Dietary conjugated linoleic acid (CLA) has comparable effects to ractopamine on the growth performance, meat quality and fatty acid profiles of loin muscles of finishing pigs under commercial husbandry

Araceli Pinelli-Saavedra, Humberto González-Ríos, José Luis Dávila-Ramírez, Thalia Yamileth Islava-Lagarda and Ingrid Rebeca Esquerra-Brauer

Department of Nutrition and Metabolism, Centro de Investigación en Alimentación y Desarrollo, Research Center for Food and Development, Hermosillo, Mexico; Department of Science of Animal Technology, Centro de Investigación en Alimentación y Desarrollo, Research Center for Food and Development, Hermosillo, Mexico

ABSTRACT
The effects of conjugated linoleic acid (CLA) supplementation on pig loin muscle growth performance, meat quality and fatty acid profiles were compared with the effects of ractopamine supplementation. Two hundred seventy commercial Landrace-Yorkshire pigs (135 barrows and 135 gilts/BW = 75 kg) were randomly assigned to one of three treatments. Ninety animals per treatment were allocated into groups of 30 animals (15 barrows and 15 gilts) with three replicates. The treatments were as follows: RAC (diet given on the farm and included ractopamine and mixed vegetable oils); CLA 0.5% (basal diet + CLA 0.5%); and CLA 1% (basal diet + 1% CLA). Final weight and daily gain were affected by treatment/sex in gilts supplemented with CLA 0.5% but not in barrows. Feed intake was reduced in animals supplemented with CLA at 0.5%. Supplementation with CLA did not affect the meat quality parameters (p > .05). CLA 1% delayed the lipid oxidation of the pork meat. CLA 1% decreased the contents of 18:1, 18:2, 20:5 n3, and 22:6 n3 in intramuscular fat compared to those in unsupplemented animals (p < .05). In conclusion, CLA 0.5% in the diet showed a better effect than the use of ractopamine on growth performance in gilts but not in barrows. CLA 0.5% evoked a similar response as ractopamine in the PUFA content of intramuscular fat and meat quality. Thus, CLA could replace this commonly used additive, which has been restricted in several countries, in the pork industry.

HIGHLIGHTS
- Dietary CLA 0.5% produced an improvement in growth performance in gilts.
- Dietary CLA 1% delayed the lipid oxidation of pork meat.
- CLA is an alternative supplement to ractopamine due to their similar effects on meat quality parameters.

Introduction
Pigs are susceptible to changes in the fatty acid composition of adipose tissue and muscle due to the effects of the source of fat in the diet. Thus, the proportion of fatty acids in the diet is linearly associated with deposits in muscle and adipose tissue. This relation allows the modification of the profile of fatty acids in adipose and intramuscular tissues (Wood 1984; Wood et al. 2008). Therefore, the pig is a good candidate for supplementation with CLA, which is deposited in these tissues with relatively high efficiency. Thus, pork meat can be a physiologically significant source of CLA for human consumption (Dugan et al. 2004). The conjugated linoleic acid occurs in vivo as an intermediary during the partial bio-hydrogenation linoleate of bacteria by Butyrivibrio fibrisolvens in the bacterial flora of ruminant animals, and it is produced in higher quantities than in non-ruminants (Pariza et al. 2001). In contrast, monogastic (pig) hydrogenation of fatty acids is limited and CLA production is also limited. However, commercial use of CLA as an additive in pig diets is not frequent, despite the properties in the redistribution of body fat and improvements in weight gain found in most pig studies and those previously observed in other species. Several
investigations have demonstrated the practicality of using CLA to affect growth with improvements in the feed conversion and reduction in the fat content of the carcase in pigs (Dugan et al. 1997; Thiel-Cooper et al. 1998, 2001; Ostrowska et al. 1999). Several studies have shown that CLA administered in the diet for pigs is a promoter of growth, improving feed conversion and decreasing fat deposition (Thiel-Cooper et al. 1998; 2001). Dugan et al. (1997) reported that 60-kg pigs supplemented with CLA tend to have improved feed conversion and reduced subcutaneous fat. Ostrowska et al. (1999) reported similar results, finding a significant effect on feed conversion. Additionally, Weber et al. (2001) reported a better performance of pigs fed with CLA (weight gain, subcutaneous fat or feed conversion). In contrast, other studies showed no change in feed conversion or a nonsignificant decrease in fat (Tous et al. 2013). These differences may be related to the age/weight of the animal, the level of supplementation, differences in response to different isomers or the proportion of the mixtures of isomers used in diet supplementation (Fernández-Figares et al. 2008; Schiavon et al. 2010). In addition, it has been reported that fatty acid composition of intramuscular fat was modified by CLA, increasing saturated fatty acids in the pork loin, and that lipid oxidation was inhibited when CLA concentration was increased in meat (Wiegand et al. 2002; Cordero et al. 2010; Barnes et al. 2012).

However, pig farms currently use ractopamine hydrochloride (RAC), which is an additive that promotes growth performance and increases in lean meat percentage of the carcase. According to reports, CLA has similar properties as ractopamine, as described above. This additive is an analogue of catecholamines, hormones that regulate many physiological processes by activating adrenergic receptors (Ramos and Silveira 2002). However, a growing number of countries have banned the use of RAC, including European Economic Community countries, China and Russia. Furthermore, recent reports have indicated that the use of RAC negatively affects the quality of meat (high WHSF values and low values of colour parameters (Puls et al. 2014; Needham and Hoffman 2015). Thus, the aim of this study was to compare the effect of dietary CLA with that of RAC as a growth promoter in finishing pig diets on the fatty acid profile of loin muscle and parameters of meat quality in commercial husbandry.

**Materials and methods**

All procedures involving animal handling were performed within the official Mexican guidelines for animal care (Norma Oficial Mexicana 1995a). Humanitarian care of animals during mobilisation; Norma Oficial Mexicana (1995b): slaughter of domestic and wild animals.

**Animals and diet**

The experiment was performed on a pig farm in Sonora, Mexico, under commercial pig production conditions. A total of 270 crossbred Landrace × Yorkshire finishing pigs were used starting from a weight of 75 kg. Ninety animals per treatment were allocated into groups of 30 animals (15 gilts and 15 barrows) with three replicates. The animals were selected from the start of the fattening process with uniform weight and age. All animals were individually identified using an ear tag.Supplementation with CLA was performed within the last 30 days of the finishing phase. For assessments of fatty acid profiles, 10 samples were used per treatment. The treatments were as follows: RAC (diet given on the farm, which included ractopamine and mixed soft acid oils, without CLA); CLA 0.5% (basal diet + 0.5% CLA); and CLA 1% (basal diet + 1% CLA). Diets were isocaloric and isoproteic with variation only in the source of fat, and they were formulated according to the recommendations of the National Research Council (NRC 1998) as shown in Table 1. The feed and water were provided ad libitum. A commercial product (Lutalin™) BASF was used as a source of CLA, consisting of 30% cis-9, trans-11 isomer and 30% trans-10, cis-12 isomer. Table 1 shows the contents of fat, protein and CLA analysed in the laboratory to confirm that the calculated values in the formulation of the diet (proportion of each ingredient of the diet) corresponded to the actual values present in the diet. The fatty acid profiles analysed are shown in Table 1 and confirm the CLA content in the experimental diets.

**Growth performance**

All animals were weighed individually at the beginning and end of the experiment using a Model E 500 M Accu-Arm OSBORNE (Osborne Ks, USA) electronic balance. The average daily gain (ADG) was estimated by the difference between initial and final body weight (FBW) divided by the 30 days of the feeding period. Feed intake was evaluated from the difference in weight between the feed offered and the feed rejected. The feed conversion ratio (FCR) per pen/treatment was based on average feed intake and ADG. Animals were also observed daily throughout the study, and no adverse effects of CLA were observed.
Table 1. Diet composition on a dry basis, finishing pigs (kg ton⁻¹).

| Ingredients                      | RAC   | CLA 0.5% | CLA 1.0% |
|----------------------------------|-------|----------|----------|
| Wheat 12% crude protein          | 835.75| 836.70   | 836.70   |
| Soybean meal 47% crude protein   | 124.00| 124.00   | 124.00   |
| Mixed fatty acids                | 20.00 |          |          |
| Soybean oil                      | 0.00  | 14.300   | 9.300    |
| CLA-Lutalin®¹⁺                   | 0.00  | 5.000    | 10.000   |
| Mic. End LP²                      | 20.00 | 20.000   | 20.000   |
| Ractopamine Hydrochloride⁵        | 0.250 | 0.000    | 0.000    |
| Analysis calculated              |       |          |          |
| Metabolizable energy (MCal/kg)   | 3341  | 3341     | 3341     |
| Protein (%)                      | 16.580| 16.580   | 16.580   |
| Fat (%)                          | 3.660 | 3.660    | 3.660    |
| Lysine (%)                       | 0.947 | 0.947    | 0.947    |
| Fibre (%)                        | 2.336 | 2.336    | 2.336    |
| Total phosphorus (%)             | 0.442 | 0.442    | 0.442    |
| Calcium (%)                      | 0.474 | 0.474    | 0.474    |
| Analysis                         |       |          |          |
| Protein (%)                      | 15.040| 15.060   | 14.840   |
| Ether fat (%)                    | 3.090 | 3.450    | 3.880    |
| Fatty acid composition (mg⁻¹/100 mg total FA) |       |          |          |
| SFA⁴                             | 22.980| 17.580   | 18.190   |
| C14:0                            | 1.140 | 0.070    | 0.190    |
| C16:0                            | 16.510| 13.980   | 13.740   |
| C18:0                            | 4.390 | 3.520    | 4.060    |
| MUFA®                            | 32.190| 24.090   | 26.410   |
| C18:1, c9                        | 28.560| 24.090   | 25.850   |
| PUFA®                            | 44.830| 58.330   | 55.400   |
| C18:2, n-6                       | 1.590 | 1.280    | 1.230    |
| C18:2, n-6                       | 39.210| 48.140   | 44.030   |
| C18:3, n-3                       | 3.510 | 5.090    | 4.300    |
| Conjugated linoleic acid         | 0.000 | 2.960    | 5.830    |

¹BasF, composed of 30% cis-9, trans-11 isomer and 30% trans-11, cis-12 isomer.
²Premix of minerals and vitamins.
³Racmina 2%, 2 g ractopamine hydrochloride in 100 g c.b.p PISA, Agropecuaria, Mexico.
⁴SFA: Saturated fatty acids.
⁵Monounsaturated fatty acids.
⁶Polyunsaturated fatty acids.
⁷Treatments: RAC, animals receiving a diet with ractopamine (control group); CLA 0.5%, animals supplemented with 0.5% of conjugated linoleic acid; CLA 1%, animals supplemented with 1% of conjugated linoleic acid.

Slaughter and longissimus thoracis muscle (LTM) dissection

After recording the final body weight, all pigs were slaughtered in the slaughterhouse of Navojoa, México, in compliance with current regulations (Norma Oficial Mexicana 1995a). At 24 h post-mortem, the LTM was removed (4th until 12th intercostal space) from the carcass (10 per treatment), vacuum packed, stored on ice and transported to the laboratory of Meat Science and Technology at CIAD AC (Centro de Investigación en Alimentación y Desarrollo AC) in Hermosillo, México. Upon arrival at the laboratory, the samples were stored in a freezer at −18°C until further analysis.

Sample sectioning

Before analyses, samples were thawed for 24 h at 4°C and then sectioned following the same order in which the anatomical position of each cut was recorded to carry out the analyses of meat quality and fatty acid profile. Sectioning started from the distal end (12th rib interface) and proceeded cranially towards the chuck end of the rib. The 1st steak (2.5 cm) was used for fatty acid composition, and the following two pairs (2.5 cm each) were used for Warner Bratzler Shear Force (WBSF) and cooking loss, respectively. The other two steaks (2.5 cm each) were used to evaluate the colour, pH, TBARS and metmyoglobin (MetMb). All measurements were made immediately after sample sectioning.

Meat quality

Ph

The pH determination was performed at three points at the centre of the steak at 4°C using a portable digital pH metre, which was previously calibrated with pH 4 and 7 standards (Hanna, Model HI 98140, Woonsocket, RI).

Meat colour

Fresh colour measurements of LTM surfaces after 30 min blooming at controlled room temperature (4°C) were performed using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Japan) with D65 illuminant, with 10° and 8 mm of aperture in the observer. Colour parameters evaluated included L* (lightness), a* (redness) and b* (yellowness) (Cassens et al. 1995). Hue angle (Hue) was calculated with the following formula: Hue = \( \tan^{-1}\left(b^* / a^*ight) \). Colour determinations were made at 5 different locations of the surface (perpendicular to fibres) of the cold samples (4°C).

Warner Bratzler shear force (WBSF)

WBSF was measured using a Texture Analyzer T.A.X.T. Plus (Texture Technologies Corp., Scarsdale, NY). Sections of LTM 2.5 cm thick were cooked in an electric skillet (Cook Master Oster, model 3222-3, Mississauga, Ontario, Canada) until a final internal temperature of 71°C was reached. A thermocouple (type T copper-constantan [ECKLUND-HARRISON]) was inserted into the geometric centre of each steak, and the temperature was monitored throughout the cooking process with a thermometer sweep (Barnant, 692-0000, USA). Cooked samples were cooled (20–25°C) and chilled at 4°C for 24 h. Subsequently, the meat was cut into pieces of 1.27 cm diameter in the longitudinal direction of the muscle fibres, and WBSF was determined with a Warner Bratzler attachment cutter.
on 10 specimens per sample. The value WBSF was expressed as the mean of 10 determinations in 1 kg.

**Cooking loss**

Cooking loss was evaluated in LTM sections 2.5 cm thick, which were cooked in an electric skillet, under the same cooking conditions used for WBSF analysis as described above. Samples were weighed in the raw state and immediately after reaching the final cooking temperature (71 °C).

**Shelf life study in refrigeration (4 °C)**

The steaks of LTM were placed on polypropylene plates, covered with PVC film (~17 cm³/m² d of oxygen permeability and <5 g/m² d of moisture permeability) and stored at 4 °C for 15 d in the presence of light (~70 candles). The instrumental colour parameters as well as lipid oxidation using the thiobarbituric acid reactive substances assay (TBARS) and percentage of metmyoglobin were evaluated on days 1, 5, 10 and 15 of storage. The colour determinations were performed as indicated above in the section on ‘Meat colour’.

**Thiobarbituric acid reactive substances (TBARS)**

The state of oxidation of the lipids was evaluated by determining the TBARS as previously described by Pfalzgraf et al. (1995). Meat samples (5 g) were mixed with 20 ml 10% (w/v) trichloroacetic acid and centrifuged (2300 × g) for 20 min at 5 °C, and the supernatants were filtered through Whatman 4 filter paper. Next, 2 ml 20 mM 2-TBA was added to 2 ml of filtrate. The mixture was homogenised, placed in a water bath at 97 °C for 20 min, and subsequently cooled. The absorbance values were measured at 531 nm using a UV-vis spectrophotometer. TBARS values were calculated using a standard calibration curve of 1, 1, 3, 3-tetramethoxypropane and expressed as mg of malondialdehyde (MDA)/kg of meat.

**Metmyoglobin (MetMb)**

Sample preparation was performed as described by Lee et al. (1998). Briefly, 5 g of sample was weighed and placed in polypropylene tube with 50 ml capacity, and 20 ml of iced phosphate buffer (pH = 6.8, 40 mM) was added. The sample was homogenised at 11,300 rpm for 30 s (Ultra Turrax T25, IKA-Werke, USA). Subsequently, it was centrifuged (Beckman refrigerated J2-21) at 5000 rpm for 30 min at 4 °C. The supernatant was filtered and read in a spectrophotometer (Spectronic Genesis 5, Thermo Electron Corporation, USA) at 700, 572 and 525 nm. The MetMb percentage was determined using the formula of Krzywicki (1982):

\[
\text{%MetMb} = \left( \frac{A_{572} - A_{700}}{1.395} \right) \times 100
\]

where %MetMb = Percentage of metmyoglobin, 1.395 = Constant, A572 = Absorbance at 572 nm, A700 = Absorbance at 700 nm, A525 = Absorbance at 525 nm.

**Fatty acid profile**

The fatty acid profile was evaluated in subcutaneous and intramuscular fat of the longissimus thoracis muscle. The extraction of the lipid fraction of the thawed samples was performed as described by Bligh and Dyer (1959). Briefly, 20 g of previously ground meat was added to 20 ml of chloroform and 40 ml of methanol (Sigma-Aldrich, Missouri, USA) and homogenised for 2 min (11,000 rpm) using an Ultraturrax IKA (model T25) disperser. Next, 20 ml of chloroform, 20 ml of the potassium hydroxide solution (0.8%) and 16 ml of distilled water were added. The samples were homogenised for 2 min and centrifuged at 3500 rpm for 10 min at 0 °C (Beckman model J2-21). Next, the precipitate was removed, and the lower phase (chloroform + fatty acids) was obtained and transferred into a tube with a few drops (~10 µl) of butylated hydroxytoluene (BHT 1 g/10 ml of ethanol, Sigma-Aldrich, Missouri, USA). The samples were incubated in a water bath (34–36 °C), and the air was removed using a nitrogen stream to continue the methylation.

The methylation of the fatty acids was performed as described by Park and Goins (1994). Briefly, fatty acids (tube with cap) were placed into a water bath at 40 °C, and 100 µl of dichloromethane (Aldrich-Chemical, Wisconsin, USA) and 1 ml of sodium hydroxide were added. Next, the tubes were heated in a water bath at 90 °C for 10 min. After the samples were cooled, 1 ml of boron trifluoride (Sigma-Aldrich, Missouri, USA), 1 ml of distilled water and 500 µl of hexane (Aldrich-Chemical, Wisconsin, USA) and 1 mg of internal standard (Tridecanoic, Sigma-Aldrich, Missouri, USA) were added. The upper phase was removed to obtain the fatty acid methyl esters (FAMEs) including 9-cis, 11-trans-CLA and 10-trans, 12-cis-CLA and frozen at –40 °C until further analysis.

The fatty acid composition was analysed by gas chromatography using a Hewlett Packard 6890 Series (Palo Alto, CA, USA) with a flame ionisation detector (FID) and an auto-sampler 6890 m and Supelco SP2560 capillary column (100 m × 0.25 mm). The temperature of the injection port was maintained at 250 °C, and the
temperature of the detector was maintained at 300 °C. The identification of fatty acids was performed according to the retention time, and the elution pattern showed the FAME standards (No. 2 Animal Source, Supelco 4-7015-U) and CLA standards isomers (Matreya Inc., PA). The quantification was performed by integrating the area under the curve of the peak, and it was expressed as the percentage of total fatty acid present in the sample. Additionally, the amounts of saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) were calculated. The relationship between PUFAs/SFAs and the n-6/n-3 ratios were also calculated.

### Experimental design and statistical analysis

Data of growth performance were analysed under a completely randomised design with a 3 × 2 factorial arrangement. The model included treatments, sex and their interaction as fixed effects and the initial weight of animal as a covariate. For final body weight (FBW) and the average daily weight (ADG) variables, each animal was the experimental unit, and for feed intake and feed conversion, the pen was the experimental unit. Data regarding meat quality and fatty acid profiles were analysed using one-way analysis of variance, adjusting the mean factor for the treatments. Shelf life data were analysed using a model that included storage time and the interaction effects.

### Results

#### Growth performance

Data regarding growth performance are shown in Table 2. After 30 days, the FBW and ADG were affected by sex and treatment and the treatment × sex interaction (p < .05). With regard to the final weight of the gilts, those supplemented with CLA 0.5% were heavier (p < .05) than those in the RAC and CLA 1% groups. For ADG, CLA 0.5% led to higher ADG than RAC but not CLA 1%. In the barrows, the FBW and ADG were similar between treatments (p > .05). The feed intake was affected by treatments in both sexes (p < .05), where a lower intake was observed in the RAC and CLA 0.5% groups with respect to CLA 1%. In contrast, the feed conversion was not affected by any of the factors or their interaction (p > .05). However, a trend (p = .08) towards better feed conversion was observed in animals supplemented with CLA 0.5% in both sexes.

### Meat quality

The effects of CLA supplementation on the meat quality of the finishing pigs are presented in Table 3. Colour parameters L*, a*, b* and Hue angle, pH, cooking loss, WBSF, TBARS and MetMb were not affected (p > .05) by the addition of CLA (0.5 and 1.0%) in the diet. Although it was not statistically significant, the use of CLA 1% decreased the WBSF (12.67%) and TBARS (27.78%) values compared to the values of meat obtained from animals fed with the RAC diet. In the pork steak shelf life study (Table 4), it was observed that a*, b* and Hue angle colour parameters, were affected by the storage time (p < .05) but not by the treatments (p > .05). These colour parameters decreased throughout the storage time (p < .05).

Regarding the lipid oxidation of pork meat (Table 5), an effect of storage time and treatments was observed (p < .05). All treatments showed low and similar levels of lipid oxidation in the first days of sampling (p > .05); however, on day 15 of sampling, the meat obtained from animals supplemented with CLA 1% showed less oxidation (64%) than the meat from animals that received RAC and CLA 0.5% treatments.

### Table 2. Productive performance of gilts and barrows supplemented with CLA for 30 d.

| Variable  | Gilts | Barrows |
|-----------|-------|---------|
| IBW, kg   | RAC   | CLA 0.5% | CLA 1% |
|           | 73.300 | 71.400  | 71.700 |
|           | 78.400 | 72.500  | 77.600 |
| FBW, kg   | 101.200 | 104.300 | 102.600 |
|           | 106.400 | 105.100 | 106.300 |
| ADG, kg   | 0.899c | 1.005b  | 0.949ab |
| Feed intake, kg | 2.500a | 2.460a  | 2.600b |
| Feed conversion, kg | 2.750 | 2.390  | 2.690 |

### Table 5. The effects of CLA supplementation on the lipid oxidation of pork meat.

| Variable | Gilts | Barrows |
|----------|-------|---------|
| IBW, kg | RAC | CLA 0.5% | CLA 1% |
|          | 73.300 | 71.400  | 71.700 |
|          | 78.400 | 72.500  | 77.600 |
| FBW, kg | 101.200 | 104.300 | 102.600 |
|          | 106.400 | 105.100 | 106.300 |
| ADG, kg | 0.899c | 1.005b  | 0.949ab |
| Feed intake, kg | 2.500a | 2.460a  | 2.600b |
| Feed conversion, kg | 2.750 | 2.390  | 2.690 |

### Table 4. Growth performance of gilts and barrows supplemented with CLA for 30 d.

| Variable | Gilts | Barrows |
|----------|-------|---------|
| IBW, kg | RAC | CLA 0.5% | CLA 1% |
|          | 73.300 | 71.400  | 71.700 |
|          | 78.400 | 72.500  | 77.600 |
| FBW, kg | 101.200 | 104.300 | 102.600 |
|          | 106.400 | 105.100 | 106.300 |
| ADG, kg | 0.899c | 1.005b  | 0.949ab |
| Feed intake, kg | 2.500a | 2.460a  | 2.600b |
| Feed conversion, kg | 2.750 | 2.390  | 2.690 |

SEM: Standard error of the mean.

**Means with different superscripts within the row are different (p < .05).**
Table 3. Meat quality of longissimus thoracis (LT) muscle from pigs supplemented with RAC and CLA for 30 d.

| Variable           | RAC          | CLA 0.5%     | CLA 1%      | SEMd | p value |
|--------------------|--------------|--------------|-------------|------|---------|
| pH                 | 5.950        | 5.760        | 5.770       | 0.120| .340    |
| Lightness (L<sup>+</sup>) | 55.370       | 51.570       | 56.670      | 1.820| .190    |
| Redness (a<sup>+</sup>) | 9.230        | 7.760        | 8.960       | 0.670| .130    |
| Yellowness (b<sup>+</sup>) | 8.780        | 7.640        | 9.460       | 0.780| .100    |
| Hue angle<sup>a</sup> | 43.970       | 44.010       | 46.600      | 2.850| .740    |
| Cooking loss %     | 13.890       | 18.150       | 17.040      | 2.290| .620    |
| WBSF, kg<sup>f</sup> | 7.180        | 7.070        | 6.270       | 0.570| .560    |
| TBARS value<sup>g</sup> | 0.288        | 0.324        | 0.208       | 0.150| .470    |
| MetMb<sup>b</sup>   | 47.330       | 50.860       | 48.450      | 3.010| .800    |

<sup>a</sup>Means with different superscripts within a row are different (<i>p < .05</i>).  
<sup>c</sup>Treatments: RAC: animals receiving a diet with ractopamine (control group); CLA 0.5%, animals supplemented with 0.5% of conjugated linoleic acid; CLA 1%, animals supplemented with 1% of conjugated linoleic acid.  
<sup>d</sup>Standard error of mean.  
<sup>f</sup>WBSF: Warner Bratzler shear force.  
<sup>g</sup>TBARS: Thiobarbituric acid reactive substances.  
<sup>b</sup>MetMb: Metmyoglobin.

However, the % MetMb formation (Table 5) was only affected by the storage time (<i>p < .05</i>), where the meat of all treatments presented values above 60% at the end of the refrigeration period.

### Intramuscular fatty acid profile

Table 6 shows the results of the fatty acid profile of intramuscular fat of the longissimus thoracis muscle. Supplementation with CLA 1% increased the palmitic acid content (<i>p < .05</i>) compared with that in animals fed with RAC diets and the CLA 0.5% treatment. However, supplementation with CLA 0.5 and 1% did not have an effect (<i>p > .05</i>) on stearic acid content, and CLA 1% decreased the oleic acid content (<i>p < .05</i>) compared with that in RAC. In addition, the CLA 1% dietary treatment led to a one-fold decrease in the

Table 5. Effect of dietary CLA on lipid oxidation (TBARS) and metmyoglobin (MetMb) of pork meat during 15 d of storage at 4°C.

| Variable        | Days   | RAC     | CLA 0.5% | CLA 1%     | SEM<sup>d</sup> | p value |
|-----------------|--------|---------|----------|------------|-----------------|---------|
| TBARS<sup>c</sup>, mg/kg | 1      | 1.480   | 1.410    | 1.500      | 0.130           | .900    |
|                  | 5      | 25.360  | 25.640   | 27.820<sup>a</sup> | 0.360           | .001    |
|                  | 10     | 0.380   | 0.320    | 0.800      | 0.330           | .550    |
|                  | 15     | 10.600  | 12.560   | 13.190     | 0.820           | .090    |
|                  | 5      | 0.120   | 0.170    | 0.120      | 0.020           | .290    |
|                  | 10     | 0.130   | 0.190    | 0.320      | 0.060           | .100    |
|                  | 15     | 0.100   | 0.100    | 0.080      | 0.020           | .660    |
| MetMb<sup>b</sup> | 1      | 1.390   | 1.620    | 0.690<sup>a</sup> | 0.160           | .001    |
|                  | 5      | 3.790   | 3.350    | 3.920      | 0.380           | .560    |
|                  | 10     | 4.540   | 4.320    | 4.620<sup>a</sup> | 0.670           | .020    |
|                  | 15     | 0.220   | 0.440    | 0.390      | 0.090           | .200    |
|                  | 5      | 0.250   | 0.180<sup>ab</sup> | 0.080<sup>ab</sup> | 0.030           | .020    |

<sup>a</sup>Means with different superscripts within a row are different (<i>p < .05</i>).  
<sup>c</sup>Treatments: RAC: animals receiving diet with ractopamine (control group); CLA 0.5%, animals supplemented with 0.5% of conjugated linoleic acid; CLA 1%, animals supplemented with 1% of conjugated linoleic acid.  
<sup>d</sup>Standard error of the mean.  
<sup>b</sup>MetMb: Metmyoglobin.  
<sup>ab</sup>Saturated fatty acids.  
<sup>abc</sup>Monounsaturated fatty acids.  
<sup>ac</sup>Polyunsaturated fatty acids.
tricosanoic acid content (0.69) compared with that in the RAC (1.59) and CLA 0.5% (1.62) (p < .05) treatments. Moreover, CLA 1% decreased the linoleic acid content (27.34%) and ~50% of the eicosapentaenoic and docosahexaenoic acid content compared to CLA 0.5% (p < .05).

The partial sum of fatty acids and nutritional value of intramuscular fat are presented in Table 7. Supplementation with CLA, irrespective of the level used, increased saturated fatty acid contents (p < .05) compared with those in the RAC group. In contrast, the addition of CLA decreased the total content of monounsaturated fatty acids (p < .05) compared to that in RAC. Regarding the total PUFA content, we found that CLA 1% decreased the content of PUFAs by 24.6% and the total n-3 by 55%, as well as the ratios of MUFA/SFA and PUFA/SFA (p < .05), compared with the values in animals fed the RAC diet. The total amount of n-3 was lower in CLA 0.5% than in CLA 1% and RAC. No differences were observed in total n-6 among the treatments. However, the n-6/n-3 ratio was modified by the addition of CLA to the diets (p < .05).

**Discussion**

**Growth performance**

This study provides information about CLA supplementation in finishing pig diets under commercial husbandry, whereas previous studies have been carried out under experimental conditions. However, the effect of CLA as a growth promoter is still contradictory. Our results showed that feed conversion tended to be lower (p = .08) and ADG higher (p > .001) in the group of animals with CLA 0.5%, and these results were similar to those found by Stanimirovic et al. (2012) under environmentally controlled conditions in gilts observed over 44 days. Previous studies reported by Dugan et al. (1997) and Thiel-Cooper et al. (2001) found an effect of CLA on ADG and feed conversion. In contrast, Morel et al. (2008) and Ivanovic et al. (2015) reported no effect at 2% CLA. Moreover, the positive effect of CLA in our study on ADG and FBW was found only on gilts, which is a response similar to that reported by Stanimirovic et al. (2012), which was carried out in gilts, the FCR and ADG was enhanced by CLA 0.5%, suggesting an effect related to sex. In contrast, Pompeu et al. (2013) reported an interactive effect of CLA 1% with corn dried distiller’s grains with solubles (DDGS) on average daily feed intake and carcass fat in finishing pigs, but this effect was not found for the interaction of RAC and CLA with DDGS. They also reported that CLA counteracted the negative effects of DDGS on fatty acid profiles, whereas RAC did not have this effect. Thus, these discrepancies in response to CLA may be related to the age/weight of the animal, genetic factors, the level of supplementation, differences in response to the different isomers or proportion of isomers used in supplementation of diets or the duration of supplementation with CLA. Moreover, differences in response to the different isomers of conjugated linoleic acid (CLA) may be due to an increase in hormone thyroid T3 in barrows, reported by Sechman et al. (2007). This hormone was increased in the blood of barrows, but not gilts, fed a diet supplemented with CLA 0.5%, but no effect was found with CLA 1.0%, which may be related to the higher metabolic rate in barrows according to Danfort and Burguer (1989). However, this possibility warrants additional study.

**Meat quality**

Regarding the meat quality parameters, no effects due to the use of CLA were found on pH, colour, WBSF, or cooking loss when compared to RAC. These results are consistent with those reported by Matak et al. (2013), who evaluated CLA 1% for 6 weeks in 20 barrows and did not find an effect on these meat quality characteristics. In the pork steak shelf life study, initial TBARs values were low (0.208–0.324); however, at the end of the storage period, only meat from animals treated with CLA 1% had values below 1.4 mg of MDA, which has been considered the lower limit to detect unpleasant odours characteristic of meat lipid rancidity by consumers (Wenjiao et al. 2014). In contrast to our
study, Mata et al. (2013) found no effect on the addition of 1% of CLA in the finishing diets of pigs on lipid oxidation of meat. Similarly, in the present study, meat obtained from animals supplemented with CLA had a higher percentage of some SFAs, which promoted a decrease in the susceptibility to oxidation. In this sense, Wiegand et al. (2002) reported that pigs fed with CLA diets produced meat that had lower TBARS values than animals fed with a traditional diet. They attributed this improvement to the reduction in PUFAs and an increase in SFAs in meat with CLA because unsaturated fatty acids are more susceptible to oxidation (Cardenia et al. 2011).

**Fatty acid analysis in subcutaneous and intramuscular tissue**

CLA diets promote or increase the amount of SFAs and reduce MUFA (Tous et al. 2013). Consistent with our findings, the composition of fatty acids in the intramuscular fat was modified by CLA increasing the SFAs in the loin of pork with the concomitant inhibition of lipid oxidation according to the increased concentration of CLA in the meat (Wiegand et al. 2002; Cordero et al. 2010; Barnes et al. 2012). Moreover, Dunshea et al. (2005) and Morel et al. (2013) reported similar data to those in our study regarding the higher efficiency in CLA transfer in subcutaneous tissue compared to intramuscular LT, with the cis-9, trans-11 CLA isomer being more efficiently transferred than the trans-10, cis-12 CLA isomer in both tissues. Ivanovic et al. (2015) and Morel et al. (2013) reported a similar response in the FA profile, in which SFAs are increased by the effect of CLA supplementation irrespective of the level of CLA used. Lipid characteristics are important for meat quality as soft fat meat shows rancidity and develops an oily appearance (Maw et al. 2003). These authors reported that the softness of fat is associated with a decreased percentage of palmitic and stearic acid and increased percentage of linoleic and α-linolenic acid. Thus in our study, meat obtained from groups of animals supplemented with CLA has less softness because it contains a higher percentage of palmitic and stearic with respect to the RAC group. The relationship of PUFA/SFA and n-6/n-3 showed values similar to those reported by Dugan et al. (2015), Kasprzyk et al. (2015) and Yang and Lien (2016) for pork meat. However, from the perspective of human health associated with fatty acids, these values exceed the recommendation according to the UK Department of Health (1994) since they must be above of 0.4 for PUFA/SFA and lower than 4 for n-6/n-3 (Wood et al. 2008).

**Conclusions**

This study was carried out under commercial conditions and showed that CLA could be an alternative to ractopamine, a compound that has been restricted in several countries, in the pork industry due to the similarity in the responses of meat quality parameters found between CLA and ractopamine. In addition, we found an improvement in productive performance (gain weight, final body weight) with supplementation with CLA in low doses in gilts; however, this improvement was not observed in barrows.

**Acknowledgements**

The authors are grateful to the owner of the farm for allowing this experiment to be performed under commercial conditions.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**ORCID**

Araceli Pinelli-Saavedra http://orcid.org/0000-0003-1487-5767

**References**

Barnes KM, Winslow NR, Shelton AG, Hlusko KC, Azain MJ. 2012. Effect of dietary conjugated linoleic acid on marbling and intramuscular adipocytes in pork. J Anim Sci. 90:1142–1149.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 37:911–917.

Cardenia V, Rodriguez-Estrada MT, Cumella F, Sardi L, Della Casa G, Lercker G. 2011. Oxidative stability of pork meat lipids as related to high-oleic sunflower oil and vitamin E diet supplementation and storage conditions. Meat Sci. 88:271–279.

Cassens RG, Demeyer D, Eikelenboom G, Honikel KO, Johanson G, Nielsen T. 1995. Recommendations of reference methods for assessment of meat colour. San Antonio (TX): Proceedings of the 41st international congress of meat science and technology.

Cordero G, Isabel B, Menoyo D, Daza A, Morales J, Piñeiro C, López-Bote CJ. 2010. Dietary CLA alters intramuscular fat
and fatty acid composition of pig skeletal muscle and subcutaneous adipose tissue. Meat Sci. 85:235–239.

Danfort E, Burguer AG. 1989. The impact of nutrition on thyroid hormone physiology and action. Annu Rev Nutr. 9: 201–227.

Department of Health. 1994. Nutritional aspects of cardiovascular disease. Report on health and social subjects No.46. London (UK): HMSO.

Dugan M, Vahmani P, Turner T, Maplye C, Juárez M, Prieto N, Beaulieu A, Zijlstra R, Patience J, Aalhus J. 2015. Pork as a source of omega-3 (n-3) fatty acids. J Clin Med. 4: 1999–2011.

Dugan MER, Aalhus JL, Kramer JKG. 2004. Conjugated linoleic acid pork research. Am J Clin Nutr. 79:1212S–1216S.

Dugan MER, Aalhus JL, Schaefer AL, Kramer JKG. 1997. The effect of conjugated linoleic acid on fat to lean repartitioning and feeding conversion in pigs. Can J Anim Sci. 77: 723–725.

Dunshea FR, D’Souza DN, Pethick DW, Harper GS, Warner RD. 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci. 71:8–38.

Fernández-Figares I, Conde-Aguilera JA, Nieto R, Lachica M, Aguilera JF. 2008. Synergistic effects of betaine and conjugated linoleic acid on the growth and carcass composition of growing Iberian pigs. J Anim Sci. 86: 102–111.

Hintze J. 2001. NCSS and PASS. Number Cruncher Statistical Systems. Kaysville, Utah [accessed 2018 Nov 24]. www.ncss.com

Ivanovic J, Pantic S, Dokmanovic M, Glamoclija N, Markovic R, Janjic J, Baltic MZ. 2015. Effect of conjugated linoleic acids in pig nutrition on quality of meat. Procedia Food Sci. 5:105–108.

Kasprzyk A, Tyra M, Babicz M. 2015. Fatty acid profile of pork from a local and a commercial breed. Arch Anim Breed. 58:379–385.

Krzywicki K. 1982. The determination of haem pigments in meat. Meat Sci. 7:29–36.

Lee BJ, Hendricks DG, Cornforth DP. 1998. Antioxidant effects of carnosine and phytic acid in a model beef system. J Food Sci. 63:394–398.

Matak KE, Maditz KH, Barnes KM, Beamer SK, Kenney PB. 2013. Effect of dietary inclusion of conjugated linoleic acid on quality indicators of aged pork loin. J Agric Sci. 5: 1–8.

Maw SJ, Fowler VR, Hamilton M, Petchey AM. 2003. Physical characteristics of pig fat and their relation to fatty acid composition. Meat Sci. 63:185–190.

Morel PC, Janz JA, Zou M, Purchas RW, Hendriks WH, Wilkinson BH. 2008. The influence of diets supplemented with conjugated linoleic acid, selenium, and vitamin E, with or without animal protein, on the composition of pork from female pigs. J Anim Sci. 86: 1145–1155.

Morel PCH, Leong J, Nuijten WGM, Purchas RW, Wilkinson BHP. 2013. Effect lipid type on growth performance, meat traits and the content of long chain n-3 fatty acids in pork meat. Meat Sci. 95:151–159.

National Research Council [NRC]. 1998. Nutrient requirements of swine. Washington (DC): The National Academies Press.

Needham T, Hoffman LC. 2015. Carcass traits and cutting yields of entire and immunocastrated pigs fed increasing protein levels with and without ractopamine hydrochloride supplementation. J Anim Sci. 93:4545–4556.

Norma Oficial Mexicana [NOM- 033- ZOO-1995]. 1995a. Sacrificio humanitario de los animales domésticos y silvestres. México: SAGARPA.

Norma Oficial Mexicana [NOM-EM-051-ZOO-1995]. 1995b. Especificaciones técnicas para el uso de beta-agonistas en los animales. México: SAGARPA.

Ostrowska E, Muralitharan M, Cross RF, Bauman DE, Dunshea FR. 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. J Nutr. 129:2037–2042.

Pariza MW, Park Y, Cook ME. 2001. The biologically active isomers of conjugated linoleic acid. Prog Lipid Res. 40: 283–298.

Park PW, Goins RE. 1994. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J Food Sci. 59:1262–1266.

Pfalzgraf A, Frigg M, Steinhardt H. 1995. Alpha-tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage. J Agric Food Chem. 43:1339–1342.

Pompoe D, Wiegand BR, Evans HL, Rickard JW, Gerlemann GD, Hinson RB, Carr SN, Ritter MJ, Boyd RD, Allee GL. 2013. Effect of corn dried distillers grains with solubles, conjugated linoleic acid, and ractopamine (paylean) on growth performance and fat characteristics of late finishing pigs. J Anim Sci. 91:793–803.

Puls CL, Ellis M, McKeith FK, Gaines AM, Schroeder AL. 2014. Effects of ractopamine on growth performance and carcass characteristics of immunologically and physically castrated barrows and gilts. J Anim Sci. 92: 4725–4732.

Ramos F, Silveira M. 2002. Agonistas adrenérgicos β2 e produção animal: III-Efeitos zootécnicos e qualidade da carne. Rev Portuguesa Cienc Vet. 97:51–62.

Schlaxon S, Tagliapietra F, Dal Maso M, Balioni L, Bittante G. 2010. Effects of low-protein diets and rumen-protected conjugated linoleic acid on production and carcass traits of growing double-muscled Piemontese bulls. J Anim Sci. 88:3372–3383.

Sechman A, Pieszka M, Rząsa J, Migdal W, Wojtysiak D, Pustkowiak H, Zivkovic B, Paściak P. 2007. The effect of dietary conjugated linoleic acid on the level of lipids, cholesterol and iodothyronines in blood of pigs. J Animal Feed Sci. 16:193–204.

Stanimirovic M, Petrujkic B, Delic N, Djelic N, Stefanovic Z. Stanimirovic Z. 2012. Dietary conjugated linoleic acid influences the content of stearic acid in porcine adipose tissue. Veterinarni Medicina. 57:92–100.

Thiel-Coooper RL, Parrish FC, Sparks JC, Wiegand BR, Ewan RC. 2001. Conjugated linoleic acid changes swine performance and carcass composition. J Anim Sci. 79: 1821–1828.

Thiel-Coooper RL, Sparks JC, Wiegand BR, Parrish Jr FC, Ewan RC. 1998. Conjugated linoleic acid improves performances and body composition in swine. J Anim Sci. 79:1821–1828.

Tous N, Lizardo R, Vilà B, Gisbert M, Font-I-Furnols M, Esteve-Garcia E. 2013. Effect of a high dose of CLA in finishing pig diets on fat deposition and fatty acid composition in.
intramuscular fat and other fat depots. Meat Sci. 93: 517–524.

Weber TE, Schinckel AP, Houseknecht KL, Richert BT. 2001. Evaluation of conjugated linoleic acid and dietary antibiotics as growth promotants in weanling pigs. J Anim Sci. 79: 2542–2549.

Wenjiao F, Yongkui Z, Yunchuan C, Junxiu S, Yuwen Y. 2014. TBARS predictive models of pork sausages stored at different temperatures. Meat Sci. 96:1–4.

Wiegand BR, Sparks JC, Parrish FC, Zimmerman DR. 2002. Duration of feeding conjugated linoleic acid influences growth performance, carcass traits, and meat quality of finishing barrows. J Anim Sci. 80:637–643.

Wood JD. 1984. Fat deposition and the quality of fat tissue in meat animals. In: Wiseman J, editor. Fats in animal nutrition. London (UK): Butterworths. p. 407–435.

Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI, Whittington FM. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78:343–358.

Yang J-R, Lien T-F. 2016. Healthy pork production through dietary n6:n3 ratio regulation. J Agric Sci. 8:25–38.