Spanish Journal of Agricultural Research (2005) 3(2), 213-219

Effects of the use of ractopamine in pregnant sows on reproductive and blood parameters

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Abstract

The effects of the use of ractopamine (20 ppm per sow and day) in sows were evaluated during three different pregnancy stages (T1: 25 to 50; T2: 50 to 80; T3: 25 to 80 days of gestation, and T4: control-no ractopamine), assessing possible effects on reproduction, litter performance up to weaning, blood parameters, as well as on some of the biochemistry parameters at 20, 40, 60, 80, and 100 days of pregnancy. Forty sows were included in the trial for the evaluation of reproductive measures and litter performance. As to blood counts, a total of 10 sows were used. The use of 20 ppm of ractopamine during the three pregnancy stages had no effects on reproductive measures, litter performance or blood values, when compared to the control. However, total cholesterol, high density lipids and triglyceride values were different ($P < 0.05$) between treated and control sows (71.80 mg dl^{-1} *vs* 65.04 mg dl^{-1}; 42.30 mg dl^{-1} *vs* 37.00 mg dl^{-1}, and 59.40 mg dl^{-1} *vs* 53.40 mg dl^{-1}, respectively), indicating the action of the drug on protein and lipid metabolism. This experiment demonstrated that the use of ractopamine in pregnant sows did not affect the performance of the sows and the progeny until weaning.

Additional key words: beta-adrenergic, blood parameters, gestation, lipid profile, swine.

Resumen

Efectos de la utilización de ractopamina en cerdas gestantes sobre parámetros reproductivos y sanguíneos

Se analizaron los efectos de la utilización de ractopamina (20 ppm) en cerdas durante tres diferentes fases de la gestación (T1: entre los días 25 y 50 de la gestación; T2: entre 50 y 80; T3: desde el día 25 hasta el 80; T4: control, sin ractopamina) sobre los resultados reproductivos, el desarrollo de los lechones hasta el destete, los valores sanguíneos y bioquímicos a los 20, 40, 60, 80 y 100 días de gestación. Se utilizaron 40 cerdas para evaluar los índices reproductivos y el desarrollo de los lechones y 10 para los análisis de sangre. La utilización de ractopamina en los tres períodos de gestación no tuvo efectos significativos sobre los resultados reproductivos, el peso de los lechones al nacimiento y al destete y las variables sanguíneas, comparado con el grupo control. Sin embargo, los niveles de colesterol total, de los lípidos de alta densidad y de los triglicéridos fueron diferentes ($P < 0,05$) en cerdas tratadas y no tratadas. Tales resultados indican la acción de la ractopamina sobre el metabolismo lipídico y proteico (71,80 *vs* 65,04 mg dl^{-1}; 42,30 *vs* 37,00 mg dl^{-1} y 59,40 *vs* 53,40 mg dl^{-1}). Este trabajo demostró que la utilización de ractopamina en cerdas gestantes no afecta a los resultados productivos de las madres y de los lechones hasta el destete.

Palabras clave adicionales: beta adrenérgico, cerdas, gestación, perfil lipídico, valores sanguíneos.

Introduction

The growth of lightweight piglets is usually slower and less efficient than that of heavier littermates. According to Wigmore and Stickland (1983), it is possible that slower growth may result from continuous competitive disadvantage, and not necessarily be caused by reduced numbers of secondary muscle fibers, when compared to heavier littermates.

It is known that the uterine environment may affect antenatal development of secondary muscle fibers mainly due to the influence of nutritional and hormonal factors, able to stimulate increased fiber formation, thus affecting post-natal growth (Dwyer *et al.,* 1993). These factors may affect not only the number of fibers, but birth weight and growth rate of animals as well (Handel and Stickland, 1987; Dwyer and Stickland, 1991).

Low glucose or amino acid uptakes by the fetoplacental unit resulting from nutritional deficiencies will promote increased fetal catabolism (Simmons *et al*., 1978; Battaglia and Meschia, 1978, cited by Gluckman,

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Received: 03-06-04; Accepted: 22-02-05.

1986), and reduce the endocrine stimulus for cell replication. In addition, the development of the placenta is also affected (Pilistine *et al*., 1984, cited by Gluckman, 1986).

Maternal glucose crossing the placenta is the primary nutrient for fetal production. Thus, glucose is considered to be an important fetal growth-regulating factor (Bassett *et al*., 1990). In addition, high blood sugar levels promote the release of fetal IGF (Insulin-like Growth Factor) that has a direct effect on myoblast proliferation and differentiation.

From this, it can be concluded that fetal development could be influenced, and consequently, birth weight and post-natal growth could also be changed (Hegarty and Allen, 1978). There is a positive correlation between antenatal and post-natal development (Campbell and Dunkin, 1982).

During the stage when muscle fibers undergo hyperplasia, the number of membrane beta-adrenergic receptors is increased (Parent *et al*., 1980; Schonberg *et al*., 1980). Thus, the use of a beta-adrenergic agonist (ractopamine) during this stage could optimize fetal muscle fiber replication through adenosine cyclic monophosphate (cAMP) modulation. In turn, development of the piglets could be improved during the post-natal life stages.

Administration of the beta-adrenergic agonist cimaterol to rats has resulted in increased functional activity and size of mammary gland cells (Choi *et al*., 1992), suggesting that the use of ractopamine in pregnant sows could improve milk production, allowing for a better nutrition of the progeny (Kim *et al*., 1994).

Kim *et al*. (1994) treated sows with salbutamol (beta-adrenergic agonist) during three different stages of pregnancy and did not see improvements in birth weights. However, when the authors quantified muscle fibers, the results favored the treated groups, suggesting that the number of muscle fibers is not directly related to birth weight.

The goal of this trial was to evaluate the use of ractopamine in pregnant sows and to assess possible effects on reproductive measures, on the performance of the litter up to weaning and on some blood parameters.

Material and Methods

The trial was conducted at the gestation and farrowing units of a commercial 450-sow farm located in Rolândia, Parana State, Brazil.

Forty hybrid (Large White × Landrace) 3-4-parity pregnant sows were used. During pregnancy, the breeders were housed individually. After farrowing, the piglets were identified to allow follow-up according to their mothers' treatments. Piglets were maintained with the sows up to weaning (at 21 days of age).

The trial was started at artificial insemination of the sows. The semen used for insemination was obtained from hybrid boars with the same genetic background.

At 20 days of pregnancy ten sows were randomly assigned to each of 4 treatment groups. Treatments were administered via the feed as follows: T1: 20 ppm ractopamine from 25 to 50 days of pregnancy (pre-hyperplasic stage); T2: 20 ppm ractopamine from 50 to 80 days of pregnancy (hyperplasic stage); T3: 20 ppm ractopamine from 25 to 80 days of pregnancy (pre-hyperplasic stage + hyperplasic stage); T4: control (no ractopamine).

During gestation, routine management was maintained, and the sows were fed 1.8 kg of feed per day up to 80 days of pregnancy. From day 80 on, the sows were fed on average 2.8 kg of feed per day until farrowing. Pregnancy and pre-lactation feeds for all the four treatment groups were formulated according to the NRC (1998) (Table 1).

During pregnancy, blood samples were collected from two groups of 5 sows at 20, 40, 60, 80, and 100 days. Five sows belonged to the group receiving ractopamine from 25 to 80 days of pregnancy, and the other 5 sows belonged to the control group (without ractopamine). Blood tests were conducted at the Clinical Pathology Laboratory of the Londrina State University and in the Pathology Laboratory of the North of Parana.

From farrowing to weaning, sows were fed lactation feed *ad libitum*, formulated according to the NRC guidelines (1998).

The following measures were used to assess the reproductive performance of the sows and litter performance: total number of piglets born, number of piglets born alive, number of stillborns, litter weight at farrowing, number of weaned piglets and weaning weights. Blood collected from the sows was tested for the following parameters: total lipids (TL), total cholesterol (TC), low density lipids (LDL), high density lipids (HDL), triglycerides (TG), blood glucose (BG), blood urea nitrogen (BUN), creatinine (CR), total serum protein level (TP), hemoglobin (HB), hematocrit (HM), leukocyte count (LC), lymphocyte count (LF),

Table 1. Percentage and calculated composition of the experimental feeds fed during pregnancy (0 to 80 days of pregnancy), pre-lactation (81 days of pregnancy to farrowing) and lactation (from farrowing to weaning)

Ingredients (%)	Pregnancy	Pre-lactation	Lactation
Corn	54.70	64.18	54.98
Wheat bran	30.60	6.00	—
Soybean meal	11.00	23.80	31.00
Vegetable oil	—	2.04	5.02
Limestone	1.35	1.08	0.92
Dicalcium phosphate	1.35	1.90	2.08
Salt	0.50	0.50	0.50
Sugar	—	—	5.00
Premix[1]	0.40	0.40	—
Premix[2]	—	—	0.40
Premix[3]	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated values[4]			
Crude protein (%)	14.07	16.89	18.49
Metab. En. (kcal kg^{-1})	2,865	3,170	3,375
Crude fiber (%)	5.06	3.90	3.59
Ether extract (%)	3.43	5.09	7.54
Calcium (%)	0.95	0.95	0.94
Total phosphorus(%)	0.75	0.70	0.69
Lysine (%)	0.63	0.88	1.03

[1] Vitamin and mineral supplement per kg of product: vit. A, 1,250,000 IU; vit.D3, 250,000 IU; vit. E, 8,750 IU; vit. K3, 150 mg; vit. B1, 125 mg; vit. B2, 1,125 mg; vit. B6, 150 mg; vit. B12, 4,500 mcg; folic acid, 400 mg; calcium pantothenate, 3,250 mg; niacin, 3,750 mg; biotin, 50 mg; choline, 70,000 mg; iron, 12,250 mg; copper, 5,250 mg; manganese, 8,750; zinc, 26,250 mg; iodine, 350 mg; selenium, 75 mg; antioxidants, 1,000 mg. [2] Vitamin and mineral supplement per kg of product: vit. A, 1,000,000 IU; vit.D3, 250,000 IU; vit. E, 8,750 IU; vit. K3, 163 mg; vit. B1, 125 mg; vit. B2, 1,125 mg; vit. B6, 150 mg; vit. B12, 4,500 mcg; folic acid, 400 mg; calcium pantothenate, 3,000 mg; niacin, 3,500 mg; biotin, 45 mg; choline, 70,000 mg; iron, 10,500 mg; copper, 4,500 mg; manganese, 7,500; zinc, 22,500 mg; iodine, 300 mg; selenium, 75 mg; antioxidants, 1,000 mg. [3] Mineral supplement per kg of product: Fe, 60,000 mg; Cu, 120,000 mg; Zn, 60,000 mg. [4] As-fed basis.

neutrophil count (NC), monocyte count (MC), rod count (RC), and eosinophil count (EC).

For reproductive and litter performance measures, the experimental design was completely randomized, with 4 treatments and 10 replicates per treatment.

For blood parameters, the experimental design was completely randomized using a 2×5 factorial arrangement of treatments (2 treatments and 5 blood collection periods), with 5 replicates, each sow representing a treatment repetition.

Variance analysis and Tukey's test were used in the GLM procedure as outlined by SAS (1999) to evaluate the results obtained in the trial. Blood parameters were tested by regression analysis and Student's *t* Test according to the sample collection schedules.

Results

Table 2 shows the results related to the total number of piglets born, piglets born alive and weights at birth and at weaning. No differences were detected among treatments ($P > 0.05$).

Table 3 shows the results obtained for blood counts. Total cholesterol (TC), HDL and triglycerides (TG) were significantly different ($P < 0.05$) among treatments. There were no significant differences seen for the other measures ($P > 0.05$).

Table 4 shows the results of blood cell counts: hemoglobin (HB), hematocrit (HM), leukocyte (LC), lymphocyte (LF), neutrophil (NC), monocyte (MC), rod (RC) and eosinophil (EC) counts. There were no significant differences ($P > 0.05$) among treatments. It can, therefore, be concluded that the drug had no harmful effects on any of the blood parameters evaluated.

When blood parameters were analyzed by sampling period for both treatments, it was noted that total lipids showed no response to regression analysis in ractopamine-treated animals, but in untreated controls (Fig. 1) there was a linear effect ($Y = 160.02 + 0.689X$; $R^2 = 0.70$; $P < 0.01$).

Low density lipids (LDL) were positive for regression analysis in both treatments (Fig. 2), with a cubic effect in ractopamine-treated sows ($Y = 59.6451$

Table 2. Treatment of pregnant sows with ractopamine at different gestational ages: effects on total number of piglets born (TB), piglets born alive (BA), birth weight (BW), and weaning weight (WW)

Treatments	Measures			
	TB	BA	BW (kg)	WW (kg)
25 to 50 days	11.81	11.24	1.67 ± 0.14	6.08 ± 0.51
50 to 80 days	11.00	10.80	1.60 ± 0.29	5.74 ± 1.03
25 to 80 days	13.11	11.13	1.52 ± 0.20	5.42 ± 1.06
Controls	11.32	10.39	1.55 ± 0.22	5.74 ± 0.48
CV (%)	11.61	12.21	13.50	13.37

There was no significant difference between treatments ($P > 0.05$).

Table 3. Serum biochemistry results: total lipids (TL), total cholesterol (TC), low density lipids (LDL), high density lipids (HDL), triglycerides (TG), blood glucose (BG), blood urea nitrogen (BUN), creatinine (CR), total protein (TP). Samples obtained from ractopamine-treated (25 to 80 days of pregnancy) and untreated sows

Treatment	TL (mg dl^{-1})	TC (mg dl^{-1})	LDL (mg dl^{-1})	HDL (mg dl^{-1})	TG (mg dl^{-1})	BG (mg dl^{-1})	BUN (mg dl^{-1})	CR (mg dl^{-1})	TP (g dl^{-1})
Ractopamine	223.05a ± 53.87	71.80a ± 12.11	17.65a ± 7.74	42.30a ± 10.01	59.40a ± 25.03	71.79a ± 11.68	22.86a ± 12.83	2.11a ± 0.41	6.19a ± 0.54
Controls	201.36a ± 32.86	65.04b ± 8.19	17.40a ± 6.91	37.00b ± 7.57	53.40b ± 17.33	67.79a ± 14.19	25.38a ± 17.03	2.17a ± 0.28	6.11a ± 0.61

Averages in the same column with different superscripts are significantly different ($P < 0.05$).

Table 4. Blood counts results: hemoglobin (HB), hematocrit (HM), leukocyte (LC), lymphocyte (LF), neutrophil (NC), monocyte (MC), rod (RC) and eosinophil (EC) counts. Samples obtained from ractopamine-treated (25 to 80 days of pregnancy) and untreated sows

Treatment	HB (g dl^{-1})	HM (%)	LC (number µl^{-1})	LF (%)	NT (%)	MN (%)	BT (%)	ES (%)
Ractopamine	14.04 ± 1.66	37.35 ± 3.54	12,555 ± 3,953	52.75 ± 15.38	43.30 ± 15.17	0.75 ± 1.16	0.10 ± 0.31	3.05 ± 2.39
Controls	13.93 ± 1.88	37.32 ± 3.82	14,264 ± 4,485	56.68 ± 16.22	39.44 ± 16.45	0.52 ± 0.96	0.28 ± 0.68	3.00 ± 2.61

There was no significant difference between treatments ($P > 0.05$).

$- 2.75594X + 0.0529631X^2 - 0.000304656X^3$; $R^2 = 0.97$; $P < 0.01$) and a linear effect in untreated controls ($Y = 27.36 - 0.166X$; $R^2 = 0.85$; $P < 0.01$).

High density lipids (HDL) showed a positive response to regression analysis for both treatments (Fig. 3), with a cubic effect in ractopamine-treated sows ($Y = -22.6946 + 3.96957X - 0.06769X^2 + 0.000346316X^3$; $R^2 = 0.99$; $P < 0.05$) and a linear effect in untreated controls ($Y = 30.28 + 0.112X$; $R^2 = 0.41$; $P < 0.05$).

Triglycerides showed no response to regression analysis in ractopamine-treated animals, but in untreated controls (Fig. 4) there was a linear effect ($Y = 30.48 + 0.382X$; $R^2 = 0.83$; $P < 0.01$).

BUN showed a positive response to regression analysis for both treatments (Fig. 5). For ractopamine-treated sows, the equation is $Y = 50.395 - 1.48241X + 0.0139571X^2$ ($R^2 = 0.80$; $P < 0.01$), and for the untreated group, $Y = 51.6705 - 1.64148X + 0.0164076X^2$ ($R^2 = 0.91$; $P < 0.01$). Although no interaction was observed between the treatments and days, according to the Student's *t* test only the means at 100 days were different ($P < 0.05$) between the control and the test group.

Total protein levels were also positive for regression analysis in both groups (Fig. 6). For ractopamine-treated sows, the equation is $Y = 4.509 + 0.0641089X - 0.000493303X^2$ ($R^2 = 0.90$; $P < 0.01$), and for the untreated controls, $Y = 4.4328 + 0.0650859X -$

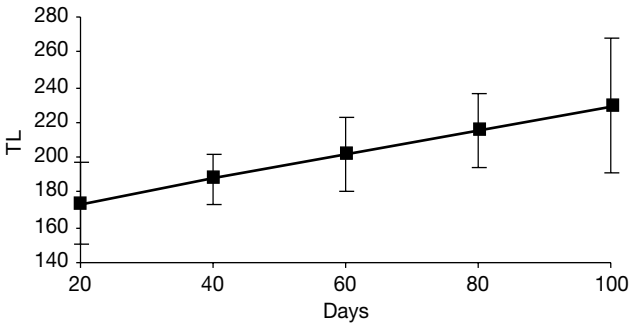

Figure 1. Total serum lipid levels (TL) (mg dl^{-1}) in untreated sows, shown by sampling day.

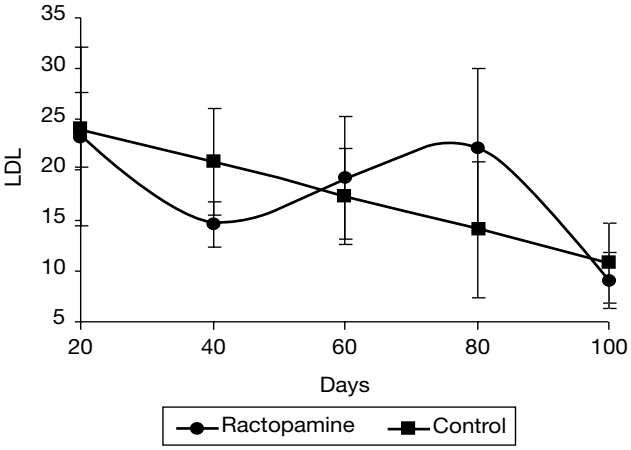

Figure 2. Serum low density lipid levels (LDL) (mg dl^{-1}) by sampling day, for ractopamine-treated (25 to 80 days of pregnancy) and untreated sows.

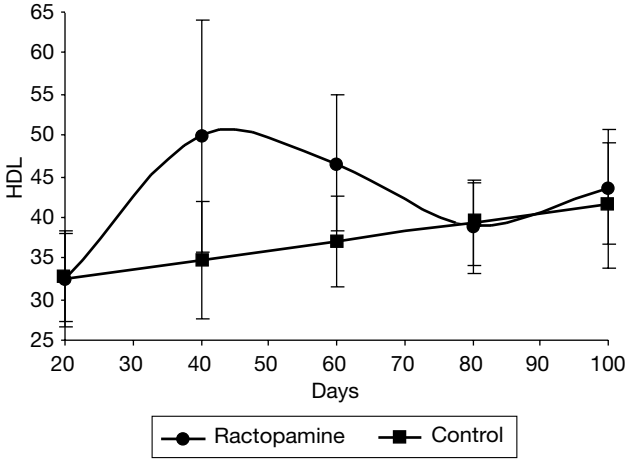

Figure 3. Serum high density lipid levels (HDL) (mg dl^{-1}) by sampling day, for ractopamine-treated (25 to 80 days of pregnancy) and untreated sows.

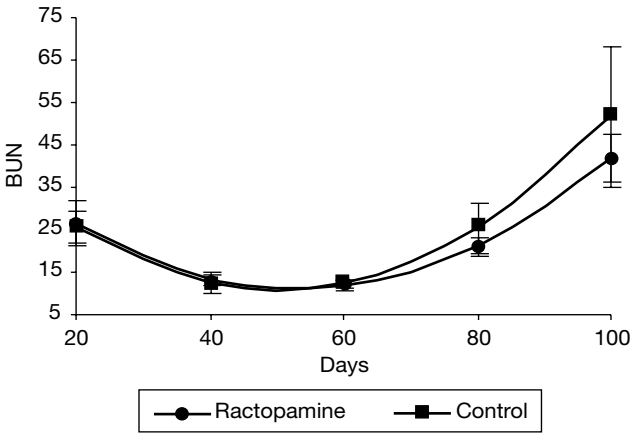

Figure 5. Serum blood urea nitrogen (BUN) levels (mg dl^{-1}) by sampling day, for ractopamine-treated (25 to 80 days of pregnancy) and untreated sows.

$0.000505716X^2$ ($R^2 = 0.90$; $P < 0.01$). By using the Student's *t* test, the means of the treatments for each period evaluated (20, 40, 60, 80 and 100) were similar ($P > 0.05$).

Discussion

Ractopamine did not affect piglet performance up to weaning or the reproductive performance of the treated sows. These results agree with those obtained by Kim *et al*. (1994), who used salbutamol, a beta-adrenergic agonist in pregnant sows and did not see any effect on birth and weaning weights in piglets produced by treated sows compared to the controls.

Although birth weights were not improved by ractopamine, this does not necessarily mean that the number of muscle fibers remained unchanged. According to Handel and Stickland (1988), birth weight is not a good indicator of total number of muscle fibers. In pigs with a high number of muscle fibers, these fibers are of smaller diameter than in animals with fewer fibers (Dwyer *et al.,* 1993). Higher numbers of muscle fibers only became evident when pigs reached 70 days old or weighed 25 kg, compared to animals with a lower number of fibers (Dwyer *et al.,* 1993). Thus, growth rate up to 25 kg of bodyweight is independent of the number of fibers.

If we analyze only birth and weaning weights, the absence of significant differences shows that when sows are treated in periods other than the final third of pregnancy, there is no effect on piglet growth. Based on weaning weights, it can be hypothesized that the treatments had no effect on milk production, in contrast to the hypothesis of Kim *et al*. (1994), and findings by

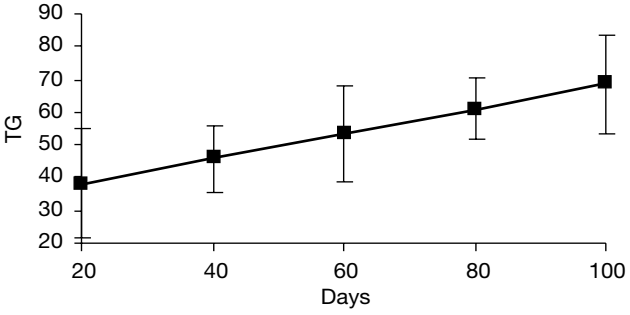

Figure 4. Serum triglyceride levels (TG) (mg dl^{-1}) by sampling day for untreated sows.

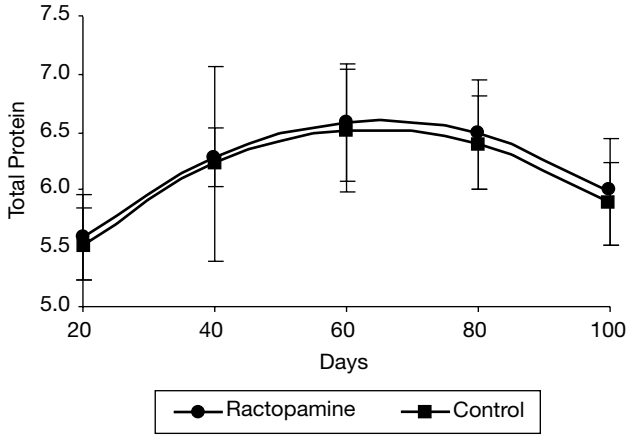

Figure 6. Serum total protein (TP) levels (g dl^{-1}) by sampling day, for ractopamine-treated (25 to 80 days of pregnancy) and untreated sows.

Choi *et al*. (1992), who worked with the beta-adrenergic drug cimaterol and did not see improvements in the activity and diameter of mammary gland cells in rats.

Total cholesterol and triglyceride levels were increased in ractopamine-treated sows, probably because of the lipolytic effect of the drug. This also resulted in increased HDL levels, since these molecules are responsible for the transport of cholesterol from the adipose tissue to the liver (Lehninger *et al*., 1993).

Blood urea nitrogen levels remained unchanged ($P > 0.05$) among treatments.

Creatinine is a product of muscle contraction, and its synthesis is increased in more active animals. The results obtained in this trial did not reveal significant differences ($P > 0.05$) among treatments. This indicates that beta-adrenergic drugs have a negligible action on muscle contraction, even when the drug is from the same family as adrenaline and noradrenaline (Marsden and Meadows, 1970; Bülbring, 1976, cited by Ingram and Dauncey, 1986).

The serum lipid data showed that ractopamine-treated sows presented different levels of compounds related to lipid metabolism. This difference was caused by the action of the drug on the lipid metabolism, through the stimulation of the adenyl cyclase system that promotes cAMP production, increasing the activation of kinases which are responsible for the phosphorylation and modification of the activities of several enzymes, modulating metabolic processes such as muscular contraction, lipolysis and glycogenolysis.

Although BUN levels were similar for both treatments up to 60 days of pregnancy, a difference was noted at 80 days of pregnancy when the treated group was compared with the controls indicating a lower protein catabolism during the stage of secondary fiber hyperplasia. This occurrence could improve muscular development.

The total protein values were very close for both groups, probably because the test method used measures total serum protein levels, regardless of the source and use of the protein.

According to the results obtained in this trial, it can be concluded that the 20 ppm dose of ractopamine had no effect on reproduction, independently of the treatment period. As for the biochemical parameters, some were changed as a result of the action of the drug on lipid and protein metabolism.

In conclusion, the use of ractopamine at 20 ppm during the pregnancy stages studied did not affect the number of piglets born or the birth or weaning weights. The lipid profile of ractopamine-treated pregnant sows was changed, with elevation of total cholesterol, triglyceride and HDL values, when compared to the control group (71.80 mg dl^{-1} *vs* 65.04 mg dl^{-1}; 42.30 mg dl^{-1} *vs* 37.00 mg dl^{-1}, and 59.40 mg dl^{-1} *vs* 53.40 mg dl^{-1}, respectively). Immune cells or blood values were not affected in ractopamine-treated pregnant sows.

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