

# Effects of production system and growth promotants on the physiological maturity scores in steers

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López-Campos, Ó., Aalhus, J. L., Larsen, I. L., Juárez, M. and Basarab, J. A. 2014. **Effects of production system and growth promotants on the physiological maturity scores in steers.** *Can. J. Anim. Sci.* **94**: 607–617. Over a 2-yr period, 224 crossbred steers were allotted to a 2 × 2 × 2 factorial arrangement of treatments to determine the effect of the production system (calf-fed vs. yearling-fed), growth implant strategy (non-implanted vs. implanted) and β-agonist supplementation (no ractopamine vs. ractopamine) on physiological indicators of maturity. Dentition and ossification scores along the vertebral column were collected post-mortem during head inspection and grading. Dentition score was significantly affected ( $P < 0.001$ ) by production system, but not by implant ( $P = 0.68$ ) or β-agonist ( $P = 0.31$ ). There were significant interactions ( $P < 0.001$ ) between production system and implant strategy on the frequencies of carcasses showing ossification in the thoracic, lumbar and sacral vertebral processes. There was a significant interaction ( $P < 0.0001$ ) between the production system and implant strategy on the frequencies of the carcasses considered as <21 or >21 mo of age based on a segregation model using only physiological maturity assessments. These data emphasize the inability of physiological scores to accurately reflect chronological age, with overall classification accuracies of 0.68 and 0.53 for dentition and ossification scores. The highest overall classification accuracies were obtained using the thoracic (0.74) or lumbar (0.69) ossification scores. Implants accelerate the ossification process, particularly in younger animals, thus having a dramatic effect on numbers of animals eligible to be categorized as <21 mo of age based on physiological maturity evaluation.

**Key words:** Age, β-adrenergic, beef, dentition, growth implant, ossification, production system

López-Campos, Ó., Aalhus, J. L., Larsen, I. L., Juárez, M. et Basarab, J. A. 2014. **Les effets du système de production et des promoteurs de croissance sur les résultats physiologiques de maturité chez les bouvillons.** *Can. J. Anim. Sci.* **94**: 607–617. Pendant une période de deux ans, 224 bouvillons croisés ont été attribués à un plan factoriel 2 × 2 × 2 pour déterminer les effets du système de production (nourri au stade veau *c.* nourri au stade d'un an), de la stratégie d'implants de croissance (non implanté *c.* implanté) et de la supplémentation au β-agoniste (sans ractopamine *c.* avec ractopamine) sur les indicateurs physiologiques de la maturité. Les résultats de la dentition et de l'ossification le long de la colonne vertébrale ont été évalués après abattage pendant l'inspection de la tête et du classement. Le système de production a eu un effet significatif ( $P < 0,001$ ) sur la cote de dentition, mais non sur l'implant ( $P = 0,68$ ) ou le β-agoniste ( $P = 0,31$ ). Il y a des interactions significatives ( $P < 0,001$ ) entre le système de production et la stratégie d'implants sur les fréquences des carcasses montrant de l'ossification dans les apophyses vertébrales thoraciques, lombaires et sacrées. Il y a interaction significative ( $P < 0,0001$ ) entre le système de production et la stratégie d'implants sur les fréquences des carcasses considérées <21 ou >21 mois d'âge selon un modèle de ségrégation qui utilise seulement les évaluations physiologiques de la maturité. Ces données démontrent l'incapacité des cotes physiologiques à refléter de façon exacte l'âge chronologique, avec des précisions globales de classement de 0,68 et 0,53 pour les résultats de dentition et d'ossification. Les plus grandes précisions de classement ont été obtenues au moyen des résultats d'ossification thoraciques (0,74) ou lombaires (0,69). Les implants accélèrent le processus d'ossification, particulièrement dans les animaux plus jeunes, ayant donc un effet dramatique sur le nombre d'animaux admissibles au classement de <21 mois d'âge selon l'évaluation physiologique de la maturité.

**Mots clés:** Âge, β-agoniste, boeuf, dentition, implant de croissance, ossification, système de production

In the absence of verifiable chronological age, such as birth records, both dentition and carcass ossification have been used as physiological indicators. Changes in production practices may have altered the relationship between chronological age and physiological maturity (Shackelford et al. 1995). These changes to the

physiological age associated with different production strategies may impact the proportion of carcasses that can qualify for export markets that have imposed

**Abbreviations:** OTM, over 30 months of age; UTM, under 30 months of age

chronological age restrictions. For example, following the identification of bovine spongiform encephalopathy in the Canadian cattle herd in 2003, several countries restricted imports to cattle under 30 mo of age (UTM; e.g. United States, Mexico, Macau, Hong Kong, Taiwan) whereas Japan restricted imports to cattle less than 21 mo of age (Canfax 2009). On the other hand, maturity is also an important consideration in the determination of meat quality, as it is generally accepted that beef tenderness decreases with increasing maturity (Purslow 2005). For this reason, maturity is also considered a key factor in most of the beef quality grading systems (Polkinghorne and Thompson 2010).

In North America, there are numerous beef production systems and cattle management strategies developed to improve efficiency, reduce input costs and enable production of differentiated beef products to satisfy market needs. However, there is often a trade-off between beef quality and production economics (Reinhardt 2007). Post-weaned calves are either directed to an intensive, calf-fed or an extensive, yearling-fed beef cattle production system. Calf-fed production requires the earlier placement of calves on high-concentrate diets following a 1-mo adjustment period. Profitability and risk tends to favour the calf-fed over the yearling-fed system (Winterholler et al. 2008; Small et al. 2009; López-Campos et al. 2013). Even though economic benefits have been widely associated with the calf-fed system, concerns have been reported regarding possible deleterious effects on carcass quality (Schoonmaker et al. 2004).

Growth implants and  $\beta$ -adrenergic agonists, commonly called repartitioning agents, have been integrated into these production systems as routine management practices; in North America over 90% of feedlot-finished slaughter cattle receive some type of growth promotant (Johnson and Hanrahan 2012). Hormonal growth promotants and  $\beta$ -adrenergic agonists work through separate mechanisms; however, both act to increase protein deposition (Apple et al. 1991; Gruber et al. 2007; Winterholler et al. 2007). Combinations of implants that contain estrogenic and androgenic hormones are a common practice in the cattle industry and they produce a greater response than single-hormone implant strategies (Reinhardt 2007). However, implanting steers and heifers with estrogenic growth-promotants, especially in combination with trenbolone acetate, also advances skeletal maturity (Apple et al. 1991; Foutz et al. 1997; Reiling and Johnson 2003). This effect is especially significant in heifers, which have more advanced skeletal maturity than steers (Boleman et al. 1998).

Thus, the objective of this study was to examine both dentition and carcass ossification in animals of known chronological age to determine the impact of calf-fed vs. yearling-fed production systems, with and without aggressive growth implant and  $\beta$ -adrenergic agonists, on physiological indicators of chronological age.

## EXPERIMENTAL DETAILS

### Animal Management

All cattle used in this research were produced, managed and slaughtered at the Lacombe Research Centre, Agriculture and Agri-Food Canada (Lacombe, AB). All dietary treatments and experimental procedures were approved by the Lacombe Research Centre Animal Care Committee. Animals were cared for as outlined under the guidelines established by the Canadian Council on Animal Care (1993). The management of the cow-calf herd has been previously described by Basarab et al. (2007, 2011). In each of 2 yr, 112 spring-born steer calves were assigned at weaning to a  $2 \times 2 \times 2$  factorial arrangement of treatments to determine the effect of production system (calf-fed harvested at 11–14 mo of age; yearling-fed harvested at 19–23 mo of age), growth implant (non-implant; implant) and  $\beta$ -adrenergic agonist (no  $\beta$ -agonist;  $\beta$ -agonist) on physiological indicators of chronological age. Steer calves were allocated to production systems and implant groups based on birth date, calf weight (42.2 kg, SD = 6.3 kg) and dam age (4.8 yr, SD = 2.7 yr). One-half ( $n = 56$ ) of the calf-fed and yearling-fed steers were implanted at prescribed intervals with 200 mg progesterone and 20 mg estradiol benzoate (Component E-S, Elanco-Animal Health A Division of Eli Lilly Canada Inc., Toronto, ON). In both, calf-fed and yearling-fed steers, the last implant was with 120 trenbolone acetate and 24 mg estradiol (Component TE-S, Elanco-Animal Health A Division of Eli Lilly Canada Inc., Toronto, ON) approximately 90–100 d before slaughter. In addition, half of each non-implanted and implanted group were supplemented with 200 mg head<sup>-1</sup> d<sup>-1</sup> of ractopamine hydrochloride for 28 d before slaughter (Optaflexx™, Elanco-Animal Health A Division of Eli Lilly Canada Inc., Toronto, ON). Further information on the production systems, diets and experimental treatments is detailed in López-Campos et al. (2013).

All steers were targeted to be slaughtered within a commercial range and constant backfat end point of 8 to 10 mm with 14 animals in each slaughter group. At 1- to 2-wk intervals, steers were transported 3 km for slaughter, processing and fabrication at the federally inspected Lacombe Research Centre abattoir (Establishment No. 021), such that there were seven implanted and seven non-implanted steers within each slaughter group.

### Dentition and Physiological Age Evaluation

At the time of slaughter, two experienced evaluators estimated steer age using dentition pictorial standards (USDA-FSIS 2012). This pictorial system bases the aging of cattle on the eruption times for the permanent incisors. Based on this system, animal age is scored as: Score 3,  $\leq 14$  mo; Scores 4 and 5, 15–18 mo; Scores 6, 7 and 8, 18–24 mo; Scores 9 and 10, 24–30 mo; and Score 11 or higher,  $> 30$  mo. Carcasses were symmetrically

split, with special emphasis in the lumbar and sacral areas, to be eligible for detailed maturity assessment. The spinous processes and cartilaginous caps were fully intact and visible on both sides of the carcass in order to confidently assess ossification to assign a carcass to the <21 mo group. When splitting was non-symmetrical resulting in cartilage being unevenly distributed between the two sides, carcasses were considered a bad split and consequently were not evaluated. An experienced evaluator using the criteria established in the Canadian beef age verification study (Robertson et al. 2006) assessed the physiological maturity on the carcasses. The criteria for the age verification of carcasses from cattle <21 mo of age are summarized in Table 1 and Fig. 1. In short, the primary foci of the evaluations for maturity were the caps of the lumbar vertebrae, the caps of the thoracic vertebrae and the segments and caps of the sacral vertebrae. Imperfectly split areas of the carcasses were noted but not scored in the study. Both sides of the carcass were evaluated since left and right sides of even nearly perfectly split carcasses can give a different impression of the amount of cartilage and the degree of ossification, thus resulting in dissimilar evaluations. When evaluations between sides were dissimilar the highest maturity score was assigned.

After assessing all the lumbar vertebrae, the single vertebra showing the greatest maturity was used to assign a lumbar score to the carcass. The lumbar score system was: Score 0, no islands of ossification; Score 1, one short island; Score 2, two short islands; Score 3, one long or thick island, or two moderately long islands; Score 4, two long islands with short gaps between them; and Score 5, two islands fused with a single island extending nearly across the width of the cap (Fig. 1B). As determined in Robertson et al. (2006), carcasses having a lumbar score greater than 2 were rejected from the eligible pool as being >21 mo of age. Carcasses receiving lumbar scores of 2 or less were further evaluated, with

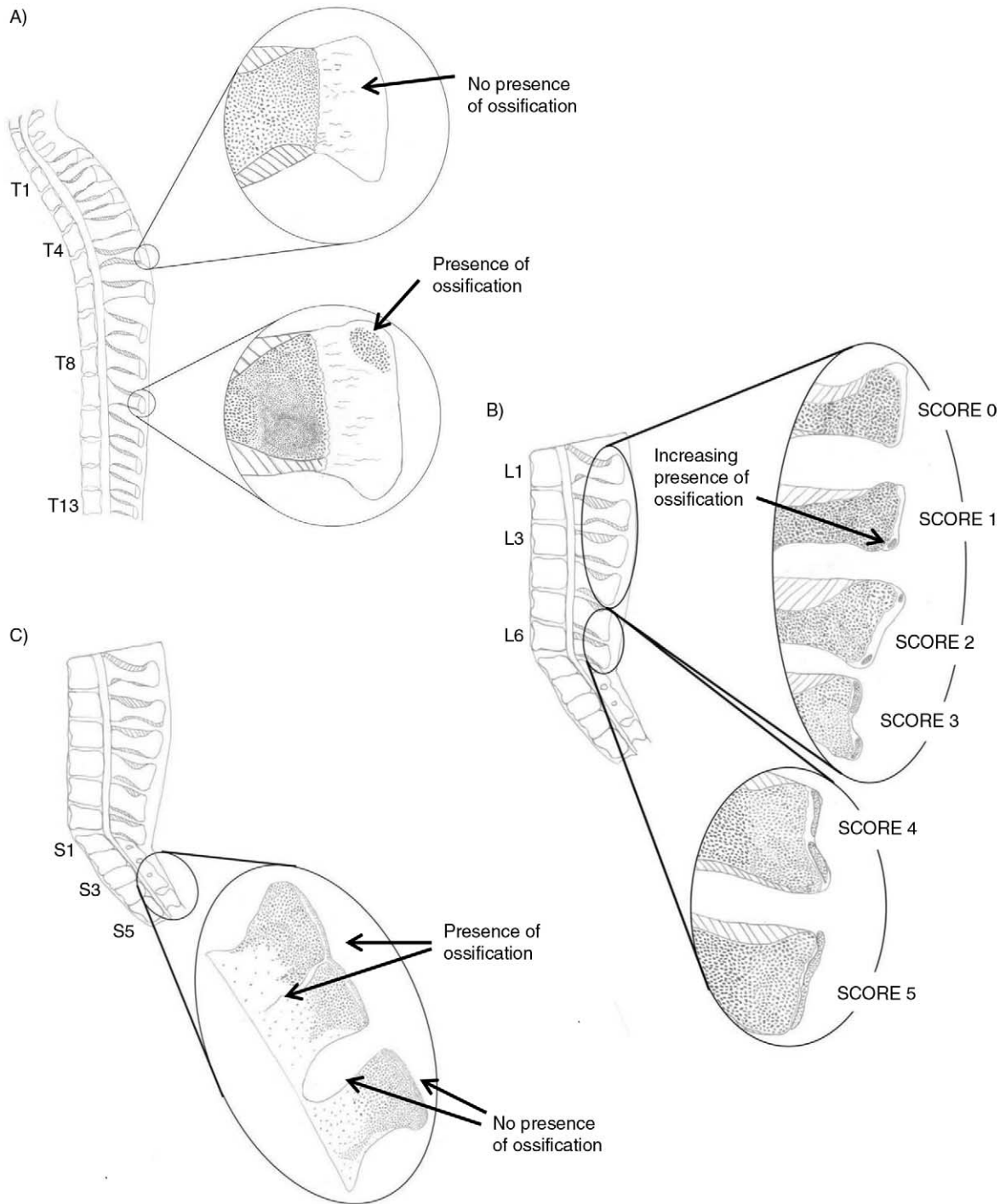
particular emphasis on the degree of separation between the sacral segments and the amount of cartilage evident in the sacrum. In order to qualify for the under 21 mo of age group, the separation between the sacral segments must show no evidence of any two segments beginning to fuse together, i.e., there must be some cartilage between the segments. Additionally, the cartilage over the segments was considered. If the ossification islands over the sacral segments were fused together, or if the islands were not fused, but were thick and extended widely over each segment, then the carcass was rejected (Fig. 1C). In the case of thoracic vertebrae, carcasses with buttons, or evidence of ossification in the cartilaginous caps were rejected from the eligible pool (Fig. 1A). Carcasses receiving a lumbar score of 2 or less (scores 0 or 1) but which were eliminated from the eligible pool based on the assessment of the thoracic vertebrae or the sacrum were scored as 0X, 1X or 2X. The number indicates the lumbar score and the X indicates that although the carcass met the criterion set for the lumbar vertebrae to qualify for being from an animal less than 21 mo of age, it did not do so for the thoracic vertebrae or sacrum. Other indicators of physiological maturity were not ignored. For example, occasionally carcasses that would otherwise pass based on the above criteria were rejected based on the general appearance of the split vertebrae, or the width, flatness and color of the ribs, which suggested to the evaluators that the carcass displayed more advanced maturity. In order to qualify for the younger group, the split vertebrae must be porous and red, while the ribs must be narrow and generally rounded and have some red coloration.

#### Statistical Analysis

Statistical analyses were performed using SAS software (SAS Institute, Inc. 2009). Scores for dentition, and ossification, and further classification into above and below 21 mo of age were analyzed with the PROC FREQ

**Table 1. Criteria for the age verification of carcasses from fed cattle less than 21 mo of age using physiological maturity characteristics (Robertson et al. 2006)**

Group	Lumbar vertebrae	Thoracic vertebrae	Sacrum
<21 months	<u>Score 2 or less</u> 0 = no islands of ossification. 1 = 1 short island. 2 = 2 short islands. Split vertebrae soft, porous and red. Ribs narrow, generally rounded and red in coloration.	No ossification in caps.	Distinct separation of vertebrae with cartilage between (open). Caps with no more than short, thin islands of ossification. Islands of ossification distinct, not fused together.
21 months or older	<u>Score 3 or higher</u> 3 = 1 long or thick island, or 2 mod. long islands. 4 = 2 islands with short gap between. 5 = islands fused together. Score of 2 or less Split vertebrae hard, flinty or white. Ribs tending to be wide, flat and less red in color.	Ossification evident in cap(s)	Any two vertebrae starting to fuse together, no cartilage between (not open). Islands of ossification extensive or thick or fused together.



**Fig. 1.** Overview of the criteria established in the Canadian beef age verification study (Robertson et al. 2006) for the thoracic (A), lumbar (B) and sacral regions (C). Technical drawings by Mr. Christopher Villacorta-López.

procedure using a chi-square ( $\chi^2$ ) option and Fisher's exact test (SAS Institute, Inc. 2009). Accuracy of classification, overall and within each group, using dentition or ossification scores was calculated according to the confusion matrix procedure described by Kohavi and

Provost (1998). Briefly, the confusion matrix procedure is used to assess the performance of a classifier. This procedure contains information about actual and predicted classifications obtained by the different methods or criteria applied. In this study, birthdate was the actual

classifier while dentition and ossification scores were the predicted. Accuracy is widely used as a metric for the evaluation of classification systems; higher accuracy means better performance. Partial least squares discriminant analysis (PLS2-DA) was applied to segregate the carcasses into <21 mo of age (eligible) or >21 mo of age (non-eligible). This model seeks to correlate dentition and ossification scores (X) with the eligibility of the carcass based on birth date (Y), attempting to maximize the covariance between the two types of variables for group differences and ignoring variance within a class. In this type of approach, Y is a dummy matrix with arbitrary numbers assigned to the different classes to be distinguished (eligible, carcasses <21 mo of age = 1, non-eligible, carcasses >21 mo of age = 2) (Naes et al. 2002). According to this equation, a sample was classified as belonging to a specific category (eligible or non-eligible) if the predicted value was within  $\pm 0.5$  of the dummy value. The accuracy of the models obtained was evaluated using the percentage of correctly classified samples. Cross-validation (leave one-out) was performed to validate calibrations and to restrict the number of PLS terms incorporated in the regression, to prevent overfitting. Dentition and ossification scoring data management and PLS2-DA were performed by means of The Unscrambler<sup>®</sup> software (version 10.2, Camo, Trondheim, Norway).

## RESULTS AND DISCUSSION

### Dentition Scores

In the present study, yearling-fed steers were harvested at 19–23 mo of age (average  $21.2 \pm 0.3$  mo) and were about 8 mo older than calf-fed steers that were harvested at 11–15 mo of age (average  $13.0 \pm 0.3$  mo). Based on verifiable birth dates, 100% of the calf-fed steers, and only 22% of yearling-fed steers were <21 mo of age and would meet export criteria established by Japan prior to January 2013.

As expected, the dentition score frequencies were affected by production system ( $P < 0.001$ ; Table 2). Dentition scores were the lowest (scores 4 and 5) for calf-fed steers, and spread across the full range (scores 4 to 7) for yearling-fed steers. There were no significant ( $P > 0.05$ ) interaction effects or main effects of implant

( $P = 0.68$ ) or  $\beta$ -agonist ( $P = 0.31$ ) on dentition scores. Since the dentition score equates to a range of chronological age, only scores of 4 and 5 (15–18 mo) relate to an age range below 21 mo of age, whereas scores of 6 and 7 (18–24 mo) could not be reliably used to segregate carcasses into an age range below 21 mo of age. Hence, according to dentition, 100% of carcasses from calf-fed steers would have been accurately classed as being <21 mo of age. In contrast, 83% of yearling-fed carcasses would have been classed as being <21 mo of age, which was an over-estimation compared with actual chronological age (data not shown).

Accurately determining the chronological age of cattle lacking birth records has been a challenge to the beef industry, particularly, after crises like bovine spongiform encephalopathy, when protocols to segregate cattle under 30 (UTM) and over 30 months (OTM) were issued. Using dentition as an age verification method has provided a quick and simple procedure in the past and still has a role at the slaughterhouse in cross-checking cattle age (Graham and Price 1982; Priestley 2013). However, previous studies in the literature have reported high variability among individual animals on the eruption age of the incisors (Wiener and Forster 1982), and other procedures have been shown feasible and might introduce a verifiable objectivity when predicting cattle age (Raines et al. 2008).

### Ossification Scores

In addition to dentition as an age verification method, physiological beef carcass maturity has been used to segregate UTM/OTM carcasses or to ensure there was a critical mass of age-verified cattle that could be identified and accepted by export markets. As well, a premise of many beef grading systems is that advances in physiological carcass maturity result in decreases in palatability of beef (Breidenstein et al. 1968; Smith et al. 1988).

Physiological beef carcass maturity is mainly determined by evaluating the degree of ossification in the bones and cartilages in the vertebral column. In cattle, ossification generally occurs at an earlier stage of maturity in the posterior portion of the vertebral column (sacral vertebrae) and gradually progresses towards the

Table 2. Main effects of the production system, implant strategy and  $\beta$ -agonist on dentition score frequencies (%)

Dentition	Production system <sup>z</sup>		Implant strategy <sup>y</sup>		$\beta$ -agonist strategy <sup>x</sup>	
	Calf-fed	Yearling-fed	Non implanted	Implanted	No $\beta$ -agonist	$\beta$ -agonist
4	96.4	30.6	65.8	61.3	66.7	60.4
5	3.6	52.3	27.9	27.9	26.1	29.7
6	0	14.4	5.4	9.0	7.2	7.2
7	0	2.7	0.9	1.8	0	2.7

<sup>z</sup>Production system  $\chi^2 < 0.001$ .

<sup>y</sup>Implant strategy  $\chi^2 = 0.68$ .

<sup>x</sup> $\beta$  - agonist strategy  $\chi^2 = 0.31$ .

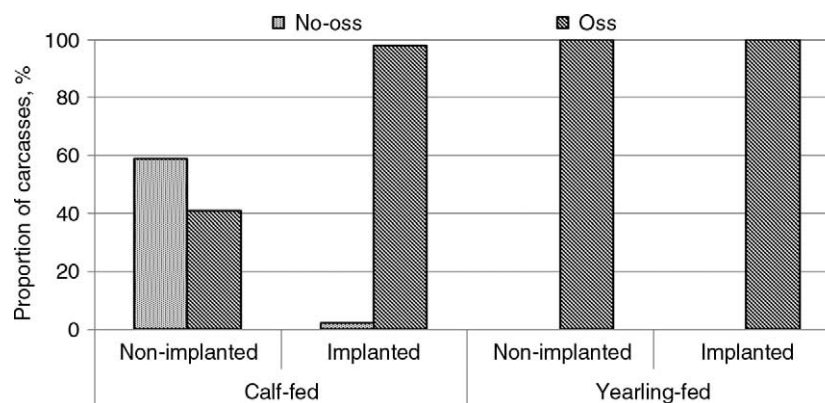
anterior portion through the lumbar, and at later stages of maturity in the thoracic vertebrae (Tatum and Collins 1997).

In practice, evaluation of ossification along the vertebral column requires a balanced carcass split, allowing visual inspection in all regions. Overall, in the present study, only 9% of the carcasses (20 carcasses out of 224) were considered as being imperfectly split and thus ineligible for assessment. In the subsequently evaluated carcasses there were significant interactions ( $P < 0.001$ ) between the production system and the implanting strategies on the frequencies of the carcasses showing ossification in the sacral, lumbar and thoracic vertebral column portions (Figs. 2, 3 and 4). All (100%) of the carcasses from the yearling-fed steers clearly showed an advanced stage of fusion between the sacral vertebrae and ossification islands over each segment, regardless of implant treatment. In contrast, in the calf-fed steers, 59% of the non-implanted and 2% of the implanted carcasses were at earlier stages of physiological maturity and did not show any evidence of fusion in the sacral portion of the vertebral column (Fig. 2). In the case of the lumbar portion of the vertebral column, none of the non-implanted calf-fed steers showed any ossification (score 0), while most of the implanted calf-fed steers (80%) showed varying degrees of ossification (scores ranged from 1 to 5). In the case of yearling-fed steers, all the carcasses from the implanted animals showed advanced ossification ranging from two islands (score 3, 17% and score 4, 15%) to a long, fused island on the vertebral cap (score 5, 67%). On the contrary, 66% of the non-implanted yearling-fed steers did not show any osseous formations in the lumbar vertebral caps (score 0) and 26% showed a single island (score 1) with minimal frequencies in the remaining scores (scores 2, 3 and 5, 3% each) (Fig. 3). As far as the thoracic portion of the vertebral column, 96% of the implanted yearling-fed steers clearly showed ossification in this vertebral

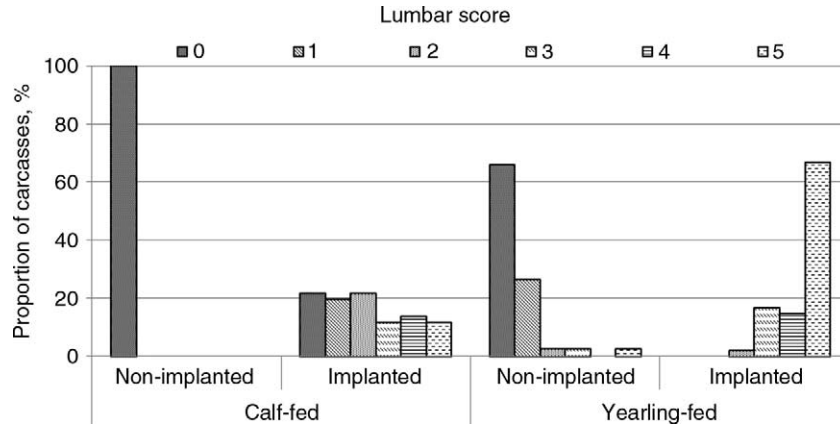
portion, while most of the non-implanted yearling-fed (98%) and both implant groups of the calf-fed steers (non-implanted 100% and implanted 91%), did not show ossification in the thoracic caps (Fig. 4). A major factor influencing skeletal maturity is estrogen concentration in the animal (Silberberg and Silberberg 1939) and implanting with estrogenic growth-promoting hormones advances skeletal maturity. Turner (1981) reported more advanced maturity scores for steers implanted with estradiol benzoate. Likewise, Foutz et al. (1997) and Roeber et al. (2000) reported advanced skeletal maturity scores for steers implanted with combined estradiol benzoate and trenbolone acetate. Moreover, Apple et al. (1991) and Reiling and Johnson (2003) also found more advanced skeletal maturity scores in implanted steer carcasses. The results of the present study are in agreement that implanting practices advance physiological maturity.

Studies in the literature have reported no effects of  $\beta$ -adrenergic agonist on physiological maturity. For instance, Beckett et al. (2009) and Holmer et al. (2009), in studies conducted to evaluate the effects of the  $\beta$ -adrenergic agonist zilpaterol hydrochloride on calf-feeding Holstein steers, reported no effects on the skeletal maturity score. Also, Allen et al. (2009) reported no effects on the lean and skeletal maturity of Holstein cows using ractopamine hydrochloride. Similarly, in the present study there were no significant effects ( $P > 0.1$ ) of the  $\beta$ -agonist on the frequencies of the carcasses showing ossification at the sacral, lumbar and thoracic vertebral column portions.

The results of the present study support that the physiological age of the carcasses might be dramatically impacted depending on the combination of the production system and growth implant strategy. Advanced ossification may result in non-eligible carcasses for specific markets or branded programs with a subsequent impact on the beef industry profits. For example, until



**Fig. 2.** Interaction effects between production systems  $\times$  implant strategies (calf-fed  $\times$  implanting strategy  $\chi^2 P < 0.001$ ; yearling-fed  $\times$  implanting strategy  $\chi^2 P = 0.479$ ) on ossification processes at the sacral portion of the vertebral column. No-oss, no presence of the ossification processes; Oss, presence of the ossification processes.

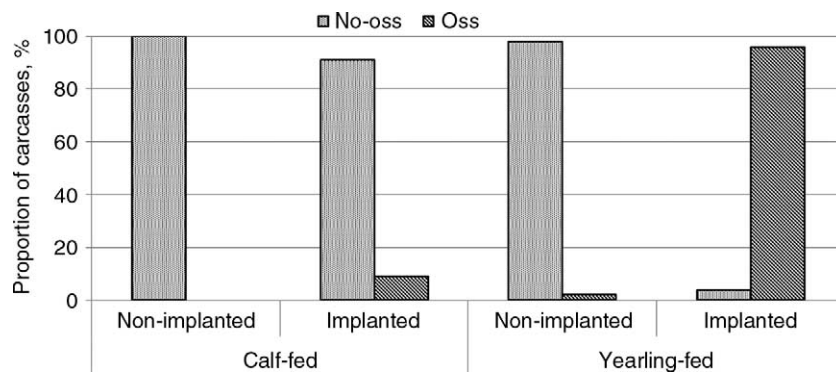


**Fig. 3.** Interaction effects between production systems × implant strategies (calf-fed × implanting strategy  $\chi^2 P < 0.001$ ; yearling-fed × implanting strategy  $\chi^2 P < 0.001$ ) on ossification processes at the lumbar portion of the vertebral column. Lumbar score (Robertson et al. 2006): Score 1, one short island; Score 2, two short islands; Score 3, one long or thick island or two moderately long islands; Score 4, two long islands with short gaps between them; and Score 5, two islands fused with a single island extending nearly across the width of the cap.

January 2013, the Japanese market only accepted beef from cattle that were less than 21 mo of age at the time of slaughter. This age requirement was verified through quality assessment programs, tracking birth date for market purposes, or verification processes such as the physiological maturity assessments developed by Robertson et al. (2006), applied in this study. As previously mentioned, in the present study, according to birthdate, 100% of calf-fed animals and 22% of yearling-fed animals were <21 mo of age. However, using physiological age estimates based on ossification as proposed by Robertson et al. (2006) only 2% of the implanted and 55% of the non-implanted calf-fed steers were considered eligible for <21 mo of age and hence eligible for the Japanese market (data not shown). At the same time, 100% of both implanted and non-implanted yearling-fed steers were considered >21 mo and non-eligible. Using ossification score criteria all carcasses would have been correctly identified as being under 30 mo of age.

A confusion matrix (Kohavi and Provost 1998) was applied in order to identify the accuracy, overall and within each group, of the different age verification methods used in the present study to predict the eligibility of carcasses for <21 mo (Table 3). The confusion matrix contains information about actual and predicted classifications obtained by the different methods or criteria applied. In this study, birthdate was the actual classifier while dentition and ossification scores were the predicted. Accuracy is widely used as a metric for the evaluation of classification systems; higher accuracy means better performance.

The accuracies obtained in the matrix were widely divergent, depending on the criteria used (dentition or ossification processes) to predict eligibility of the carcasses and the population where those criteria were applied (overall or within each group). The highest overall classification accuracies were obtained using the thoracic (0.74) or lumbar (0.69) ossification scores. Although the thoracic ossification processes were overall



**Fig. 4.** Interaction effects between production systems × implant strategies (calf-fed × implanting strategy  $\chi^2 P = 0.03$ ; yearling-fed × implanting strategy  $\chi^2 P < 0.001$ ) on ossification processes at the thoracic portion of the vertebral column. No-oss, no presence of the ossification processes; Oss, presence of the ossification processes.

Table 3. Accuracy calculated using the matrix of confusion<sup>z</sup> of physiological scores compared to chronological age

	Overall					Calf-fed		Yearling-fed	
		Calf-fed	Yearling-fed	Implant	Non-implant	Implant	Non-implant	Implant	Non-implant
Birth date	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dentition	0.68	1.00	0.37	0.68	0.68	1.00	1.00	0.38	0.36
Ossification									
Thoracic	0.74	0.95	0.53	0.85	0.63	0.91	1.00	0.80	0.26
Lumbar	0.69	0.80	0.57	0.72	0.67	0.63	1.00	0.81	0.26
Sacrum	0.54	0.28	0.80	0.41	0.68	0.02	0.57	0.82	0.77
Ossification scoring system <sup>y</sup>	0.53	0.26	0.79	0.42	0.67	0.02	0.55	0.80	0.78

<sup>z</sup>Accuracy calculated using the matrix of confusion proposed by Kohavi and Provost (1998) where birthdate was the actual classifier and dentition and ossification scores were used as predicted classifier.

<sup>y</sup>Physiological maturity assessments based on the ossification scoring system developed by Robertson et al. (2006).

the most robust age verification method, compared with chronological age the thoracic criteria over-estimated by 12% the number of overall eligible carcasses. Dentition also tended to over-estimate by 30% the number of eligible carcasses, and subsequently had lower accuracy (0.68). The lowest overall classification accuracy was obtained when using the method that integrated the thoracic, lumbar and sacral ossification scores (0.53), significantly under-estimating the number of eligible carcasses (12%). While the ossification system previously developed produced a high level of confidence, which, when appropriately applied, would result in the selection of carcasses only from cattle less than 21 mo of age, retention rates were very low in the overall population. This system provided a conservative approach required to ensure the elimination of all carcasses derived from older animals.

Lawrence et al. (2001) reported little evidence of agreement between age estimates derived from USDA ossification-based maturity system, which includes overall evaluation of thoracic, lumbar and sacral vertebrae, and counting the number of incisors present at slaughter. These authors also suggested that objectively counting the number of incisors provides a more accurate method of age estimation.

In the present study, the accuracy of the sacral ossification criteria in the calf-fed was lower (0.28) than in the yearling-fed steers (0.80). Given the pattern of ossification during maturation moves from posterior to anterior, in the calf-fed steers including the sacral vertebrae criteria in the eligibility system makes it too exigent. In this sense, at earlier stages of maturity (calf-fed), the ossification scoring system (thoracic, lumbar and sacral vertebrae) showed a poor performance and hence the accuracies were low (calf-fed, 0.26; implanted calf fed, 0.02 and non-implanted calf-fed, 0.55). Conversely, the ossification scoring system performance improved and accuracies were higher (yearling-fed, 0.76; implanted yearling-fed, 0.80 and non-implanted yearling-fed, 0.78) when these criteria were applied to the yearling-fed steers. In the calf-fed steers, the inclusion of dentition, thoracic and lumbar criteria showed better

performance (accuracies: 1.00, 0.80 and 0.95, respectively) than in the yearling-fed (accuracies: 0.37, 0.57 and 0.53, respectively).

These results suggest that when birth date documentation is not available, a compendium of descriptors (e.g., dentition and ossification processes) should be taken into consideration in order to establish the eligibility of the carcass to meet certain age criteria. In addition, since dentition score equates to a range of chronological age, there are potential gaps that dentition criteria may not cover, and in these cases ossification criteria may provide additional information. On the other hand, based on the accuracies obtained in the present study, some descriptors may have more impact than others depending on the maturity stage. For instance, when evaluating a yearling-fed carcass (dentition score > 5), thoracic, lumbar and sacral might be the main descriptors to establish the age eligibility of that carcass. In cattle populations within a range of age between 11 mo to 23 mo (overall population in the present study), the use of this holistic approach might provide not only a higher level of confidence but also a higher retention of carcasses to meet market criteria.

When the dentition and all the ossification scores (thoracic, lumbar, and sacrum) were used to discriminate into eligible (<21 mo of age) or non-eligible (>21 mo of age) carcasses in the overall population, the regression model developed using a PLS2-DA and including 2 PLS terms correctly classified 88.2% of the <21 mo of age carcasses and 63.6% of the >21 mo of age (Table 4). Similar results were observed when the calibration model used dentition, lumbar and thoracic scores (87.8 and 63.2% eligible and non-eligible carcasses correctly classified, respectively), which indicated that the sacrum score did not provide much information for discrimination. The percentage of misclassified carcasses over 21 mo of age was high (36.4%); further examination showed that all carcasses corresponded to the non-implanted yearling-fed. As previously discussed, implanting practices have an impact on physiological ossification, thus introducing a treatment variable which reduces the ability of the model to discriminate on a chronological age



**Table 4. Results (%) obtained by partial least squares discriminant analysis using dentition and ossification scores (thoracic, lumbar and sacrum) to segregate into eligible (<21 mo of age) or non-eligible (>21 mo of age) carcasses on the overall population**

Physiological criteria	PLS terms <sup>z</sup>	Carcass eligibility	Classified (%)	
			Cross-validation	
			Eligible	Non-eligible
Dentition + thoracic + lumbar + sacrum	2	Eligible Non-eligible	<b>88.2</b> 36.4	11.8 <b>63.6</b>
Dentition + thoracic + lumbar	2	Eligible Non-eligible	<b>87.8</b> 36.8	12.2 <b>63.2</b>
Thoracic + lumbar	2	Eligible Non-eligible	<b>87.8</b> 44.1	12.8 <b>55.9</b>
Dentition + thoracic	1	Eligible Non-eligible	<b>87.8</b> 44.1	12.2 <b>55.9</b>

<sup>z</sup>PLS terms = partial least square terms.

basis. In addition, both development and verification stages of the ossification scoring criteria used in the present study (Robertson et al. 2006) used cattle from commercial sources, which mostly (90%) receive some type of growth promotant (Johnson and Hanrahan 2012).

When only two criteria were included in the discrimination model, either thoracic and lumbar ossification or dentition and thoracic scores, the percentage of <21 mo of age carcasses correctly classified was similar (87.8%) to that found for the overall population (88.2%). However, a decrease in the number of >21 mo of age carcasses correctly classified (55.9% vs. 63.6%) was observed, supporting the need to include multiple criteria for greater accuracy (Table 4).

Increasing market demand for accurate age determination in Canadian cattle has led to an effort to develop and improve a livestock tracking system. The Canadian Cattle Identification Agency (CCIA) has an efficient age verification process to track livestock from birth to slaughter. Compared with systems worldwide that only have dentition or ossification available, the age verification process is effective and internationally recognized.

### CONCLUSION

The results of the present study confirm that production system and growth promotants affect ossification. Use of growth implants in a calf-fed production system accelerated the ossification process in younger animals, thus having a dramatic effect on numbers of animals eligible to be categorized as 21 mo of age based on physiological maturity evaluation. In the present study, although thoracic ossification scores were a comparatively effective age verification method to estimate the chronological age of cattle lacking birth records they still, over-estimated the number of eligible carcasses. Thus, a compendium of descriptors based on dentition and ossification processes at the thoracic, lumbar and sacral vertebrae should be taken into consideration in order to ensure accurate estimation of chronological age of cattle when birth date documentation is not available.

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