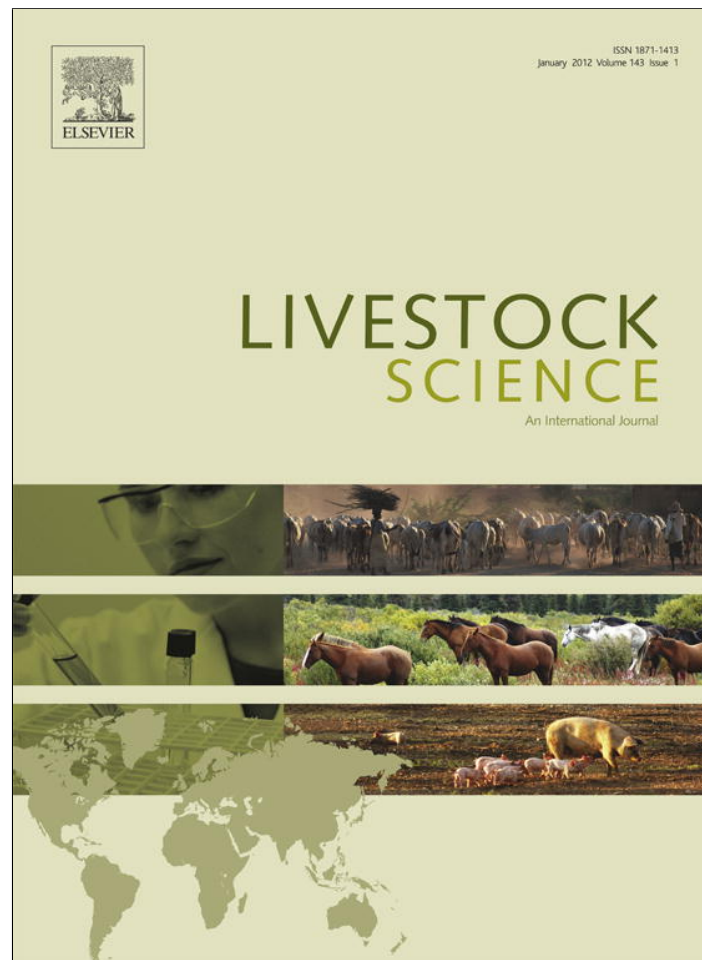


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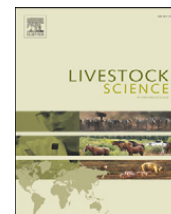
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Meta-analysis of the relationship between ractopamine and dietary lysine levels on carcass characteristics in pigs

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ABSTRACT

A meta-analysis was carried out to evaluate the relationship between ractopamine and dietary lysine levels on carcass characteristics in pigs. The database was composed by 29 articles published in international journals from 1990 to 2007, totalizing 155 treatments and 3786 pigs. Average inclusion of ractopamine was 15.3 ppm (ranging from 0 to 30 ppm) and daily average intake of ractopamine was 24.9 mg. Ractopamine addition increased ($P < 0.05$) hot carcass weight in 4%, loin area in 12% and lean meat content in 4%. Pigs supplemented with ractopamine presented decrease ($P < 0.05$) of 8% in backfat thickness at the tenth rib, 3% in backfat thickness at the last rib and 5% in mean backfat thickness. Each increase in 1 mg of ractopamine intake represented a reduction of 0.3 mm in tenth-rib ($Y = 29.61 - 0.308 \text{ RAC} + 0.025 \text{ RAC}^2$, $R^2 = 0.81$, RAC: ractopamine intake expressed in mg) and 0.5 mm at last-rib backfat thickness ($Y = 30.52 + 0.519 \text{ RAC} - 0.0054 \text{ RAC}^2$, $R^2 = 0.94$). The use of ractopamine affected ($P > 0.05$) neither carcass length and dressing, nor meat marbling and color. Loin area was positively correlated ($r = 0.27$, $P < 0.05$) and mean backfat thickness was negatively correlated ($r = -0.27$, $P < 0.05$) to dietary lysine concentration. Pigs supplemented with ractopamine whose daily intake of lysine per unit of metabolic weight was more than 195 mg presented ($P < 0.05$) loin area 4% higher and backfat thickness 10% lower than other animals. Supplemented pigs that received diets with lysine content superior to their calculated amino acid requirement presented weight gain 14% higher, lean meat content 17% higher, leaf fat 34% lower and loin area 6% higher when compared to other supplemented animals. Ractopamine increases lean meat content and reduces backfat thickness in carcass, however, the interaction between additive and nutritional components must be considered in diet formulation.

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1. Introduction

For many years, one of the industry's main objectives was to increase lean:fat ratio of pork carcasses. Major improvements in pig body composition have been made through genetic selection. Nutritional programs also have helped producers make real progress in reducing fat, which has a direct impact on consumer perception. As a result, there has been an increased interest in nutritional alternatives that could manipulate carcass characteristics.

Some additives are used to minimize fat deposition and maximize muscle deposition in order to answer market demand without productive and economic losses (Mersmann, 1998). One of these alternatives is ractopamine, a phenethanolamine β -adrenergic agonist. Ractopamine is an orally active synthetic analog of catecholamines, hormones that regulate various physiological processes by activating specific adrenergic receptors (Ramos and Silveira, 2002). This exogenous substance alters the partition of nutrients, hence a reduction in fat deposition and an increase in deposition to muscle (Mills, 2002). At cellular level, ractopamine may inhibit lipogenesis and stimulate lipolysis (Mills, 2002). In addition, the agonist activity is related to the modulation of several enzymes

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involved in lipid and glucose metabolism (Ramos and Silveira, 2002).

Despite the common use of ractopamine in swine production, little is known about its implications and about factors that can modulate its effect, especially regarding interaction between the additive and other nutritional components. Considering that ractopamine acts increasing lean meat deposition (Armstrong et al., 2004), there would be an increase in these animal's amino acid requirements. Thus, it is necessary to know the nutritional requirements of pigs fed with this nutritional additive, to produce better carcass traits and meat. In this context, the use of meta-analysis could incorporate different variables from previously published articles and establish systemic responses adjusted to the experimental diversity (Lovatto et al., 2007). Therefore, this study was carried out to investigate, through a meta-analysis, the effects of ractopamine on carcass characteristics in pigs, and the relationship between the additive and dietary lysine requirements.

2. Material and methods

Indexed publications containing results from experiments with pigs fed diets containing ractopamine were selected from different online databases. Main criteria selections were: (a) addition of ractopamine in diets, (b) finishing pigs and (c) results that included carcass evaluation. After the paper selection and subsequent exploratory analysis, information on the theoretical model and other variables were tabulated, to allow descriptive analysis of base studies. These information have been selected from material and methods and results sections, and tabulated in an electronic spreadsheet.

The database used for the meta-analysis was composed of 29 articles published between 1990 and 2007 in peer-reviewed journals (Adeola et al., 1990; Armstrong et al., 2004; Bark et al., 1992; Bellaver et al., 1991; Bridi et al., 2006; Carr et al., 2005a, 2005b; Crome et al., 1996; Dunshea et al., 1993a, 1993b; Gu et al., 1991; Marchant-Forde et al., 2003; Marinho et al., 2007a, 2007b; Mimbs et al., 2005; Mitchell et al., 1990, 1991; Schinckel et al., 2003a; See et al., 2004; Stites et al., 1991; Stoller et al., 2003; Uttaro et al., 1993; Watkins et al., 1990; Weber et al., 2006; Williams et al., 1994; Xiao et al., 1999; Xiong et al., 2006; Yen et al., 1990, 1991). The studies were conducted in United States (20), Brazil (4), Australia (2), Canada (2) and China (1).

A total of 3786 animals (females and castrated males) and 155 treatments composed the database. Average live weight of animals was 71.3 kg at the beginning of the experiments (coefficient of variation: 18%) and 103.3 kg at slaughter (coefficient of variation: 12%). Genetics were addressed in 97% of the articles (from this, 93% of the animals used were commercial genetics and 7% used pure bred). Corn and soybean meal were the main energy and protein sources, used in 55 and 64% of the diets. The mean inclusion of soybean meal in experimental diets was 20.5% (standard deviation: 5.7) and of corn was 73.2% (standard deviation: 6.5). Mean nutrient density of diets was 3.381 kcal of metabolizable energy (2.960 to 3.770 kcal; coefficient of variation: 4%) and 17.6% of crude protein (8.4 to 24%; coefficient of variation: 16%).

The average content of total lysine in diets was 1.04%, with a median of 1.12% and coefficient of variation of 12.4%. In groups fed diets with ractopamine, total lysine levels were classified as

lower or higher depending on the average lysine supply and the estimated lysine requirement. For the first classification (average supply), lysine intake per unit (kg) of metabolic weight ($BW^{0.6}$) was classified in relation to database average (195 mg lysine/kg $BW^{0.6}$). The mean body weight (average between initial and final body weight in the period under analysis) and the mean intake of total lysine (calculated considering daily feed intake and lysine level in diets) were used. For the second classification (estimated requirement), lysine requirements estimated using equations proposed by National Research Council (NRC, 1998) were classified in relation to lysine density in diets. The exponent for the metabolic weight estimation was chosen based on previous publications (Brown and Mount, 1982; Noblet et al., 1999).

The average concentration of ractopamine in diets was 15.3 ppm (ranging from 0 to 30 ppm). Considering feed intake, the calculated daily consumption of ractopamine ranged from 0 to 84 mg, with an average of 24.9 mg. Experimental diets were provided for an average period of 38 days (from 6 to 88).

The methodology for the definition of dependent and independent variables and for coding the data followed the proposals described in the literature (Lovatto et al., 2007; Sauvante et al., 2008). Some encodings were used as qualitative criteria of grouping, as a resource to associate homogeneous groups on certain criteria and include them in the analytical models as a source of variation. In this context, the main encodings were presence of the supplement (control diets or diets with ractopamine). Other encodings were used as moderator variables in the analysis, in order to consider the variability of the studies reviewed (general, *inter* and *intra* effects). For general encoding (article effect) was assigned a unique sequential number for each article in the database. *Inter* encoding was formed by the union of general codification and sequence numbers, in order to assign a specific code for each treatment in the database. *Intra* encoding was similar to *inter*, but was attributed to groups with repeated measures (time and concentrations).

The analyzed variables were experimental features (age, weight and sex of animals; concentration of ractopamine in diets and feeding period), nutritional composition of diets (metabolizable energy, crude protein and total lysine), nutrient intake, carcass characteristics (hot carcass weight, dressing, length, lean meat content, leaf fat, loin area and backfat thickness) and meat (marbling and color). To facilitate the comparison, some variables were corrected for metabolic weight ($BW^{0.6}$). Ractopamine intake expressed in mg (indicated by RAC) was estimated from ractopamine concentration in the commercial product, the inclusion of this product in diets, the feed period and the average feed intake.

Meta-analysis followed three sequential analyses: graphic (to control the database quality and to observe biological data consistency), correlation and variance-covariance. The factors with highest correlation coefficients and encoding for the general, *inter* or *intra* effect were used in models to analyze variance-covariance (Lovatto et al., 2007). All response variables were analyzed for the effect of ractopamine and only the variables influenced by the additive were analyzed for the effect of lysine levels. Regression equations were obtained by variance-covariance analysis using GLM procedure. All analyses were performed using Minitab 15 (2007).

3. Results

Ractopamine did not affect ($P>0.05$) carcass length and dressing, marbling and visual color of meat in pigs (Table 1). For the same variables, there were no differences ($P>0.05$) between groups supplemented or not supplemented with ractopamine when the data were adjusted by covariance for constant live weight at slaughter.

Pigs fed diets containing ractopamine showed hot carcass weight 4% higher ($P<0.05$) when compared to those not supplemented. Loin area was 12% higher ($P<0.05$) in pigs fed diets containing ractopamine. Each increase of 1 mg in ractopamine intake represented an increase of 0.04 cm^2 ($Y = 31.75 + 0.043 \text{ RAC}$, $R^2 = 0.78$, RAC: ractopamine intake expressed in mg) or a variation of 0.71% in loin area ($Y = 0.708 + 0.740 \text{ RAC} - 0.032 \text{ RAC}^2$, $R^2 = 0.91$, Fig. 1).

Mean backfat thickness was inversely correlated to duration of supply period ($r = -0.24$, $P<0.05$), level of ractopamine inclusion in diets ($r = -0.27$, $P<0.05$) and to estimated ractopamine intake ($r = -0.22$, $P<0.05$). Ractopamine decreased ($P<0.05$) backfat thickness at the tenth rib in 8%, backfat thickness at the last rib in 3% and mean backfat thickness in 5%. When data were adjusted for similar live weight at slaughter, the additive decreased ($P<0.05$) tenth-rib backfat thickness in 11%, last-rib backfat thickness in 8% and mean backfat thickness in 2%. Each increase of 1 mg in ractopamine intake represents a reduction of 0.3 mm at the tenth-rib ($Y = 29.61 - 0.308 \text{ RAC} + 0.025 \text{ RAC}^2$, $R^2 = 0.81$, Fig. 2) and 0.5 mm at the last-rib backfat thickness ($Y = 30.52 + 0.519 \text{ RAC} - 0.0054 \text{ RAC}^2$, $R^2 = 0.94$).

Leaf fat was 11% lower ($P<0.05$) in ractopamine supplemented pigs when compared to control animals. Carcass lean meat content was positively correlated to the estimated ractopamine intake ($r = 0.51$, $P>0.05$). Diet supplementation with ractopamine increased ($P<0.05$) content of lean meat in 4%, independent of the adjustments for final weight. Increasing ractopamine intake in a unit resulted in an increase of 0.6% in lean meat content ($Y = 0.632 + 0.660 \text{ RAC} - 0.027 \text{ RAC}^2$, $R^2 = 0.84$).

Table 1

Carcass characteristics, obtained by meta-analysis, in control animals (CTL) or in pigs fed diets containing ractopamine (RAC).

	Mean				Adjusted mean ^a			
	CTL	RAC	P ^b	RSD ^c	CTL	RAC	P	RSD
Hot carcass weight, kg	76.6	79.6	*	9.2	77.1	78.4	*	1.4
Carcass dressing, %	75.5	75.9	ns	6.8	72.2	72.1	ns	4.2
Carcass length, cm	80.6	79.6	ns	2.1	80.0	79.3	ns	2.7
Lean meat, %	51.4	53.3	*	8.7	47.2	49.2	*	3.9
Leaf fat, g	1.55	1.38	*	0.6	1.66	1.59	*	0.5
Loin area, cm ²	35.2	39.6	*	2.4	32.2	36.2	**	2.2
Tenth-rib BT ^d , mm	24.8	22.7	*	2.5	27.1	24.1	**	2.5
Last-rib BT, mm	19.9	19.3	*	1.9	25.4	23.3	*	2.3
Mean BT, mm	35.8	34.1	*	1.9	32.6	31.8	*	2.5
Marbling, %	2.10	2.04	ns	0.5	2.11	2.18	ns	0.1
Visual color, score	3.17	3.18	ns	0.4	2.98	2.99	ns	0.1

^a Variables were adjusted by covariance for a constant final live weight at slaughter.

^b Significance: * $P<0.05$, ** $P<0.01$, ns not significant ($P>0.05$).

^c Residual standard deviation.

^d Backfat thickness.

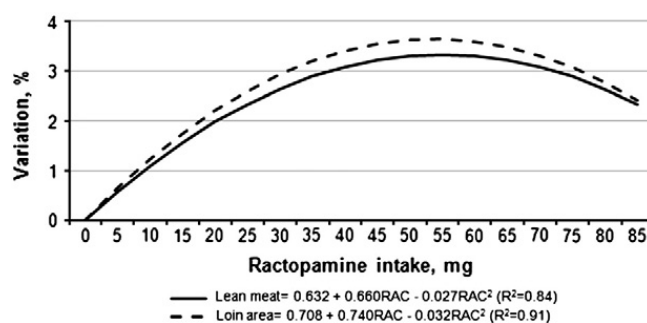


Fig. 1. Variation (expressed in %) in lean meat and loin area according to ractopamine intake (RAC, expressed in mg) in relation to non supplemented animals.

Lean meat content was positively correlated to duration of ractopamine supply period ($r = 0.50$, $P<0.05$). Increasing ractopamine supply duration (expressed by D, in days) in 1 day resulted in an increase of 0.29% in lean meat content, provided that ractopamine concentration in diets (expressed by RACc) were maintained constant ($Y = 66.15 + 0.139 \text{ RACc} + 0.295 \text{ D}$; $R^2 = 0.95$).

In pigs supplemented with ractopamine, loin area was positively correlated ($r = 0.27$, $P<0.05$) and mean backfat thickness was negatively correlated ($r = -0.27$, $P<0.05$) to dietary lysine concentration. Supplemented pigs whose daily intake of lysine was more than $195 \text{ mg/kg BW}^{0.6}$ presented average daily weight gain, loin area and lean meat content higher ($P<0.05$) than other animals (Table 2). Supplemented pigs that received diets with lysine content superior to their amino acid requirement presented weight gain 14% higher, lean meat content 17% higher, leaf fat 34% lower and loin area 6% higher when compared to other animals.

Backfat thickness measurements also varied depending on the supply of lysine in pigs supplemented with ractopamine. Animals with daily lysine intake above $195 \text{ mg/kg BW}^{0.6}$ presented a decrease ($P<0.05$) of 15% in backfat thickness measured at the tenth rib, 14% at the last rib and 10% of mean backfat thickness. Similarly, animals that received diets with more than their lysine requirements presented a reduction ($P<0.05$) of 17% in backfat thickness measured at the tenth rib, 27% at the last rib and 14% of mean backfat thickness. Each total lysine unit (%) in ractopamine supplemented diets represented an increase of 2.4 cm^2 at the loin area ($Y = 37.31 + 2.45 \text{ LYS}$, $R^2 = 0.62$) and a reduction of 14.7 mm at mean backfat thickness ($Y = 37.91 - 14.73 \text{ LYS}$, $R^2 = 0.58$).

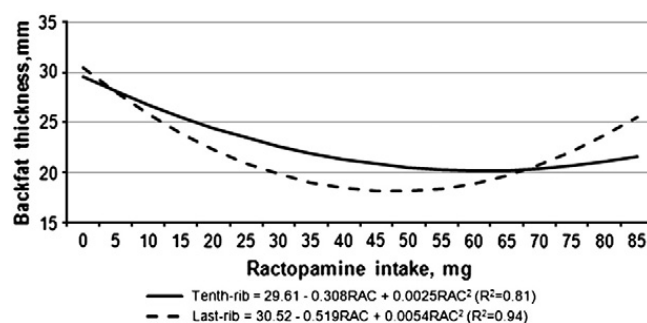


Fig. 2. Backfat thickness at tenth-rib and last-rib (expressed in mm) according to ractopamine intake (RAC, expressed in mg).

Table 2

Carcass characteristics, obtained by meta-analysis, in pigs fed diets containing ractopamine according to higher or lower lysine supply in relation to average intake or estimated requirement.

	Relation to the average intake ^a				Relation to the requirement ^b			
	Lower	Higher	P ^c	RSD ^d	Lower	Higher	P	RSD
Weight gain, g/day	0.94	1.04	*	0.1	0.88	1.00	**	0.2
Lean meat, %	57.4	62.7	*	1.3	55.2	64.4	*	2.1
Leaf fat, g	1.33	0.81	**	0.1	1.31	0.86	**	1.3
Hot carcass weight, kg	80.5	80.2	ns	5.3	81.5	79.4	ns	2.4
Loin area, cm ²	40.1	41.8	*	2.6	41.0	43.5	*	4.6
Tenth-rib BT ^e , mm	23.1	19.7	*	2.9	22.0	18.3	**	3.3
Last-rib BT, mm	22.9	19.6	*	2.6	20.7	15.1	**	2.6
Mean BT, mm	36.7	32.9	*	3.1	35.3	30.5	**	2.9

^a Overall average daily intake of lysine, calculated from the database (195 mg lysine/kg BW^{0.6}).

^b Requirements in lysine, estimated based on NRC (1998).

^c Significance: *P<0.05, **P<0.01, ns not significant (P>0.05).

^d Residual standard deviation.

^e Backfat thickness.

4. Discussion

Several studies observed the relationship between ractopamine and improvements in variables, such as loin area (Weber et al., 2006, Xiong et al. 2006) or backfat thickness (Mimbs et al., 2005). An increase in muscle protein synthesis in supplemented pigs may explain this interference. Ractopamine influence in variables associated with muscle or fat deposition may be related with cellular responses, including lipolysis, gluconeogenesis and glycogenolysis stimulation (Mills, 2002). In adipose tissue, the activation of β -adrenergic receptors may promote lipid degradation, reducing body fat content (Mersmann, 1998). Thus, in general, increase in lean meat content may be related to both, the reduction of fatty acids synthesis in adipose tissue, and a corresponding increase of protein synthesis in muscle tissue.

Results observed in this study for hot carcass weight are similar to those found by Armstrong et al. (2004), Crome et al. (1996), Marchant-Forde et al. (2003), See et al. (2004), Weber et al. (2006) and Xiong et al. (2006). However, other studies (Dunshea et al., 1993b; Watkins et al., 1990) reported no differences on this variable for pigs supplemented or not with ractopamine. In previous studies, and similarly in present meta-analysis, ractopamine did not affect carcass dressing (Armstrong et al., 2004; Bark et al., 1992; Marchant-Forde et al., 2003; Marinho et al., 2007a, 2007b; Xiao et al., 1999; Xiong et al., 2006) and length (Bellaver et al., 1991; Carr et al., 2005a, 2005b; Crome et al., 1996; Dunshea et al., 1993b; See et al., 2004; Stites et al., 1991).

Several papers included in the database reported an increase in loin area in pigs fed diets containing ractopamine (Adeola et al., 1990; Bark et al., 1992; Carr et al., 2005a, 2005b; Crome et al., 1996; Mimbs et al., 2005; See et al., 2004; Stites et al., 1991; Watkins et al., 1990; Weber et al., 2006; Xiao et al., 1999). However, some studies of the database found that ractopamine supplementation does not affect this variable in pigs (Bellaver et al., 1991; Dunshea et al., 1993b).

Results observed for backfat thickness at tenth-rib are in agree with that reported previously by *in vivo* (Bark et al., 1992; Bellaver et al., 1991; Carr et al., 2005b; Crome et al., 1996; Dunshea et al., 1993b; Mimbs et al., 2005; See et al., 2004; Uttaro et al., 1993; Xiao et al., 1999) or meta-analytical studies (Kiefer and Sanches, 2009). The increase in lean meat

content, observed in the present meta-analysis, was similar to that reported in previous studies by Armstrong et al. (2004), Weber et al. (2006) and Xiong et al. (2006). Similarly, the reduction in leaf fat was already reported by Adeola et al. (1990) and Watkins et al. (1990).

In mice, β -adrenergic agonists may increase muscle mass from 6 to 18% and reduce body fat at rates ranging from 6 to 33% (Ramos and Silveira, 2002). In pigs, this relationship is expressed at rates ranging from 1 to 16% of fat reduction and about 10% of protein content increase in carcasses (Ramos and Silveira, 2002). However, additive action may be influenced by some factors, like duration of supply period. Beyond the supplementation period, the animal age increase may cooperate with enlarged ractopamine effect. This is due to the fact that physiologically immature animals have less β -adrenergic receptors and less receptor differentiation in skeletal and adipose tissues (Mersmann, 1998).

In the database used for this meta-analytical study, the correlations coefficients observed between mean backfat thickness and duration of supply period, level of ractopamine inclusion in diets and estimated ractopamine intake were significant. However, these coefficients were low, indicating that other factors beyond the supply period or ractopamine concentration can interfere in backfat thickness and in another carcass characteristics of supplemented pigs.

Some dietary factors may also influence the ractopamine action. Dietary energy, protein and lysine contents may affect animal response to ractopamine (Apple et al., 2004). The amino acid profile deposited in pig muscle tissue can be changed by ractopamine action (Reeds and Mersmann, 1991). Lysine concentration in protein deposited by pigs fed diets supplemented with ractopamine increases from 6.8 to 7.1% (Schinckel et al., 2003b). Thus, it is probable that ractopamine action in nutrient partition also change animal nutrient requirements, which would determine the need for diet adjustments (Schinckel et al. 2003b; Webster et al., 2007).

Lean meat content increases with the dietary density of protein (Cromwell et al., 1978) and lysine (Dourmad et al., 1996). This relationship appears to be maintained when animals are supplemented with ractopamine. Some studies suggest that the protein level interferes on the ractopamine efficiency by promoting nitrogen retention, and it is more effective when the protein intake is higher (Reeds and Mersmann, 1991). In

another meta-analytic study, it was observed that the total lysine content can modulate performance responses of pigs fed diets containing ractopamine (Andretta et al., 2011). In agreement with the current results, Kiefer and Sanches (2009), also using meta-analysis, reported the effect from lysine level on loin area in pigs feeding diets containing 10 and 20 ppm of ractopamine. Daily protein accretion and fat-free lean growth were also described as functions of dietary lysine intake, as reported by Schinckel et al. (2003b).

In supplemented animals, the increase in dietary lysine density can be associated with improvement in carcass characteristics (Webster et al., 2007). Backfat depth and lean meat carcass content in pigs fed diets containing ractopamine are probably optimized with 1% of true digestible lysine (Kiefer and Sanches, 2009). However, these recommendations are still varied. Thus, results of the present meta-analysis suggest that more studies are necessary to adjust the relationship between ractopamine and nutritional requirements, in order to improve important carcass characteristics in supplemented pigs.

5. Conclusion

Many studies have been conducted to evaluate the ractopamine effects in body composition of farm animals. The association between ractopamine and metabolic changes has also been the subject of several studies. This meta-analysis allowed the theme to be studied systematically. Results obtained showed that ractopamine addition in pig diets increases the content of lean meat, reduces backfat thickness and does not alter variables studied for meat quality (marbling and visual color). The meta-analysis also indicated the need for future research relating ractopamine and lysine content in pig diets. Animals supplemented with ractopamine and fed diets containing higher lysine levels present higher loin area and lower backfat thickness than other pigs. Meta-analysis has proven to be an important tool for combining results from previous studies in animal nutrition.

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