00
L – Lys 78%	0.02	0.09
L – Thr 98%	0.08	0.03
DL – Met 99%	-	0.02
Calcitic limestone	-	0.26
Calculated values		
ME (Kcal/kg)	3150	3400
CP (%)	15.00	21.30
Lys (%)	0.60	1.10
Met (%)	0.21	0.30
Thr (%)	0.54	0.70
Calcium (%)	0.81	0.78
Phosphorus (%)	0.43	0.44
Crude fibre (%)	3.28	3.24
Ether extract (%)	3.44	4.64
Ash (%)	5.30	5.60

Lys=lysine; Thr=threonine; Met=methionine; ME=metabolizable energy;.
[1] Swine concentrate (De Heus Animal Nutrition, Rio Claro, Brasil), composition: calcium 630.00 mg/kg; ether extract 175.50 g/kg; crude fibre 57.20 g/kg; phosphorus 3420.00 mg/kg; Lys 3060.00 mg/kg, crude protein 82.00 g/kg; ash 33.00 g/kg; Met 1260.00 mg/kg; humidity 120.00 g/kg.
[2] Swine Nucleus (Invivo Nutrição e Saúde Animal Ltd, Descalvado, Brasil), composition: calcium 243.3 g/kg; phosphorus 37.52 g/kg; sodium 67.00 g/kg; iron 3333.3 mg/kg; copper 333.3 mg/kg; zinc 333.33 mg/kg; manganese 1333.33 mg/kg; iodine 23.33 mg/kg; selenium 10.00 mg/kg; vitamin A 33333.33 UI/kg; folic acid 50.00 mg/kg; biotin 8.33 mg/kg; choline 11.66 g/kg; niacin 833.33 mg/kg; pantothenic acid 466.66 mg/kg; thiamine 33.33 mg/kg; riboflavin 143.33 mg/kg; pyridoxine 50.00 mg/kg; cobalamine 667.00 mcg/kg; phytase 16600.00 Ftu/kg; xylanase 2320.00 u/kg; β glucanase 3320.00 u/kg.

Vitality score

The piglets were not assisted at farrowing and during the vitality evaluation, in order to avoid interference with vitality criteria. Neonates were evaluated for vitality by two persons previously trained using the Apgar score described by Randall (1971), adapted by Zaleski and Hacker (1993) and modified by Mota-Rojas *et al.* (2005).

The following variables were quantified: time from birth to breathing: >1 min, between 16 s and 1 min and <15 s; heart rate: bradycardia (<120 bpm), normal (between 121 and 160 bpm) and tachycardia (>161 bpm); meconium stain: severe, mild or absent (Mota-Rojas *et al.*, 2006); colour of the skin on the snout: cyanotic, pale or pink; and attempts to stand on all four legs: >5 min, between 1 and 5 min and <1 min. The score for each variable was from 0 (the least favorable) to 2 (the most favorable) and the sum of them in an Apgar score ranging from 1 to 10 for each animal. Within each litter, all piglets were classified into three groups of Apgar scores: low vitality (scores ≤5); medium vitality (scores between 6 and 7); and high vitality (scores ≥8) (Trujillo-Ortega *et al.*, 2011). After all vitality measurements, each piglet was individually weighed and tagged according to their mother's treatment. Twenty-four hours after birth, litter size was standardised by cross-fostering 12 piglets according to their mother's treatment.

Blood analysis

At birth, blood samples from six piglets of each litter were collected from the jugular vein in a heparinised syringe, according to their birth order (first, second, fifth, sixth, ninth and tenth). The samples were immediately analysed for partial pressure of carbon dioxide (**pCO$_2$**) and oxygen (**pO$_2$**), oxygen saturation (**sO$_2$**), total carbon dioxide (**TCO$_2$**), bicarbonate (**HCO$_3$**), base excess (**BEecf**), pH, glucose and lactate levels using a portable blood analyser i-Stat® with the EC4+ cartridge (Abbot Point of Care Inc., Princeton, NJ, USA).

On days 1, 7, 14, 21 of age, three piglets from each litter were selected according to the average litter weight to collect blood samples from the jugular vein, using 5-ml tubes with ethylenediaminetetraacetic acid. Samples were analysed for complete blood count: haemoglobin, haematocrit, red blood cells, mean corpuscular volume (**MCV**), mean corpuscular haemoglobin (**MCH**), mean corpuscular haemoglobin concentration (**MCHC**) and leukocyte count with automated haematological equipment (BC-2800Vet; Mindray, Shenzhen, China). Lymphocyte count, neutrophil count, monocyte count, eosinophil count and basophil count were analysed manually in the blood smear stained with May–Grünwald/Giemsa stain. In addition, 5-ml blood samples were collected using VACUETTE® (Greiner Bio-One, Kremsmünster, Austria) tubes without anticoagulant and were kept at room temperature for 1 h to ensure complete coagulation. Serum was collected after centrifuged (10 min, 3500 rpm), and stored at −20°C until biochemical analysis. Serum samples were analysed for the following parameters: total cholesterol, high-density lipoprotein (**HDL**), very low-density lipoprotein (**VLDL**), low-density lipoprotein (**LDL**), triglycerides, blood glucose, blood urea nitrogen (**BUN**), creatinine, total serum protein level using commercial kits (Labtest, Lagoa Santa, Brazil) in an automated spectrophotometer (BS120; Mindray), and the Friedewald et al.'s (1972) method was used to estimate the concentration of VLDL and LDL. Blood tests were conducted at the Clinical Veterinary Laboratory of the Faculty of Animal Science and Food Engineering of University of São Paulo.

Piglets' performance characteristics

On days 1 (birth), 7, 14 and 21 (weaning), all the piglets were individually weighed. These values were used for calculating average daily gain (**ADG**) according to their mother's treatment, in three periods of age: 1 to 7, 7 to 14 and 14 to 21 days.

Histological analysis

At farrowing, one piglet from each litter with an average birth weight of the litter was euthanised to harvest *semitendinosus muscle*. A 1-cm-thick transverse slice of the *Semitendinosus muscle* was collected from each animal and stored for 24 h in

Table 2 *Effects of supplementing ractopamine during 25 to 50 days of pregnancy sows on percent of litter vitality classified according to the Apgar score of newborn piglets*

Vitality[1] (%)	Treatments[2]			SEM	P-value[3]		
	Control ($n = 161$)	Rac10 ($n = 171$)	Rac20 ($n = 206$)		Treatment	C_1	C_2
Low vitality	0.00 (0)	3.19 (6)	1.62(3)	0.43	*	*	Ns
Medium vitality	22.76 (38)	20.23 (40)	15.03 (33)	5.39	Ns	Ns	Ns
High vitality	77.24 (123)	76.90 (125)	83.35 (170)	1.62	Ns	Ns	Ns

Ns=not significant.
[1] Low vitality=score Apgar 1 to 5; Medium vitality=score Apgar 6 to 7; High vitality=score Apgar 8 to 10.
[2] Control=unsupplemented; Rac10=10 mg/kg ractopamine; Rac20=20 mg/kg ractopamine.
[3] Probability values for the effects of the dietary treatment; Contrast 1 (C_1) = Control × Rac10 + Rac20; Contrast 2 (C_2) = Rac10 × Rac20.
*$P < 0.05$.

10% neutral buffered formalin, and then routinely processed for histology.

Four-micrometer sections of the medium portion of the muscle were obtained and stained with haematoxylin–eosin (Prophet *et al.*, 1992). Each section was examined using a Nikon Eclipse Ni-U 80i® microscope (Nikon Instruments Inc., Melville, NY, USA). Four low-power fields (20 × objective) were evaluated for muscle fibre counting (area=0.0836 mm^2 per field). The number of muscle fibres per square millimeter was estimated between the area measured by field and the number of muscle fibres counted. Fibre diameter and perimeter were measured (a total of 240 fibres per piglet) using four low-power fields (40× objective). The muscle analysis was measured in a software image analyser system ImageJ IJ 1.46r (Rasband and Ferreira 2012).

Statistical analysis

Data were analysed using the MIXED procedure of SAS software (SAS, 2011), according to a block design containing treatments as fix effect and parity order and room as random effects. Each sow was considered as one experimental unit. The effect of treatments was evaluated using orthogonal contrasts; contrast 1 (C_1): effect of supplementation (control *v.* ractopamine) and contrast 2 (C_2): effect of Rac level (Rac10 *v.* Rac20). The time effect was evaluated by the Tukey–Krammer's test. Effects were considered significant when $P<0.05$.

Results

Overall reproductive performance

The total number of piglets born, number of piglets born alive, stillborn piglets, and mummified fetuses independently of treatment were 17.94 − 0.64, 15.76 − 0.65, 1.05 − 0.21 and 0.88 − 0.15, respectively. In addition, the number of piglets weaned and the percentage of mortality were 11.10 − 0.28 and 8.49 − 1.22, respectively.

Vitality score

Piglets' vitality for each treatment is presented in Table 2. There was no statistical difference ($P>0.05$) among treatments regarding the percent of high-vitality piglets and medium-vitality piglets. A higher number of low-vitality piglets was observed in Rac-fed sows, regardless of dose, compared with the control group ($P<0.05$). However, no differences were detected between the Rac10 and Rac20 treatments ($P >0.05$).

Furthermore, it was observed that in low-vitality piglets, 66.6% of piglets were low birth weight (<1000 g) and 22.2% were higher birth weight (>1500 g). However, the percentage of light piglets born (<800 g) did not differ ($P>0.05$) between control (7.261 ± 1.595%), Rac10 (7.634 ± 2.475%) and Rac20 (5.395 ± 2.136%) treatments.

Blood analysis

Blood pH was lower in the Rac10 treatment compared with that in the Rac20 ($P<0.05$); however, no differences were found between piglets from the control treatment and Rac-fed sows. No differences ($P>0.05$) were found between the groups regarding pCO_2, pO_2, sO_2, TCO_2, HCO_3, BEecf, glucose and lactate levels (Table 3). The complete blood count (haemoglobin, red blood cells, MCV, MCH, platelets count, leukocyte count, lymphocyte count, neutrophil count, monocyte count and basophil count) was not different ($P >0.05$) among treatments (Supplementary Tables S1 and S2). Eosinophil count in the Rac10 treatment was lower than that in the Rac20 treatment ($P<0.05$).

An interaction between treatment and days ($P<0.05$) for haematocrit and MCHC was observed. Although haematocrit was greater in piglets from Rac20 sows, statistically significant difference ($P<0.05$) was only observed on day 14, when this group was compared with Rac10 (Figure 1). The MCHC was lower ($P<0.05$) in piglets from Rac-fed sows and between Rac20 and Rac10 sows on day 7 (Figure 1). MCHC was lower on day 14 in piglets from Rac20 compared with Rac10.

With respect to serum biochemical indices, BUN, total cholesterol, triglycerides, creatinine, total protein, LDL, HDL and blood glucose did not differ among treatments (Supplementary Table S3). VLDL was lower in the Rac10 group when compared with the Rac20 ($P<0.05$) group on day 21 (Supplementary Table S3).

Time effects of piglets' complete blood count and serum biochemical indices for each treatment are presented in Supplementary Table S4. Higher values of haemoglobin,

Table 3 *Effects of supplementing ractopamine during 25 to 50 days of pregnancy sows on blood gasometry of newborn piglets*

| Item | Treatments[1] | | | SEM | P-value[2] | | |
	Control ($n = 40$)	Rac10 ($n = 45$)	Rac20 ($n = 46$)		Treatment	C_1	C_2
pH	7.30	7.28	7.30	0.01	*	Ns	**
pCO_2	55.64	57.55	56.78	0.64	Ns	Ns	Ns
pO_2	17.88	18.85	18.92	0.51	Ns	Ns	Ns
BEecf	1.31	−0.10	0.80	0.40	Ns	Ns	Ns
HCO_3	27.70	26.24	27.58	0.35	Ns	Ns	Ns
TCO_2	29.36	28.34	29.26	0.35	Ns	Ns	Ns
sO_2	21.00	21.90	21.59	0.78	Ns	Ns	Ns
Lactate	4.88	4.82	4.59	0.15	Ns	Ns	Ns

pCO_2=partial pressure of carbon dioxide; Ns=not significant; pO_2=partial pressure of oxygen; BEecf=base excess; HCO_3=bicarbonate; TCO_2=total carbon dioxide; sO_2=oxygen saturation.
[1] Control=unsupplemented; Rac10=10 mg/kg ractopamine; Rac20=20 mg/kg ractopamine.
[2] Probability values for the effects of the dietary treatment; Contrast 1 (C_1) = Control × Rac10 + Rac20; Contrast 2 (C_2) = Rac10 × Rac20.
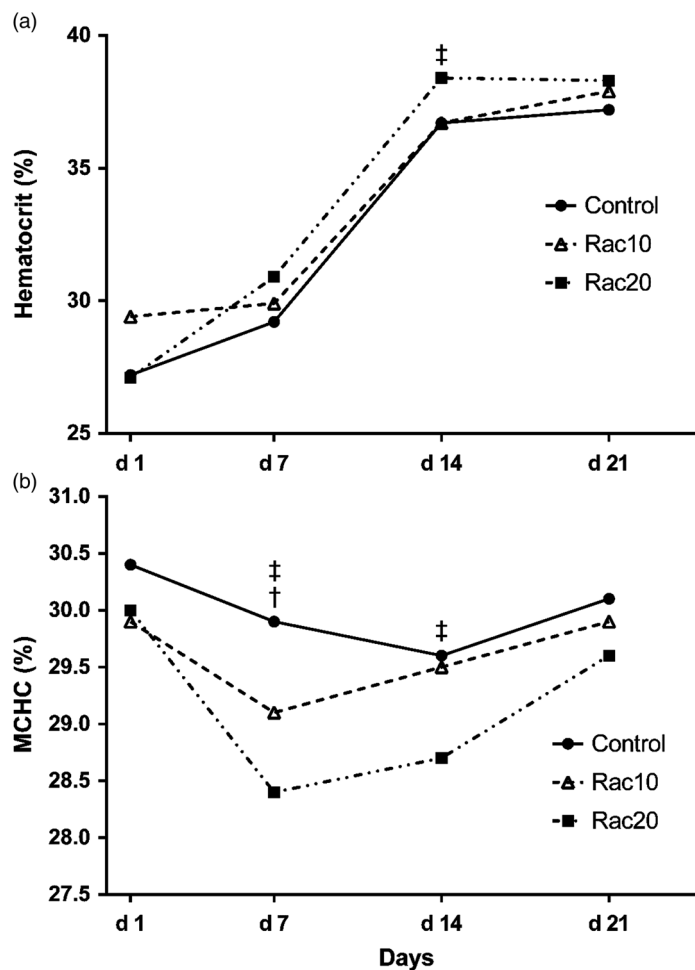*$P < 0.05$, **$P < 0.01$.

Figure 1 Effects of supplementing ractopamine during 25 to 50 days of pregnancy sows on MCHC and haematocrit values of their piglets from birth to weaning. Control=unsupplemented; Rac10=10 mg/kg ractopamine; Rac20=20 mg/kg ractopamine. Probability values for the effects of the dietary treatment; Contrast 1 (C_1)= Control × Rac10 + Rac20; Contrast 2 (C_2)=Rac10 × Rac20; †Effect C_1, $P<0.05$; ‡Effect C_2, $P<0.05$. (a) Haematocrit; interaction treatment and days, $P<0.05$; SEM: Control=0.50; Rac10=0.43; Rac20=0.4; d14, Effect C_2=$P<0.05$; (b) MCHC; interaction treatment and days, $P<0.01$; SEM: Control=0.12; Rac10=0.09; Rac20=0.11; d7, Effect C_1=$P<0.001$, Effect C_2=$P<0.05$; d14, Effect C_2=$P < 0.01$. MCHC=mean corpuscular haemoglobin concentration.

Table 4 *Effects of supplementing ractopamine during 25 to 50 days of pregnancy sows on BW and ADG of their piglets from birth to weaning*

| | Treatments[1] | | | | P-value[2] | | |
Item	Control ($n=140$)	Rac10 ($n=146$)	Rac20 ($n=159$)	SEM	Treatment	C_1	C_2
BW (kg)							
Day 1	1.370	1.427	1.407	0.015	Ns	Ns	Ns
Day 7	2.799	2.820	2.875	0.035	Ns	Ns	Ns
Day 14	4.686	4.524	4.552	0.050	Ns	Ns	Ns
Day 21	5.624	5.683	5.649	0.059	Ns	Ns	Ns
ADG (kg/dia)							
Days 1 to 7	0.207	0.207	0.213	0.004	Ns	Ns	Ns
Days 7 to 14	0.270	0.247	0.242	0.004	Ns	Ns	Ns
Days 14 to 21	0.137	0.167	0.159	0.004	Ns	Ns	Ns

BW=piglets body weight; ADG=average daily gain; Ns=not significant.
[1] Control=unsupplemented; Rac10=10 mg/kg ractopamine; Rac20=20 mg/kg ractopamine.
[2] Probability values for the effects of the dietary treatment; Contrast 1 (C_1) = Control × Rac10 + Rac20; Contrast 2 (C_2) = Rac10 × Rac20.

Table 5 *Effects of supplementing ractopamine during 25 to 50 days of pregnancy sows on muscle fibre number and fibre diameter of the semitendinosus muscle of newborn piglets*

| | Treatments[1] | | | | P-value[2] | | |
Item	Control ($n=13$)	Rac10 ($n=14$)	Rac20 ($n=14$)	SEM	Treatment	C_1	C_2
Muscle fibre number (no/mm^2)	2546.64	2468.3	2377.06	31.97	Ns	Ns	Ns
Muscle fibre diameter (μm)	9.17	8.85	8.94	0.28	Ns	Ns	Ns
Muscle fibre perimeter (μm)	28.81	27.80	28.09	0.89	Ns	Ns	Ns

Ns=not significant.
[1] Control=unsupplemented; Rac10=10 mg/kg ractopamine; Rac20=20 mg/kg ractopamine.
[2] Probability values for the effects of the dietary treatment Contrast 1 (C_1) = Control × Rac10 + Rac20; Contrast 2 (C_2) = Rac10 × Rac20.

red blood cells, MCV, MCH, platelet count, leukocyte count, neutrophil count and lymphocyte count were observed at weaning compared to birth; however, the values of MCV, MCH, platelet count, leukocyte count, neutrophil count and lymphocyte count increased to 7 day and decreased until weaning. Lower values of BUN, total protein, triglycerides and VLDL and higher values of creatinine, total cholesterol, HDL, LDL and blood glucose were observed at weaning compared to birth.

Piglets' performance characteristics
The results for piglets' performance are presented in Table 4. Body weight and ADG were not affected by maternal dietary treatment, and no differences were found from birth to weaning ($P>0.05$).

Histological analysis
The results for histological analysis are presented in Table 5. Muscle fibre number, fibre diameter and fibre perimeter from *Semitendinosus muscle* were not affected by treatments ($P>0.05$).

Discussion

Maternal dietary Rac supplementation has been shown to increase foetal weight, *semitendinosus muscle* fibre number at birth, piglet birth weight and piglet growth rates from pre-weaning to finishing, and consequently slaughter weight (Hoshi *et al.*, 2005b; Gatford *et al.*, 2009; Garbossa *et al.*, 2015).

It was observed that Rac supplementation from the 25th to the 50th day of gestation increased the percentage of low-vitality piglets (2.4%, n=4.5 piglets) compared to the control treatment. Trujillo-Ortega *et al.* (2011) observed that piglets with a low-vitality score (≤5) had the highest birth weights, and suggested that this could be related to difficulties in the passage through the birth canal and a higher incidence of ruptured umbilical cord, resulting in foetal hypoxia. In our experiment, piglets' birth weight and percentage of light piglets born (<800 g) did not differ among treatments. However, we observed a higher percentage of low-vitality piglets with low birth weight (66.6%). Therefore, we suggest that maternal supplementation did not improve the growth in the smaller piglets and would have caused a harmful effect on the smaller piglets.

On the other hand, we did not find the effects of Rac supplementation on the pCO_2, pO_2, BEecf, HCO_3, TCO_2, sO_2, lactate and pH values. Lower pH values in Rac10 piglets compared with Rac20 might be associated with the increase in the percentage of low-vitality piglets, which was numerically higher in the Rac10 treatment compared to Rac20. Neonates piglets with a low-vitality score (≤ 5) often suffer a degree of asphyxia during birth (Mota-Rojas et al., 2011). Nevertheless, we evaluated the blood gases to observe the effect of the treatment and not separately for the category of vitality; therefore, we did not find the signs of intrapartum asphyxia. Similar results were found by Trujillo-Ortega et al. (2007) in the group with mild or no evidence of intrapartum asphyxia in neonates' piglets when studied the relation between functional neurological alterations and electrolytes and metabolic disturbances

In the present work, Rac supplementation during gestation did not influence red blood cells, haemoglobin, MCV, MCH and leukocyte counts. Adrenergic agonists, at physiological levels, in vitro have a stimulatory effect on the normal growth of hematopoietic progenitor cells, but when these levels are increased to stress levels, progenitor cell growth may be inhibited (Fonseca et al., 2005), what suggests that catecholamines have a concentration dependent regulatory effect (Cosentino et al., 2015).

Marchant-Forde et al. (2003) suggested that Rac supplementation in pigs may increase serum adrenaline and noradrenaline concentrations through a regulatory mechanism from the sympathetic nervous system. This could cause changes in blood values and possibly interfere in hematopoiesis in Rac-fed swine. However, although higher values of haematocrit were observed and lower values of MCHC and eosinophils in piglets from sows supplemented with Rac, the possibility that the major effects were not observed on blood parameters was caused by the interruption of maternal Rac supplementation 65 days prior to birth.

Haematological parameters are affected by a variety of factors including age, sex, nutritional and health status; however, increases in piglets' haemoglobin, red blood cells, MCV, MCH, platelet count, leukocyte count, neutrophil count and lymphocyte count until weaning were a result expected of physiological changes, colostrum intake, age and rapid rate of growth of piglets (Thorn, 2010).

Rac supplementation is also known to cause changes in swine metabolism, stimulating lipid catabolism, thus having a direct impact on the serum levels of triacylglycerols, cholesterol and HDL levels in Rac-fed swine (Hoshi et al., 2005a Araújo et al., 2014). The lipolysis stimulation caused by dietary Rac supplementation increases the utilization of fat for oxidation and results in blocking lipogenesis, and decreases fat deposition. Furthermore, the Rac may have decreased in the uptake of chylomicron remnants and circulating lipoproteins, as well as cholesterol uptake by the liver, which may have affected the levels of serum triglycerides and lipoproteins (Araújo et al., 2014). Therefore, it is expected that alterations on the lipid metabolism should be associated with altered concentrations of lipids in tissues and plasma.

We did not find differences in total cholesterol, triglycerides, HDL and LDL blood levels among treatments; however, higher values were detected for VLDL in piglets from Rac-fed sows.

In prenatal piglets, the fat deposition begins at the foetal period; however, after 69 days of gestation this foetal fat growth is accelerated (McPherson et al., 2004). In newborn piglets, heat generation from fat is a potential strategy in thermogenesis beyond thermogenesis of tremor; moreover, piglets' fat deposition is a rapid process that fluctuates from 3% to 16% at 21-day weaning (McPherson et al., 2004; He et al., 2018). Although significant differences were not detected, triglycerides and HDL values were higher. We suggest that the maternal dietary Rac supplementation could be associated with alterations of the piglets' lipid metabolism in the long term; however, the associated mechanisms were not identified in this research. The maternal Rac supplementation was discontinued at 50 days of pregnancy, and this may be an explanation that major effects were not observed on lipid metabolism at birth.

Lower values of triglycerides and VLDL and higher values of total cholesterol, HDL, LDL and blood glucose were observed until weaning. This is the result of the fast fat deposition, growing rate and the gradual supplementation of solid diet throughout the lactation period (McPherson et al., 2004). Factors such as age, breed, sex, physiological condition, amino acid catabolism and dietary protein intake can exert influences in serum urea and protein levels (Padilha et al., 2017). In addition, changes in muscular metabolism can alter serum creatinine and glucose concentration. In the present study, we did not find changes in piglets' BUN, total protein, creatinine and glucose values by maternal Rac supplementation. As expected, higher values of creatinine and lower values of BUN and total protein were observed on day 21 indicating a lower protein catabolism and greater muscle tissue formation (Padilha et al., 2017).

Body weight and ADG values of piglets were also not influenced by maternal Rac supplementation from birth to weaning. Similar results were found by Hoshi et al. (2005a) who supplemented 20 mg of Rac at different periods of gestation. Garbossa et al. (2015) observed an increase of 11% in the birth weight of piglets from Rac-fed sows, receiving 20 mg/kg from the 25th to the 53rd day of gestation. Although no statistical difference was detected, we showed that birth weight was 3.43% higher in pigs from Rac-fed sows.

We did not find differences in muscle fibres for number per square millimeter, diameter or perimeter of the semitendinosus muscle in newborn piglets from Rac-fed sows. Our results corroborate findings by Hoshi et al. (2005b). However, Garbossa et al. (2015) observed a reduction in the number of muscle cells and an increase in muscle fibre diameter in the semitendinosus muscle in pigs from sows supplemented with Rac and Rac+Arginine when compared to the control group.

Ractopamine supplementation during the 25 to 50 days of gestation has been shown to exert positive effects on

prenatal muscle development (Hoshi *et al.*, 2005a; Garford *et al.*, 2009), increasing the size and area of primary muscle fibres, that gives support for the subsequent development of secondary fibres (Wigmore and Stickland, 1983). A positive correlation between the number of muscle fibres and the weight at slaughter, carcass weight and depth of the *Longissimus dorsi* muscle was also described (Hoshi *et al.*, 2005b). However, those differences in weight are evident after 70 days of life (Dwyer *et al.*, 1993), making it impossible to confirm with our experiment design.

The current experiment showed that maternal Rac supplementation may cause changes in piglets' vitality without signs of asphyxia. Moreover, haematological parameter and lipid metabolism were not negatively influenced by maternal Rac supplementation.

In conclusion, the use of ractopamine from 25 to 50 days of pregnancy could increase the percentage of low-vitality piglets, did not increase the number of fibres per square millimeter in *semitendinosus muscle* at birth and did not improve BW and ADG from birth until weaning. However, more trials should be performed to better understand the effect of maternal Rac supplementation on weight, vitality and piglets' metabolism using higher numbers of animals per treatment and economic impacts until finishing pigs.

Declaration of interest

None.

Ethics statement

None.

Software and data repository resources

None of the data were deposited in an official repository.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731119001496

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