Effects of florfenicol injection on the meat characteristics of the cervical muscles in cattle

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Objective—To determine the effects of florfenicol injection on the meat characteristics of the cervical muscles in cattle.

Animals—100 steers (mean weight, 380 kg).

Procedure—In 50 calves, florfenicol (25 ml, twice) was injected into the cervical muscles of 1 side of the neck, and saline (0.9% NaCl) solution (25 ml, twice) was injected into the cervical muscles of the other side of the neck. In the remaining 50 calves, florfenicol was injected into the cervical muscles of 1 side of the neck, and nothing was injected into the cervical muscles of the other side of the neck. Animals were slaughtered 132 days later, and samples of the cervical muscles were submitted for histologic evaluation and measurement of shear forces.

Results—2 injection sites used in the present study had extensive lesions, and both of these were sites where florfenicol had been injected. However, histologic scores for the florfenicol injection sites were not significantly different from scores for the contralateral saline solution injection sites and uninjected control sites. In addition, shear force values were not significantly different between sites in which florfenicol had been injected and the contralateral sites.

Conclusions and Clinical Relevance—Results suggest that few reactions should be expected with injection of florfenicol into the cervical muscles in steers and that reactions that do occur will consist mainly of fibrosis and infiltration of adipose tissue. However, shear force values, a measure of tenderness of the meat, should not be affected. (*Am J Vet Res* 2002;63:64–68)

Studies[1-3] sponsored by the **National Cattlemen's Beef Association (NCBA)** of carcasses in packing plants and retail meat outlets have suggested that injection-site lesions continue to be a major problem for the beef industry in the United States. Similar audits of Canadian cattle carcasses have found that injection-site lesions are a problem in Canada as well.[4,5] In 1 study,[6] IM administration of various products to 48-day-old calves caused damage to the muscle tissue at the injection site, and lesions could be identified in the meat from 51.2% of the animals when the carcasses were processed 380 days later. These lesions resulted in 138 g of trim and cost $2.00/animal. Injection-site lesions have been estimated to result in losses to the fed-beef industry of approximately $55 million annually.[3,7] In nonfed beef, 1 of every 4 rounds contains injection-site lesions, costing $0.66/animal.[8] Previous studies,[1,3,6-9] have evaluated the effects of injections into the gluteus medius (top sirloin butt) or biceps femoris (outside round) muscle. However, producers are concerned about potential damage to other muscles, the effects injections may have on performance of the animals during the feeding period, and the potential lesions at the injection sites in the meat obtained when the carcasses are processed. The NCBA has suggested in a policy resolution adopted in 1994 that the only products that should be injected IM are those for which data documenting a low rate of injection-site reactivity are available.

Tenderness is a very important characteristic of meat for consumers, as consumers subjectively assess the tenderness of meat as they eat it. As a result, veterinarians working in food animal practice, teaching in veterinary schools, and working in the pharmaceutical industry should be concerned not only with efficacy of medications but also with how these medications alter the consumable product (meat). Therefore, meat tenderness should be included in studies of injection-site lesions in food animals.

Because the beef industry has an interest in the effects of IM injections into beef animals and florfenicol is extensively used to treat bovine respiratory tract disease, we chose to examine the effects of IM injection of florfenicol. The purpose of the study reported here was to determine the effects of florfenicol injection on the meat characteristics of the cervical muscles in cattle. Muscles in which florfenicol had been injected were compared with muscles in which isotonic saline (0.9% NaCl) solution had been injected and with muscles that had not received any injections.

Materials and Methods

One hundred steers (mean weight, 380 kg) purchased from sale barns were used in the study. Steers were individually identified and hot-branded in the midcervical region with a 4 × 8-cm rectangle. Brands were used to identify the locations for all injections and were positioned so that the long axis was aligned in an anterior-to-posterior direction.

At the time of processing, steers were assigned to 1 of 2 groups on an alternate basis. Steers in group 1 received an injection of florfenicol[a] in 1 side of the neck and an injection of isotonic saline solution in the other side of the neck. Steers in group 2 received an injection of florfenicol in 1 side of the neck but did not receive any injections in the other side of the neck. For both groups, the side of the neck in which florfenicol would be injected was determined for the first steer enrolled in the group by the flip of a coin. The side of the

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neck in which florfenicol was injected was then alternated for each subsequent animal that was enrolled. This procedure was used to minimize any potential bias associated with previous injections into the cervical muscles, as information on whether these animals had received any injections in the cervical muscles prior to this study was not available.

Florfenicol injection consisted of administration of 7,500 mg (25 ml containing 300 mg/ml) twice, 48 hours apart; for injection of saline solution, 25 ml of 0.9% NaCl was injected twice, 48 hours apart. Injections were given with a 16-gauge 1-in needle attached to a sterile (35-ml) plastic syringe. Animals were confined in a squeeze chute during injections. A halter was used during the first injections to minimize movements associated with branding; the second injections were given without the use of a halter.

One individual (LLH) branded all of the calves and gave the first injections; a second individual gave the second injections. Brands and injections were in the midcervical region, dorsal to the transverse processes of the vertebrae and ventral to the nuchal ligament. The omotransversarius muscle was the target muscle. No other injections were given in the cervical muscles or cervical subcutaneous tissues of any of the animals during the feeding period.

Animals were pen-fed for 132 days to a final weight of 580 kg. At the end of the feeding period, the animals were slaughtered in a commercial plant, and carcasses were allowed to cool for 24 hours. The cervical muscles (ie, the neck portion of the chuck) were then collected by packing plant personnel under the supervision of plant supervisors and the first author, vacuum packaged, and transported to the Kansas State University Meat Science Laboratory. Specimens weighed 2.84 kg, on average, and measured approximately 7.5 × 15 × 20 cm. In the laboratory, 3 muscles (the cleido-occipitalis, omotransversarius, and trapezius muscles) were dissected from the neck and examined individually for gross lesions by the first author and 2 assistants. In all but a few instances, the cutaneous musculature had been removed with the hide. However, when the cutaneous musculature was present, it was also examined.

The only muscle in which there was any evidence of gross lesions was the omotransversarius muscle, indicating that injections had been made into the target muscle. Therefore, samples (3 × 5 × 8 cm) of the omotransversarius muscle were saved for histologic and shear force evaluations. Personnel removing the samples were blinded to the treatments administered at the time the samples were removed. A section of each sample was placed in neutral-buffered 10% formalin for histologic evaluation; a second sample was vacuum packaged for shear force determinations. When a grossly visible lesion was identified, the lesion was divided to ensure that samples submitted for histologic and shear force determinations both contained part of the lesion. Samples lacking gross lesions were divided longitudinally; therefore, both samples contained a portion of the muscle length.

Warner-Bratzler shear force measurement—The Warner-Bratzler shear force measurement is generally accepted as a highly repeatable measure of meat tenderness.[10-12] The test protocol follows a standardized procedure developed by the American Meat Science Association in 1995.[13] It has been reported that restaurant quality steaks should have shear force values < 3.86 kg and that retail trade steaks should have values < 4.45 kg.[14] Previously reported Warner-Bratzler shear force data for injection sites in the semimembranosus and gluteal muscles[2,9,15] indicate that samples as much as 7.62 cm from the injection site had shear force values 40% greater than values for the uninjected contralateral muscle. In the absence of published data for cervical muscles, we used those data to conclude that increases in shear force values ≥ 10% would reduce tenderness to the point that consumers could detect the lack of tenderness. Therefore, we designed the study to detect a 10% increase in shear force with 95% confidence. To do so, we estimated that 40 animals would be needed per group, and we elected to use Warner-Bratzler shear force as an objective method of evaluating the local tissue response to florfenicol.

Vacuum-packaged samples for Warner-Bratzler shear force evaluations[13] were frozen at –29 C and later thawed at 2 to 4 C for approximately 24 hours prior to cooking. Copper-constantan 30-gauge thermocouple wires attached to a temperature recorder[b] were inserted into the geometric center of the samples for temperature monitoring. Samples were cooked in a gas convection oven[c] at 163 C to an internal temperature of 70 C. Samples were then held for 24 hours at 4 C, and cores (1.27 cm in diameter) were then collected parallel to the muscle fibers. Cores were sheared perpendicular to the fiber orientation with a Warner-Bratzler shear force attachment with a V-blade attached to a materials testing machine.[d] A 50-kg compression load cell was used with a crosshead speed of 250 mm/min.

Because meat is not a homogeneous tissue, variations within a muscle are expected. Shackelford et al[10] reported that Warner-Bratzler shear force measurements for the longissimus muscle of 400 young crossbred steers and heifers ranged from 2.7 to 14.7 kg (SD, 1.6 kg). To account for this variation, multiple core samples were obtained from each specimen in the present study, and the mean value was used for that specimen.

Histologic examination—After fixation, 2 sections approximately 2 cm^2 were taken from each muscle specimen for histologic examination. If a gross lesion was evident, 1 or more sections were taken from that area. If no gross lesions were evident, sections were taken randomly along the length of the omotransversarius muscle. Muscle sections were processed by standard procedures, sectioned to a thickness of 5 µm, and stained with H&E stain. A modification of the scoring scheme used by Glock et al[16] was used to assign histologic grades from 0 (no lesion) to 3 (severe lesion) for myodegeneration, inflammation, fibrosis, and fatty infiltration (**Appendix**). A board-certified pathologist blinded to treatment assignments graded all sections.

Data analysis—Differences between florfenicol and saline solution injection sites and between florfenicol and uninjected control sites were evaluated by use of paired *t*-tests for shear force measurement and Mantel-Haenszel methods[17-19] for histologic scores. Analyses were performed with commercial software[e]; values of $P \leq 0.05$ were considered significant.

Results

Animal identification tags were lost from 2 animals in the feed yard, and these 2 animals were removed from the study. In addition, identification tags for specimens from 2 additional animals were lost at the packing plant, and these 2 animals were also removed from the study. Thus, specimens from 96 animals were analyzed.

Specimen size and, thus, the number of cores available for shear force evaluation were dependent on the processing plant workers removing the neck muscles. Packing plant personnel tended to pull the chuck laterally with a meat hook and cut around it at an angle, sometimes resulting in a cone-shaped piece of meat instead of the desired cube. This resulted in only a small portion of the omotransversarius muscle being included in samples from some animals, and few cores could be obtained for shear force measurements.

Overall, for 1 sample, only 2 cores were obtained; for 12 samples, 3 cores were obtained; for 31 samples, 4 cores were obtained; for 62 samples, 5 cores were obtained; for 76 samples, 6 cores were obtained; for 7 samples, 7 cores were obtained; and for 3 samples, 8 cores were obtained. For each sample, the mean shear force value for all cores was used. Analysis of the data without values for the 9 animals (18 samples) with the largest sample SD and the fewest number of cores did not alter the outcome. Therefore, results for all 96 paired samples were analyzed.

For the 48 group-1 steers (florfenicol vs saline solution), shear force for the florfenicol injection sites ranged from 4.03 to 7.39 kg (mean, 5.78 kg), whereas shear force for the saline solution injection sites ranged from 4.27 to 7.60 kg (mean, 5.69 kg). Mean ± SD difference in shear force values between sides was 0.09 ± 0.08 kg, which was not significantly (P = 0.49) different from 0. For the 48 group-2 steers (florfenicol vs uninjected control), shear force for the florfenicol injection sites ranged from 4.57 to 8.05 kg (mean, 6.12 kg), whereas shear force for the uninjected control sites ranged from 4.42 to 8.07 kg (mean, 5.95 kg). Mean ± SD difference in shear force values between sides was 0.17 ± 0.10 kg, which was not significantly (P = 0.11) different from 0.

Histologically, many tissue sections contained small foci of myofibers or scattered individual myofibers with clear vacuoles, considered evidence of early degeneration. In addition, there were small clusters of individual myofibers with segmentally fragmented sarcoplasm or floccular degeneration, which appeared to be a recent change (**Fig 1**). These changes were found in sections from uninjected control sites as well as from sites where florfenicol or saline solution had been injected and, therefore, were not considered to be the result of injection reactions.

With the exception of 2 steers, inflammatory changes, when present, consisted of small foci of lymphocytes, histiocytes, and neutrophils with a few eosinophils (**Fig 2**). These findings often were associated with individual degenerate fibers. Florfenicol injection sites from 2 steers had grade-3 histologic evidence of inflammation, myodegeneration, and fibrosis. One of these steers also had a grossly evident 1.5-cm spherical nodule on the lateral aspect of the omotransversarius muscle and had grade-2 histologic evidence of infiltration of adipose tissue in and around the lesion. The adipose tissue appeared to be replacing muscle fibers. For both steers, histologic grades for the contralateral sites were 0. For 1 of these steers, shear force for the florfenicol injection site was 6.88 kg, and shear force for the saline solution injection site was 7.03 kg. For the other steer, shear force for the florfenicol injection site was 5.64 kg, and shear force for the uninjected control site was 5.88 kg.

Sarcosporidia cysts were commonly seen in muscle fibers of many sections. In 1 section, a sarcocyst within a fiber was surrounded by infiltration of inflamma-

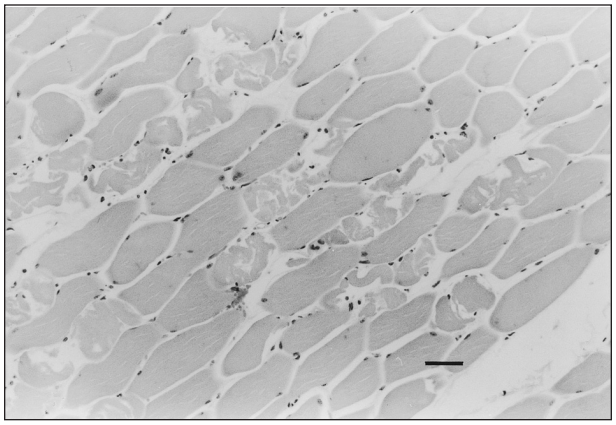

Figure 1—Photomicrograph of a section of cervical muscle from a steer that had received an injection of florfenicol in this area 132 days earlier. Notice the scattered individual fibers with myodegeneration consisting of fragmentation of the sarcoplasm (grade-1 myodegeneration). H&E stain; bar = 50 μm.

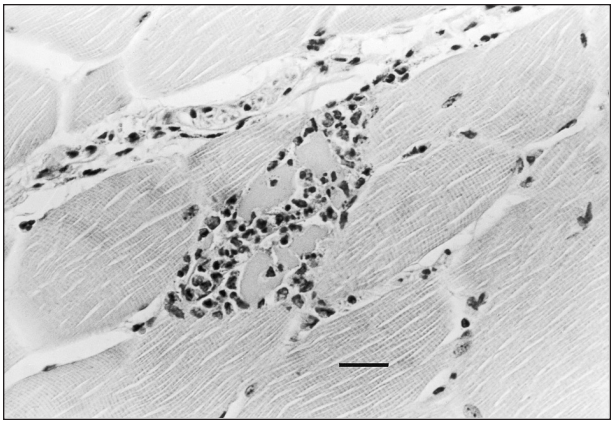

Figure 2—Photomicrograph of a section of cervical muscle from a steer that had received an injection of saline solution (0.9% NaCl) in this area 132 days earlier. Notice the small focus of inflammation consisting of infiltration of leukocytes, primarily neutrophils, into the area around a hyalinized muscle fiber (grade-1 inflammation). H&E stain; bar = 25 μm.

Table 1—Comparison of histologic scores for samples of cervical muscles from 48 steers (group 1) in which florfenicol (25 ml, twice) was injected in 1 side of the neck and saline solution (0.9% NaCl; 25 ml, twice) was injected in the other side of the neck and from 48 steers (group 2) in which florfenicol was injected in 1 side of the neck and nothing was injected in the other side of the neck

	Group 1				Group 2			
Histologic score	Florfenicol sites	Saline solution sites	Difference (mean ± SD)	*P* value	Florfenicol sites	Control sites	Difference (mean ± SD)	*P* value
Myodegeneration	0.19	0.15	0.04 ± 0.06	0.63	0.22	0.19	0.03 ± 0.08	0.81
Fibrosis	0.10	0	0.10 ± 0.07	0.81	0.13	0.01	0.12 ± 0.07	0.27
Inflammation	0.15	0.08	0.07 ± 0.07	0.81	0.20	0.09	0.11 ± 0.07	0.27
Fatty infiltration	0.04	0	0.04 ± 0.04	NA	0.04	0	0.04 ± 0.03	NA

NA = Not applicable; fewer than 2 categories were observed among the control sites.

tory cells, and the fiber was undergoing myodegeneration. Sarcocysts were found in sections from uninjected control sites as well as from sites where florfenicol or saline solution had been injected, suggesting that they were not associated with the injections.

Histologic scores for the florfenicol injection sites were not significantly different from scores for the contralateral saline solution injection sites and uninjected control sites (**Table 1**). However, fibrosis score for the florfenicol injection sites was significantly ($P = 0.02$) higher than score for the contralateral sites when data for the 2 groups were combined and pairing of treatments was controlled in the analysis.

Discussion

As data from the NCBA have become available,[3,8] many producers have moved IM injections from the top butt and round to the cervical area. Results of the present study suggest that few reactions should be expected with injection of florfenicol into the cervical muscles in steers. Reactions that occur may consist of fibrosis and infiltration of adipose tissue. The fact that only 2 steers in the present study had lesions in areas where florfenicol was injected supports our conclusion that reactions to florfenicol were generally mild. In addition, because we did not have any information on whether these animals had received any injections prior to inclusion in this study, we cannot rule out the possibility that lesions in these steers were a result of injection of some other product prior to inclusion in the present study.

The label indicates that the maximum amount of florfenicol that should be given at any site is 10 ml. Although larger doses per site are not recommended, in a previous study[20] in which larger volumes were injected into a single site, more lesions were produced 28 and 55 days after injection. Therefore, we elected to inject a large volume (25 ml) into a single site to maximize the possibility that we would identify lesions if they were going to occur. No attempt was made to compare reactions to florfenicol to reactions to other products that might be injected IM into the cervical muscles.

Initially, we planned to incise the skin in each animal and tag the injection site to assist in locating the exact site. However, this was not possible at the packing plant because of the number of animals processed (> 400 animals/h). Thus, we elected to have the neck portion of the chuck removed and then dissected each specimen muscle by muscle so that each muscle could be observed and palpated to detect injection-site lesions. This was similar to the procedure used by George et al.[21] In future studies, the neck portion of the chuck could be removed with the branded skin, as described,[20] to aid in locating the actual site.

In the present study, histologic scores for the florfenicol injection sites were not significantly different from scores for the contralateral saline solution injection sites and uninjected control sites. We had expected that florfenicol would cause some reaction in the tissue, because lesions were found in a previous study[20] in which 16 calves received IM injections of 10 ml of florfenicol in 1 side of the neck and 20 ml of florfenicol in the other side of the neck. Lesions consisting of muscle discoloration and small granulomas were seen 28 days after injection in 3 of 8 sites in which 10 ml of florfenicol had been injected and in 5 of 8 sites in which 20 ml of florfenicol had been injected. However, 55 days after injection, lesions were seen in only 1 of 8 sites in which 20 ml of florfenicol had been injected and were not seen in any of the sites in which 10 ml of florfenicol had been injected. In the present study, only 1 of the 96 steers had gross and microscopic lesions at the site of the florfenicol injection, and 1 additional steer had microscopic lesions. Other antimicrobials that have pyrrolidone or propylene glycol carriers often cause some reaction in the muscle tissue.[6,7,9,21]

It seems logical that some injection-site lesions will resolve with time.[6,9,20,21] Thus, finding only 2 lesions among the 96 florfenicol injection sites 132 days after injection is not unexpected, and the long time between injection and slaughter probably accounts for our lack of any significant differences between histologic scores for florfenicol sites and scores for the contralateral sites.

In contrast to a previous study[9] in which lesions were evident in 22% of cattle receiving injections of saline solution, we did not identify lesions after injection of saline solution in the present study. This discrepancy is likely attributable to differences in age and size of the animals used and in the use of the cervical muscles instead of the gluteus medius, biceps femoris, and semimembranosus muscles. Calves used in the previous study were 48 days old and weighed 89 kg or were 200 days old and weighed 242 kg. Lesions in the calves that received injections at 48 days of age were larger than those in calves that received injections at 200 days of age, even though all calves were slaughtered at 424 days of age. This suggests that age of the animal, size of the muscle at the time of injection, or both may contribute to the size of the lesion at slaughter. In contrast, animals in the present study were yearling steers that weighed 380 kg at the time of injection.

Also in contrast to previous studies,[2,6,7,9,15,21] all injections in the present study were given in the cervical muscles. The region of the body where injections are given can affect bioavailability by as much as 30% following SC injection, and IM injections do not consistently yield better bioavailability than do SC ones.[22] Previous data indicate that IM injection of amoxicillin into the neck will result in 26% higher peak serum concentrations and a 29% greater area under the curve than injection into the gluteus medius.[22] These differences have been suggested to be attributable to differences in blood flow.[23,24] The greater absorption rate following injection into the cervical muscle, combined with the possibility that injections may have been intermuscular as opposed to IM, may help account for the low number of lesions in the present study.

Small inflammatory foci and myodegenerative changes were seen on histologic sections from many of the steers in the present study; however, in our opinion, lesions were too acute, involved too few fibers, and were too scattered to have been the result of irritation from any injection given 132 days previously. We concluded that these lesions likely were due to migration and encystment of *Sarcosporidia* parasites.

Two injection sites in the present study had extensive lesions, and both of these were sites where florfenicol had been injected. Lesions at these 2 sites consisted of histologic evidence of inflammation and muscle damage. Interestingly, the shear force values for these sites were similar to values for the contralateral sites in both animals. Both of these sites had grade-3 histologic lesions of inflammation, myodegeneration, and fibrosis, and 1 of the sites had grade-2 fatty infiltration. It is possible that the myodegeneration of fatty infiltration caused a reduction in shear force values at these sites, accounting for the lack of difference in shear force values between these sites and the contralateral sites. Alternatively, it could be that because the cervical muscle has a relatively high proportion of connective tissue, any injection lesion that developed may not have resulted in any substantial difference in the amount of connective tissue in the muscle.

For the present study, we used data reported for the gluteal and semimembranous muscles to estimate shear force values for cervical muscles and determine sample size. As expected, shear force values for cervical muscles in the present study were higher than values reported for the gluteal and semimembranous muscles. The beef industry expects that cervical muscles will not be as tender as the gluteal muscles and has used them in ground beef, whereas gluteal muscles become steaks.

[a]Nuflor, Schering-Plough Animal Health, Union, NJ.
[b]Doric Minitrend Model 205, VAS Engineering, San Francisco, Calif.
[c]DFG-100 Series, The GS Blodgett Co, Williston, Vt.
[d]Universal testing machine, model 4201, Instron Corp, Canton, Mass.
[e]SAS, version 6.12, SAS Institute Inc, Cary, NC.

Appendix

Scoring system used to evaluate the severity of histologic lesions in the cervical muscles of steers

Score	Criteria
Myodegeneration	
0	No evidence of myodegeneration
1	A few small foci of fibers or individual fibers containing clear vacuoles or segmental fragmentation of the sarcoplasm
2	Larger foci or moderate numbers of individual fibers with vacuolation or segmental fragmentation of the sarcoplasm
3	Multiple foci or numerous scattered individual fibers with vacuolation or fragmented sarcoplasm or areas of fiber necrosis
Inflammation	
0	No evidence of inflammation
1	A few scattered small foci of inflammatory cells between or within fibers
2	Numerous small foci of inflammatory cells
3	Extensive infiltration of inflammatory cells into muscle bundles
Fibrosis	
0	No evidence of fibrosis
1	Slight increase in collagenous connective tissue within or between muscle bundles
2	Moderate increase in collagenous connective tissue within or between muscle bundles
3	Substantial increase in collagenous connective tissue replacing muscle fibers
Fatty infiltration	
0	No evidence of fatty infiltration
1	Scattered small foci of adipose cells within muscle bundles
2	More or larger foci of adipose cells within muscle bundles
3	Large foci of adipose cells replacing muscle fibers

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