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Safety determination for the use of bovine milk-derived lactoferrin as a component of an antimicrobial beef carcass spray

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Abstract

Bovine milk-derived lactoferrin (BMDL), an iron-binding glycoprotein, is known to be an effective natural antimicrobial. It is used as a spray, applied electrostatically, to raw beef carcasses to detach bacteria adhering to the surface in order to reduce microbial contamination. The use of BMDL as a component (at not more than 2% by weight) of an antimicrobial spray was determined Generally Recognized As Safe (GRAS) (GRN 67) for three proposed uses (i.e., beef carcasses, subprimals, and finished cuts), provided that the ingredient statement of food products that contain milk-derived lactoferrin identifies the source of the protein. The use of BMDL spray on only beef carcasses (not subprimals or finished cuts) at a level not to exceed 0.20 ml of formulation per kg of beef was determined safe without the requirement of labeling of food products so treated. The two key components of the assessment are: (1) a determination that exogenous lactoferrin exposure (resulting from its application to beef carcasses) is in the range of existing background exposures of lactoferrin as a result of lactoferrin found naturally in beef, and (2) a determination that this potentially small incremental increase in lactoferrin is safe (i.e., there is no reasonable expectation that BMDL will become an allergen under the conditions of its intended use).
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1. Introduction

Bovine milk-derived lactoferrin (BMDL), an iron-binding glycoprotein, is known to be an effective antimicrobial. The intended technical effect associated with the use of BMDL is to detach bacteria adhering to the surface of beef carcasses in order to reduce microbial contamination of beef products produced from these carcasses. The use of BMDL as a component (at not more than 2% by weight) of an antimicrobial spray was determined Generally Recognized As Safe (GRAS) (GRN 67) for three proposed uses, beef carcasses, subprimals and finished cuts, at the following levels: (1) application to beef carcasses at a concentration not to exceed (''NTE'') 0.20 ml of formulation per kg beef; (2) application to subprimal beef parts at a concentration NTE 0.56 ml of formulation per kg beef; and (3) application to finished cuts of beef at a concentration NTE 2.5 ml of formulation per kg beef.

The cumulative exposure for all three uses was estimated to be 4.1 mg per person per day for the ''typical'' consumer of beef products and 9.1 mg per person per day for the ''heavy'' consumer of beef products (GRN 67). The U.S. Food and Drug Administration (FDA) had no questions concerning the GRAS status of BMDL for the proposed uses as described in GRN 67, however, they did require that any food products containing BMDL resulting from these proposed uses be appropriately labeled. The FDA's response to GRN 67 contained the following statement: ''... the agency has no questions at this time regarding National Beef's conclusion that milk-derived lactoferrin is GRAS under the intended conditions of use, provided that the ingredient statement of food products that contain milk-derived lactoferrin identifies the source of the protein.''

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Some evidence exists to suggest that BMDL is a minor cows' milk allergen and therefore, this labeling requirement was requested by the Agency because of potential allergenicity thought to be associated with BMDL exposures at these levels. Furthermore, this labeling requirement was implemented to ensure that consumers who are allergic to milk would be aware of the potential presence of this milk-derived ingredient in beef products that they might consume.

Section 403(i) of the Federal Food, Drug, and Cosmetic Act ("FFDCA") and 21 CFR § 101.4 require the listing of all ingredients in a food fabricated from two or more ingredients, although the Secretary may establish exemptions from this requirement under appropriate circumstances. One such exemption established by the Secretary is for incidental additives present in food at insignificant levels, such as processing aids, as defined under 21 CFR § 101.100(a)(3)(ii). In the current application under consideration, use of BMDL as a component (at not more than 2% by weight) of an antimicrobial spray electrostatically applied only to raw beef carcasses, the BMDL is removed to the extent practicable from the surface of the carcass with a lactic acid rinse. Furthermore, BMDL is not intended to have a technical or functional effect in the finished food product. As such, it is properly considered a processing aid, exempt from the requirement of inclusion in the ingredient list under 21 CFR § 101.100(a)(3). However, an evaluation of safety of BMDL, restricted to its use on beef carcasses at a concentration not to exceed 0.20 milliliters (ml) of formulation per kg beef, was completed to determine if under this condition of its intended use, there is any expectation that the product would become an allergen and should therefore be labeled with the source of the protein.

A safety analysis of BMDL, for its use as a processing aid on beef carcasses without the requirement for labeling of food products produced from carcasses so treated, compares the exogenous BMDL exposure (resulting from its application to beef carcasses) with the range of existing background exposures due to endogenous lactoferrin levels in beef to determine if this potentially small incremental increase in lactoferrin exposure is safe (i.e., there is no reasonable expectation that BMDL will become an allergen under the conditions of its intended use). A determination of the likelihood of an allergic reaction from the use of BMDL as a component of an antimicrobial carcass spray considers the following points: the structural and allergenic equivalence of bovine milk-derived and beef-derived lactoferrin; whether beef allergies are uncommon and if any evidence exists that lactoferrin is an allergen in beef, and; a comparison of the proposed intake of BMDL with typical endogenous lactoferrin exposures from meat consumption.

2. Comparison of milk and neutrophil lactoferrin

Endogenous levels of lactoferrin are present in uncooked beef, and thus people are exposed daily to this source of lactoferrin. However, it could be argued that milk- and tissue-derived (i.e., neutrophil and plasma) lactoferrins are different proteins, and might therefore have different allergenic properties. An evaluation was conducted of what is known about the structure of bovine milk versus neutrophil lactoferrins, and the impact this structure may have on allergenic properties of these proteins is presented.

The first lactoferrin protein sequence to be published was for human milk lactoferrin (Metz-Boutigue et al., 1984), using amino acid sequencing. Subsequently, human neutrophil lactoferrin was cloned and sequenced from a myeloid library (Rado et al., 1987), and the amino acid sequence so determined differed by only three amino acids from milk-derived protein, presumably arising from sequencing errors. It therefore appears, that human milk and neutrophil lactoferrins are coded by the same gene and the protein products are identical. Although different forms of lactoferrin have been proposed (Broxmeyer et al., 1980; Furmanski et al., 1989), these have not been confirmed by any subsequent work and no supporting biochemical basis for this heterogeneity has been advanced. The only structural variation in the human lactoferrin polypeptide chain so far reported is a non-secreted splice-variant form (Δ-lactoferrin) lacking the N-terminal signal sequence (Siebert and Huang, 1997). Although gene polymorphisms occur in human lactoferrin (Liu et al., 2002), it is now accepted that there is only a single functional human lactoferrin gene (Teng, 2002) located on chromosome 3 (Teng et al., 1987).

The one structural difference that does exist between human milk and neutrophil lactoferrins is in their glycosylation. Human milk lactoferrin glycans contain fucose, while neutrophil lactoferrin glycans do not (Derisbourg et al., 1990), and thus are more similar to the glycans of serum transferrin (Spik et al., 1994). However, apart from the lack of fucose, the composition of human neutrophil lactoferrin glycans is very similar to that of milk lactoferrin glycans (Derisbourg et al., 1990). Both forms were found to be functionally identical with regard to regulation of myelopoiesis (Broxmeyer et al., 1986) and neutralization of heparin (Wu et al., 1995). There are no reports of glycans affecting the immunogenicity of lactoferrin, and moreover, the glycans do not affect the rate of clearance of lactoferrin from the circulation by the liver (Moguilevsky et al., 1984).

Although there is less comparative structural data available for bovine milk and neutrophil lactoferrins, the situation seems very similar to that of human lactoferrin. Bovine milk lactoferrin was first cloned and

sequenced from an involuting mammary gland library by Mead and Tweedie (1990). The deduced amino acid sequence of the secreted protein contained 689 amino acids, and this sequence was confirmed by Goodman and Schanbacher (1991) and by Seyfert et al. (1994), both of whom also cloned the bovine milk lactoferrin gene from a mammary gland library. An identical sequence was also deduced following cloning and sequencing from a bovine submaxillary gland library (Pierce et al., 1991). These investigators noted that the sequence showed 69 and 64% homology to human and murine lactoferrins, respectively, and contained five potential glycosylation sites. The N-terminal amino acid sequence of bovine lactoferrin isolated from tears is identical to that of the milk protein (Brown et al., 1996). As with human lactoferrin, there is only a single bovine lactoferrin gene (Teng, 2002), and it has been assigned to chromosome 22 (Schwerin et al., 1994). Bovine lactoferrin gene polymorphisms have been reported (Seyfert and Kuhn, 1994; Martin-Burriel et al., 1997), again paralleling the situation with human lactoferrin. Thus, although there appears to have been no attempt to directly clone bovine neutrophil lactoferrin from a myeloid library, it is highly unlikely that cloning of neutrophil lactoferrin would result in a product with a different amino acid sequence to that reported previously, using a variety of bovine tissues. No bovine analogue of the human splice-variant, Δ-lactoferrin, has so far been reported, and attempts to detect it have so far been unsuccessful (FL Schanbacher, personal communication, 2002).

As with human lactoferrin, there appear to be glycosylation differences between bovine milk and neutrophil lactoferrins. Hurley et al. (1993) observed multiple bands with different electrophoretic mobilities for bovine milk and neutrophil lactoferrins, which resolved into a single band with similar molecular weights for both proteins following digestion with N-glycanase. This indicates that bovine milk and neutrophil lactoferrins differ only in N-glycosylation and not in the structure of the polypeptide chain. Although the structural differences between bovine milk and neutrophil lactoferrin glycans have not been determined to the same extent as for human lactoferrin, it seems clear that the situation is entirely analogous, and that functional differences between the two proteins are unlikely to occur. Indeed, bovine milk lactoferrin is itself heterogeneous, with two major forms having been identified, lactoferrin *a* and *b*, that differ in their glycosylation, but not in their polypeptide chains (Tsuji et al., 1989; Ye et al., 1997).

Thus, bovine milk and tissue (neutrophil) lactoferrins appear to be products of the same gene and possess identical polypeptide chains, any differences between them being limited to the attached glycans. Analogy to human lactoferrin suggests that these differences are unlikely to affect protein function. With regard to the question of potential allergic responses, all immunological reactions to lactoferrin so far reported are to the polypeptide chain and not to the glycan. Therefore, there appears to be no reason to suppose that residual milk-derived lactoferrin potentially found in lactoferrin-treated beef carcasses will be any more prone to inducing allergic responses than would endogenous lactoferrin found naturally in beef carcass muscle tissue.

3. Endogenous lactoferrin levels in beef

3.1. Estimating endogenous levels of lactoferrin in beef based on a review of published literature

Lactoferrin is an iron-binding protein of the transferrin family. It is found naturally in various external secretions, especially milk and colostrum, and in the secondary (specific) granules of neutrophils. Small amounts of lactoferrin are also present in blood plasma and it can bind to a variety of mammalian cells. Because lactoferrin is naturally present in blood neutrophils and plasma, and beef contains residual blood, there will be some endogenous lactoferrin present in beef carcasses. Levels could be further increased by lactoferrin bound to other cells in the carcass. Thus, exposure to bovine lactoferrin occurs regardless of whether or not the carcass is treated with BMDL. Calculation of endogenous levels of lactoferrin in the carcass can be accomplished by determining the levels of lactoferrin in residual blood and levels of residual blood in beef.

3.1.1. Neutrophil lactoferrin content

Lactoferrin was first identified as a constituent of human neutrophil secondary granules by Masson et al. (1969), and its presence in bovine neutrophils was subsequently confirmed, although in the latter case, lactoferrin is also present in an additional type of neutrophil granule (Gennaro et al., 1983). Harmon et al. (1976) cite unpublished data in Harmon's Master's Thesis (Ohio State University, Columbus, OH, 1974) indicating that the lactoferrin content of bovine neutrophils is similar to that of humans, i.e., 3.0 micrograms (μg) per 10^6 cells. Subsequently, Harmon and Newbould (1980) estimated the lactoferrin content of bovine neutrophils to be 8.63 ($\pm$1.00) μg per 10^6 cells. The cells were isolated using EDTA as anticoagulant, a relatively mild procedure that should minimize degranulation, and hence loss of lactoferrin, so the value should be reasonably reliable. The lactoferrin was quantified by electroimmunodiffusion. Gennaro et al. (1978) reported bovine neutrophil lactoferrin content as 7.4 ($\pm$7.0) μg per 10^6 cells. These investigators used citrate as an anticoagulant, and quantified lactoferrin by double diffusion. Despite the differences in methodology, the Gennaro et al. (1978)

estimate agrees very well with that of Harmon and Newbould (1980). Sato et al. (2000) reported a somewhat lower figure of 2.9 μg per 10^6 cells (with a range of 1.5–4.7), using a sandwich ELISA technique. The method for obtaining neutrophils was not stated, so the possibility of some degranulation occurring cannot be excluded, and might account for the lower figure. However, all these amounts are comparable to those reported for other species. Human neutrophil lactoferrin content has been variously reported as 4.3 μg per 10^6 cells (Venge et al., 1978), 3.0 μg per 10^6 cells (Masson et al., 1969), and 8.2 μg per 10^6 cells (Baynes et al., 1986), while a figure of from 2 to 4 μg per 10^6 cells has been reported for mouse neutrophils (Segars and Kinkade, 1977). Based on this sampling of data, it is reasonable to assume 8.0 μg lactoferrin per 10^6 bovine neutrophils, as a basis for further calculations.

3.1.2. Lactoferrin content of bovine blood

Bovine blood contains $7–10 \times 10^6$ leucocytes/ml, of which 25–30% are neutrophils (Frandson and Spurgeon, 1995). This means that bovine blood contains $1.8–3 \times 10^6$ neutrophils/ml. Assuming the figure of 8.0 μg lactoferrin per 10^6 bovine neutrophils mentioned previously, this gives 14.4–24.0 μg of neutrophil lactoferrin/ml of blood.

Lactoferrin is also present in bovine plasma, but probably only makes a minor contribution to total bovine blood lactoferrin. The lowest of the neutrophil lactoferrin amounts is 70 times higher than the most reliable amount for plasma lactoferrin reported by Talkuder et al. (2002). Even the highest plasma amount, reported by Holloway et al. (2002) and probably unreliable (as serum rather than plasma was assayed), still indicates that the contribution of plasma/serum lactoferrin is less than 20% of the lowest likely contribution from neutrophils. Finally, if one takes the amounts from the only publication reporting both neutrophil and plasma lactoferrin concentrations (Sato et al., 2000), the contribution of neutrophil lactoferrin is still at least 17-fold greater than that of plasma. This amount may be biased towards plasma lactoferrin, as Sato et al. (2000) did not state whether precautions were taken to prevent neutrophil degranulation during the separation of cells and plasma.

3.1.3. Residual blood in the bovine carcass

Residual blood is normally determined by estimating the amount of hemoglobin in tissue. Warriss (1977), reviewing earlier studies, pointed out that early results might be unreliable due to a failure of the assays used to distinguish between myoglobin and hemoglobin. Warriss developed a gel-chromatographic assay (Warriss, 1976), and in a subsequent review of more recent work, suggested that the normal hemoglobin concentration in meat of slaughtered animals ranges from 0.2 to 1 milligrams (mg) per gram (g) (Warriss, 1984). Using tissues from heifers and steers, as well as randomly purchased beef samples from retail sources, gave hemoglobin values between 0.44 and 0.65 mg/g of tissue (Warriss and Rhodes, 1977). Samples from older animals (cows and bulls) gave higher values ranging from 1.0 to 1.1 mg/g. Although the amount of blood remaining in the whole carcass varies according to the efficiency of exsanguination at time of slaughter, the amount in muscle tissue remains relatively constant (Warriss, 1984). However, delayed bleeding following slaughter can increase the hemoglobin concentration to up to 3.3 mg/g (Gregory et al., 1988).

A number of more recent reports have given hemoglobin amounts consistent with the range proposed by Warriss (1984). Field et al. (1980) measured hemoglobin in the carcasses of 18–24-month-old steers and 4–6-year-old cows using a gel-chromatographic procedure. For steers, the amounts were 0.70 and 0.39 mg/g in cervical and lumbar muscle tissues, respectively, while for cows, the corresponding amounts were 0.85 and 0.33 mg/g. Gregory et al. (1988), using the gel chromatographic method of Warriss (1976), reported values ranging from 0.39 to 0.61 mg/g in different muscles from cattle of unstated age.

Oellingrath et al. (1990) subsequently developed an HPLC method for separation of hemoglobin from myoglobin, as well as for hemoglobin quantification. Samples of minced beef gave hemoglobin values of 0.62–0.68 mg/g. Gerold and Stolle (1994) used the same method to assay hemoglobin in longissimus dorsi muscles from 16- to 18-month-old bulls, and obtained an almost identical mean amount of 0.65 mg/g (range 0.19–1.23). These most recent studies, using more modern analytical methodologies, give results comparable with the range suggested by Warriss (1984). Furthermore, unpublished data from R.A. Lawrie, an expert with over 50 years experience in the field of meat science, also fall within Warriss' range (R.A. Lawrie, personal communication). Thus, it seems reasonable to accept Warriss' range of 0.2–1 mg/g as an accurate reflection of the hemoglobin content of meat.

Given that bovine blood has a hemoglobin content of 12 g/100 milliliters (ml) (Frandson and Spurgeon, 1995), the amounts of Warriss (1984) imply that muscle tissue contains 1.7–8.3 microliters (μl) of blood/g of tissue.

3.1.4. Lactoferrin content of bovine muscle tissue calculated using literature values

From the above calculations, using a neutrophil content of between 3.1 and 24.9×10^3 cells/g of tissue, the corresponding calculated lactoferrin content is 25–199 nanograms (ng) per g of muscle tissue (parts per billion or ppb). If one takes the lower, and perhaps less reliable number, for neutrophil lactoferrin of 0.39 μg/10^6 cells provided by Sato et al. (2000), then the lactoferrin

content of muscle would be in the range of 12–97 ng/g of tissue (ppb). The above calculations assume that the neutrophil content of residual blood in muscle is the same as that in normal blood in the live animal. It could be argued that neutrophils might be more easily drained than erythrocytes from the carcass, so the tissue hemoglobin content would overestimate the number of neutrophils remaining. However, this seems highly unlikely. Most of the residual blood in muscle is in capillaries and is not depleted of neutrophils (PD Warriss, personal communication, 2002). Indeed, it is more likely that residual blood is enriched in neutrophils, as they may start to adhere to the capillary endothelia once the animal is slaughtered.

Although lactoferrin mRNA has been detected in various tissues, neutrophils appear to be the most significant source of systemic lactoferrin. A large variety of cells and tissues can bind lactoferrin, either through specific receptors, or less specific interactions involving either lectin-type recognition of lactoferrin glycan chains, or charge interactions due to lactoferrin's high *pI*. While it is not currently possible to calculate a reliable number for this contribution to the total lactoferrin content of muscle tissue, it does suggest that the lactoferrin contribution from neutrophils alone is likely to be an underestimate.

3.2. Estimating endogenous levels of lactoferrin in beef based on ELISA measurement of beef samples

3.2.1. Method

A study was conducted using ELISA to quantitatively measure lactoferrin levels in three separate analyses of beef round-bottom round steak samples (National Beef, 2002a). The ELISA method (Adonogianaki et al., 1996; Hetherington et al., 1983) was performed using an assay kit purchased from the Bethyl Laboratories (Montgomery, TX). A 0.1 ml volume of capture antibody (i.e., goat anti-bovine lactoferrin-IgG diluted in 0.05 M carbonate–bicarbonate buffer, pH 8.0) was added to coat each well of the 96-wells of a microtiter plate (Nunc MaxiSorp C bottom module). An overnight incubation was allowed to attain maximum binding of the capture antibody to the polystyrene wells. A 0.1 ml volume of dilutions of bovine lactoferrin standards or test samples (with unknown lactoferrin levels) was prepared in TBS-Tween (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0) containing 1% bovine serum albumin, and added to assigned wells. Plates were incubated at 37 °C for 2 h to allow maximum antigen-antibody interaction. Wells were washed thoroughly, four times, with TBS-Tween. A 0.1 ml volume of an enzyme-linked antibody (i.e., goat anti-bovine lactoferrin-IgG conjugated to horseradish peroxidase prepared in TBS-Tween) was added to the wells and the reaction was allowed to proceed for 2 h at 37 °C. The washing step, described above, was repeated. Finally, a 0.1 ml volume of 3,3′, 5,5′ tetramethyl benzidine enzyme substrate solution (Bethyl Laboratories, Montgomery, TX) for horseradish peroxidase was prepared and added to the wells. The enzymatic reaction was terminated within 5–15 min (based on the rapidity of the color change) with 15 μl of 2 N sulfuric acid. The color reaction was measured as absorbance at 450 nm wavelength using a microplate reader (SpectraMax, Molecular Devices, Sunnyvale, CA). A standard curve for bovine lactoferrin was plotted using a commercial preparation of lactoferrin protein from DMV International, NY. Twofold serial dilutions of lactoferrin ranging from 500 to 7.8 ng/ml were prepared in TBS-Tween buffer containing 1% bovine serum albumin and the ELISA assay was performed as described above. A standard curve was plotted using absorbance values versus dilutions of lactoferrin protein standard. For determination of lactoferrin levels in test samples, the color intensity of test samples from ELISA were correlated against the standard curve.

3.2.2. Test samples

In each of the three analyses, beef round-bottom round steak samples (sample size; $n = 4$) were used to determine the endogenous levels of lactoferrin. Beef was ground, weighed out and placed in a stomacher bag containing TBS-Tween (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0). Meat was pummeled on high for 120 s. The fluid obtained after stomaching was collected and used for lactoferrin analysis.

3.2.3. Results

Four replicate samples were analyzed in each of the three analyses yielding average (±standard deviation) lactoferrin levels of 202.22 ± 21.55, 184.41 ± 13.65, and 192.56 ± 11.68 ng of lactoferrin/g tissue (ppb), respectively. Averaging these three estimates yields an overall estimate of the endogenous level of lactoferrin in beef of 193 ng/g tissue (ppb). An exponential plot of the standard curves yielded *R*-squared values of: 0.98, 0.97, and 0.99, respectively. This empirically derived estimate agrees quite favorably with the literature-derived range presented above of 25–199 ng/g of tissue (ppb).

3.3. Summary of data used to derive endogenous lactoferrin levels in beef

Using data derived from a review of the published literature, as well as a new analytical study, it is clear that significant amounts of endogenous lactoferrin are present in uncooked beef. Using the most reliable data in the literature, as discussed above, neutrophils in beef tissue would account for a content of between 25 and 199 ng of lactoferrin/g of muscle tissue (ppb). This is likely to be increased further by lactoferrin in plasma

and lactoferrin bound to cells such as macrophages/monocytes. This range agrees quite well with empirically derived estimates from studies conducted by National Beef of 193 ng of lactoferrin/g tissue (ppb).

4. Exogenous lactoferrin levels in beef from antimicrobial spray application

BMDL is the active ingredient in an antimicrobial spray product that is to be applied to raw beef carcasses prior to a final lactic acid rinse during processing. This water-based, antimicrobial spray product contains no more than 2% BMDL by weight.

Three primary processes comprise the typical operation of a meat packing plant: (1) the slaughter process, (2) the chilling process, and (3) the fabrication process. During this meat packing operation, lactoferrin-containing spray is to be employed at the end of the slaughter process, immediatly before chilling of the carcasses begins. This spray is to be applied within an "activated lactoferrin cabinet" at a rate of 50-ml per carcass. Assuming that the spray contains two percent lactoferrin, this 50-ml application rate will yield 1 g of BMDL on each carcass, assuming a lactoferrin density of 1 g/ml. This calculation employs the worst-case assumption that the electrostatic spraying ("ESS") process used in applying the lactoferrin to beef carcasses is 100% efficient; that is, all 50-ml of spray containing lactoferrin adheres to the carcass. In reality, it is likely that this spray process is not 100% efficient. However, because no data currently exist to specifically estimate this application efficiency, the worst-case assumption of 100% efficiency was employed.

Subsequent to lactoferrin application, the beef carcasses undergo a final lactic acid rinse prior to being chilled during the meat packing process. The intent of this lactic acid rinse is to remove bacteria bound to the lactoferrin, as well as any residual lactoferrin, from the carcass surface. In order to measure the amount of lactoferrin removed as a result of this lactic acid rinse, four carcass washing studies employing a meat processing simulation ("MPS") system were conducted (National Beef, Unpublished Study, 2002). This MPS system is a scale model of the actual 12-step processing system used in a typical meat packing plant.

4.1. Method

For the Meat Processing Simulation (MPS) system, equipment consists of a spray-, process-, and drain-unit. The spray-unit has six fluid tanks of 7.6-liter capacity, connected to six separate magnetic drive (seal-less) fan cooled sanitary/chemical solution pumps. One of the fluid tanks has a built-in heating element to deliver hot water, maximum to 190 °F. The process-unit is a cabinet with a safety view glass ceiling. It has a loading chamber, five pause chambers and six spray chambers. A drive chain passes through the center of all the 12 chambers. The chain consists of a loading trolley with a removable stainless steel cassette and spaced with six timing tabs. Inside all the spray chambers, two adjustable spray nozzles are located on both sides of the drive chain to deliver a circular stream of liquid towards the middle center of the loading trolley. The drain-unit is a secured compartment located under the process-unit. All six spray chambers are individually hosed to collect the wash-effluent into 500-ml borosilicate bottles. The rest of the chambers are jointly hosed to drain into an effluent basin. The MPS system is digitally controlled through a programmable logic controller and washes the meat according to specific washing steps; the simulated steps are comparable to that of a real-time sanitizing assembly of a meat processing plant.

The lactoferrin is applied to the meat through an electrostatic spray (ESS) system. The ESS system is a pressure and charge driven instrumentation for application of lactoferrin onto a meat surface. Pressure on the ESS from an electrical air compressor is approximately 50 pounds per square inch (psi). The ESS was set up to deliver lactoferrin at an application rate of 1 ml/s. The time of the lactoferrin application is adjusted for each trial segment (i.e., 2, 4, 6, 8, and 10 s). The lactoferrin is applied to the meat surface, with fluid levels and consistency of liquid spray from ESS monitored during the application process. Meat samples are then either directly measured for lactoferrin deposition or further processed by being subjected to an additional acid wash in the MPS system to measure residual (post-wash) lactoferrin.

4.2. Test samples

For these experiments, beef round-bottom round steaks were used to simulate beef carcasses. These steaks were then processed using the MPS system and lactoferrin levels were measured on the surface of the steaks subsequent to lactoferrin application, both before and after the final lactic acid rinse. Briefly, a 50-cc sterile conical centrifuge tube containing 5 ml of TSB was pressed against the surface of the meat and agitated for 10 s by hand. Each tube covered an area of approximately 4.9 cm^2. This wash liquid was tested for lactoferrin levels. Lactoferrin levels were measured using an ELISA technique described previously (Adonogianaki et al., 1996; Hetherington et al., 1983). In each study, five different lactoferrin spray times were evaluated (i.e., 2, 4, 6, 8, and 10 s) with four replicates at each time point. For this analysis, the two-second spray application data were selected because this is the lactoferrin spray duration that will be used in actual practice.

4.3. Results

Results from the two-second lactoferrin application pre- and post-lactic acid wash are presented in Table 1. Based on these data, the average percent lactoferrin that was estimated to have been removed by a two-second lactic acid rinse across these four studies was 91, 86, 80, and 81%, respectively. Averaging these four estimates yields an overall estimate of lactoferrin removal efficiency for a lactic acid rinse of 84.5%; thus, leaving an average of 15.5% of the applied lactoferrin on the carcass surface.

4.4. Calculation of exogenous lactoferrin levels on the beef carcass

Multiplying the amount of lactoferrin applied to the carcass (i.e., 1 g) times 0.155 (the fraction of lactoferrin retained on the carcass after the lactic acid rinse) yields an estimate of the amount of BMDL remaining on the carcass surface of 0.155 g.

It is estimated that 40% of the lactoferrin-treated carcass surface (i.e., primarily the inner surface of the carcass body cavity) consists of bone and connective tissue destined for rendering. The remaining 60% of the lactoferrin-treated carcass surface ends up in edible beef, with 10% of this total going into boxed beef and 50% into trim/grind (lean trimmings and some of the fat that is removed during fabrication is designated "trim/grind," and is used for ground beef and other processed meats such as hot dogs) (National Beef, Internal Document, 2002).

Therefore, of the estimated 0.155 g of lactoferrin remaining on the carcass surface following the lactic acid rinse, about 40% of this lactoferrin residual will end up on portions of the carcass that are not used in products intended for human consumption. Of the remaining 60% of the lactoferrin that is retained on edible portions of the carcass, about 10% will end up in boxed beef and 50% will go into trim/grind. Thus, multiplying the estimated amount of lactoferrin remaining on each carcass (0.155 g) times 0.6 yields an estimated amount of lactoferrin adhering to the edible portions of the carcass of 0.093 g or 93 mg, with one-sixth of this total or 15.5 mg going into boxed beef and five-sixths or 77.5 mg into trim/grind.

Table 1
Exogenous lactoferrin levels on beef after a two-second application by ESS system

Trial No.	Levels detected pre-wash (ng/cm^2)	Levels detected post-wash (ng/cm^2)	% Lactoferrin washed off beef
NT(02)-39A	1562 ± 43	145 ± 12	91
NT(02)-39B	1465 ± 63	211 ± 11	86
NT(02)-39C	1321 ± 12	263 ± 43	80
NT(02)-39D	1362 ± 45	263 ± 55	81

For the purposes of this assessment, two different exposure assumptions are evaluated:

1. Assume that the 93 mg of residual lactoferrin is equally distributed across all edible portions of the carcass (i.e., boxed beef and trim/grind); or
2. Assume that the 93 mg of residual lactoferrin is unequally distributed across all edible portions of the carcass, with 15.5 mg going into boxed beef and 77.5 mg into trim/grind.

The average amount of edible meat that a beef carcass yields is about 650 pounds (295 kg), and is derived by summing the average boxed beef weight (415 pounds or 189 kg) and the average trim/grind weight (235 pounds or 107 kg) on a per head basis (National Beef, 2002b). Under the first exposure scenario, the estimated concentration of BMDL in edible beef can be derived by dividing the amount of lactoferrin adhering to edible portions of the carcass (93 mg) by the weight of the edible meat from each carcass (295 kg) to yield 0.32 mg/kg (ppm) or 320 ng/g (ppb). Under the second exposure scenario, the estimated concentration of BMDL in boxed beef can be generated by dividing the amount of lactoferrin potentially found in boxed beef (15.5 mg) by the total weight of boxed beef from each carcass (189 kg) to yield 0.082 mg per kg (ppm) or 82 ng/g (ppb). The estimated concentration of BMDL in trim/grind can be derived by dividing the amount of lactoferrin potentially found in trim/grind (77.5 mg) by the total weight of trim/grind from each carcass (107 kg) to yield 0.726 mg per kg (ppm) or 726 ng/g (ppb).

5. Estimates of lactoferrin consumption based on beef intake in the U.S.

5.1. Estimate of beef consumption

Consumption of beef was estimated based on data from the 1994 to 1996 USDA Continuing Survey of Food Intakes by Individuals ("CSFII 1994–96") and its Supplemental Children's Survey ("CSFII 1998") as provided on CD-ROM (USDA, 2000). The combined data from these two surveys are designated CSFII 1994–96, 1998. In the CSFII 1994–96, 1998, dietary intakes were collected through in-person interviews using 24-h recalls on two nonconsecutive days. A total of 21,662 individuals of all ages provided data for the first day; of those individuals, 20,607 provided data for a second day. Each dietary recall includes detailed information on all foods consumed the previous day, including a descriptive name and the amount (gram weight) of each food consumed. The amount of beef in each survey code was identified by linking the CSFII food codes to the beef meat, beef fat, and beef byproducts commodity codes in the Food Commodity Intake Database, Version 2.1 (USEPA, 2000). The amounts of each commodity

Table 2
Estimates of beef consumption: 2-day average intake and intake per eating occasion

Population group	Survey population[a]	%	2-Day average intake			Intake per eating occasion		
			N[b]	Percentile		N[c]	Percentile	
				50	90		50	90
				(g per day)			(g per eating occasion)	
Infants, <1 yr	1065	28	276	13	41	478	14	43
Infants, 1 yr	972	80	782	19	55	1632	16	61
Children, 2–5 yr	5437	87	4704	25	74	10,205	21	75
Children, 6–12 yr	2089	89	1867	38	99	4289	29	96
Teenagers, 13–19 yr	1222	88	1088	52	147	2617	35	137
Adults, 20+ yr	9323	81	7556	52	152	15,423	49	156

Data source. 1994–96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Food Commodity Intake Database (USDA, 2000; USEPA, 2000).

Notes. Breastfeeding infants and children were excluded from the analyses. Analyses were limited to individuals who provided 2 days of recall data. Estimates were generated with USDA sample weighting factors to adjust for differences in representation of subpopulations.

[a] Number of survey respondents who provided 2 dietary recalls.

[b] Number of respondents who reported consumption of one or more beef-containing foods during the 2 days of recall.

[c] Number of occasions on both survey days combined on which consumption of a beef-containing food (or foods) was reported.

per 100 g food code were then summed. Food codes representing veal, bison, beef parts (e.g., heart, brain, and tongue), gelatin drinks, and those containing small amounts of beef by-products (less than 2.5 g/100 g food code) were excluded from the analyses, as these foods will not be subjected to lactoferrin treatment.

Estimates of beef intake were calculated based on both 2-day average beef intakes and beef intake per eating occasion. All dietary intake analyses included only consumers of beef (i.e., "eaters only"). Therefore, the 2-day average intake estimates are based upon the mean amount of beef consumed in 2 days by all individuals who reported consumption of at least one beef-containing food during the 2-day period, and the intake per eating occasion estimates are based on the amount of beef consumed on each occasion (i.e., unique time and day) an individual reported consumption of beef and/or beef-containing food(s). The 2-day average intakes provide an estimate of usual beef intake, while the intakes per eating occasion provide estimates of the amount of beef consumed at a single meal or snack occasion. Results of the dietary analyses are presented in Table 2.

5.2. Estimates of lactoferrin intake

As previously derived from published literature values as well as a recent analytical study, the concentration of endogenous lactoferrin in beef is 193 ng/g of tissue (ppb). Ranges of background lactoferrin consumption (Table 3) were calculated from the dietary analysis of beef intake (Table 2) and concentration of lactoferrin in beef.

The concentration of exogenous BMDL in edible beef from lactoferrin-treated carcasses was estimated to be 320 ng/g of tissue (ppb), assuming equal distribution of the applied lactoferrin throughout the meat, or 82 ng/g of tissue (ppb) of boxed beef and 726 ng/g of tissue (ppb) in trim/grind if, on the assumption that the lactoferrin is unequally distributed within the edible beef. To derive an exogenous lactoferrin intake estimate under each of these two scenarios, the exogenous lactoferrin concentration was multiplied by the amount (and type) of beef consumed. For endogenous and exogenous BMDL consumption, the intakes based on 50th and 90th percentile beef intakes were calculated, which represent the exposure for both typical and heavy consumers of beef (Table 3).

In both scenarios, the values of BMDL consumption are likely to be overestimates of intake because these calculations employ the following worst-case assumptions: (1) the ESS process employed in applying lactoferrin to beef carcasses is 100% efficient, and (2) all beef an individual consumes has been treated with lactoferrin.

5.3. Conclusions

In order to evaluate the potential impact of treating beef carcasses with lactoferrin, the range of estimated intakes of endogenous lactoferrin that result from untreated beef consumption is compared against the estimated intakes of exogenous BMDL that could potentially occur from consumption of lactoferrin-treated beef. In Table 3, the ranges of usual (50th percentile) intakes of exogenous BMDL are all within the range of estimated background exposures to naturally occurring endogenous lactoferrin across all populations. There are, of course, uncertainties in both the endogenous and exogenous estimates because of the assumptions that must be used to derive these values; in particular, the estimated intakes of exogenous lactoferrin are expected

Table 3
A comparison of estimated endogenous lactoferrin intakes with estimated exogenous lactoferrin intakes from lactoferrin-treated beef carcasses

Population group	Estimated endogenous lactoferrin intakes based on 50th percentile beef consumption (μg/occasion)[a]	Estimated endogenous lactoferrin intakes based on 90th percentile beef consumption (μg/occasion)	Estimated exogenous lactoferrin intakes based on 50th percentile beef consumption (μg/occasion)		Estimated exogenous lactoferrin intakes based on 90th percentile beef consumption (μg/occasion)	
			Scenario 1[b]	Scenario 2[c]	Scenario 1	Scenario 2
Infants, <1 yr	3	8	5	2	14	20
Infants, 1 yr	3	12	5	6	20	35
Children, 2–5 yr	4	14	7	10	24	44
Children, 6–12 yr	6	19	9	12	31	62
Teenagers, 13–19 yr	7	26	11	15	44	76
Adults, 20+ yr	9	30	16	16	50	76

Data source. USDA 1994–1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and USEPA Food Commodity Intake Database (USDA, 2000; USEPA, 2000).

Notes. Breastfeeding infants and children were excluded from the analyses. Analyses were limited to individuals who provided 2 days of recall data. Estimates were generated with USDA sample weighting factors to adjust for differences in representation of subpopulations.

[a] The endogenous (background) lactoferrin intakes in columns 2 and 3 of the above table were calculated by taking the the 50th and 90th percentiles of beef consumption (in g per occasion) for each population group of interest and multiplying each of them by 193 ng bovine neutrophil lactoferrin/g beef (ppb). See the text for a further discussion of the derivation of the estimated endogenous lactoferrin concentration in beef of 193 ng/g.

[b] Under Scenario 1, the amount of bovine milk-derived lactoferrin adhering to the treated beef carcass is assumed to be equally distributed throughout the edible portions of the carcass; this assumption yields an estimated exogenous lactoferrin concentration in all beef products of 320 ng/g of beef (ppb). Then, intake values corresponding to the 50th and 90th percentiles of beef consumption (in g per occasion) for each population group of interest were multiplied by this concentration of 320 ng bovine milk-derived lactoferrin/g of beef (ppb) to yield estimates of exogenous lactoferrin intakes listed above under "Scenario 1." See the text for a further discussion of the derivation of the estimated exogenous lactoferrin concentration in lactoferrin-treated beef of 320 ng/g.

[c] Under Scenario 2, the amount of bovine milk-derived lactoferrin adhering to the treated beef carcass is assumed to be distributed differentially within the treated beef carcass based on the surface area of the carcass that ends up in "boxed beef" vs. "trim/grind"; this assumption yields an estimated exogenous lactoferrin concentration in "boxed beef" of 82 ng/g of beef (ppb) and an estimated exogenous lactoferrin concentration in "trim/grind" of 726 ng/g of beef (ppb). Then, for each beef-eating occasion, it was determined whether the beef consumed would have most likely been derived from "boxed beef" or "trim/grind." If from the "boxed beef," the amount of beef consumed was multiplied by the concentration of exogenous lactoferrin in "boxed beef" of 82 ng bovine milk-derived lactoferrin/g. If from the "trim/grind," the amount of beef consumed was multiplied by the concentration of exogenous lactoferrin in "trim/grind" of 726 ng bovine milk-derived lactoferrin per g. These exogenous lactoferrin intakes were then summed across all beef-eating occasions and 50th and 90th percentile intake values were generated. See the text for a further discussion of the derivation of the estimated exogenous lactoferrin concentrations in lactoferrin-treated beef.

to be high. Therefore, given the inherent uncertainties in both the endogenous and exogenous lactoferrin intake estimates, the intended use of BMDL as an antimicrobial spray treatment on beef carcasses will not result in a significant, measurable increase in lactoferrin intake relative to current background exposures to lactoferrin from beef consumption.

6. Bovine lactoferrin as a potential human food allergen

Little scientific and clinical information exists to document whether BMDL is a cows' milk allergen. While there is some evidence to suggest that BMDL is a minor cows' milk allergen, the existing information is difficult to interpret because of methodological problems in the manner in which much of the data were collected.

Certainly, no argument exists that the major cows' milk allergens in IgE-mediated cows' milk allergy are casein, β-lactoglobulin, and α-lactalbumin (Besler et al., 2002; Sharma et al., 2001; Wal, 1998; Wal et al., 2001). Several clinical investigators have demonstrated that some cows' milk-allergic individuals have IgE antibodies directed against lactoferrin (Baldo, 1984; Businco et al., 2000; Host et al., 1992; Wal et al., 1995; Wal, 1998; Wal et al., 2001). However, the vast majority of these milk-allergic infants displayed IgE antibodies to one or more of the major cows' milk allergens in addition to lactoferrin. Wal (1998) may have identified two milk-allergic infants out of 92 studied (2.2%) who were mono-sensitized to lactoferrin only. However, the clinical significance of this observation is unclear because the study does not indicate if these or other infants in the study had received blinded oral challenges to document their milk allergies. Without confirmation by oral challenge, the evaluation of specific IgE antibodies in patient serum can be clinically meaningless and misleading. Furthermore, in studies where quantitative estimates were obtained of the levels of specific IgE antibodies to lactoferrin (Wal et al., 1995; Wal, 1998), the levels of anti-lactoferrin IgE antibodies were far lower than the levels of specific IgE antibodies to the major milk allergens in the vast majority of the patients.

Milk-allergic patients seem to occasionally have IgE antibodies directed at other minor milk proteins (Baldo,

1984); the clinical significance of the presence of these antibodies is unproven. To demonstrate that BMDL is a clinically significant milk allergen, patients who were challenge-positive to milk ingestion would have to demonstrate specific IgE antibodies to BMDL (and possibly other milk proteins), positive skin prick tests to BMDL, and positive oral challenges to BMDL. Such information does not exist to unequivocally demonstrate that BMDL is a human allergen.

Lactoferrin is clearly present endogenously in beef, primarily arising from its presence in bovine neutrophils. Furthermore, bovine neutrophil lactoferrin is very similar, structurally and functionally, to BMDL. Only one structural difference has emerged, that involving a difference in glycosylation (Hurley et al., 1993). Differences in glycosylation are unlikely to have any effect on lactoferrin in terms of its potential allergenicity. Glycosylation has been investigated thoroughly for its impact on the IgE-binding to pollen allergens, and for possible cross-reactivity that may exist in some patients between pollen and food allergens. Cross-reacting carbohydrate determinants (''CCDs'') have been found associated with certain pollen and food allergens, particularly allergens in fresh fruits and vegetables (Aalberse et al., 2001; Vieths et al., 1994; Vieths, 1997). Certain proteins in fruits and vegetables and certain pollen allergens possess similar glycan structures that bind to IgE antibodies in the serum of some human subjects. However, the cross-reactive IgEs directed to CCDs do not appear to have any clinical significance because they have been identified in both symptomatic and asymptomatic individuals (van der Veen et al., 1997; Vieths, 1997). Thus, if these results can be extrapolated to lactoferrin, differences in glycosylation are unlikely to result in differences in the potential of these proteins to elicit allergic reactions.

In addition, given the presence of lactoferrin in beef, allergic reactions to beef are comparatively rare and are usually relatively mild, with bovine serum albumin (''BSA'') most often associated with these reactions (Fiocchi et al., 1995; Werfel et al., 1997). Thus, the evidence suggests that bovine neutrophil lactoferrin is not an allergen in beef.

The issue of whether exogenously applied BMDL could become an allergen under the conditions of its proposed use as an antimicrobial spray can be addressed by a comparison of the proposed intake with the current intake through its endogenous occurrence in beef. Beef carcasses (and meat products derived from them) already contain naturally occurring lactoferrin, primarily from neutrophils, and as described previously, no significant increase in exposure to lactoferrin would occur from this use relative to current background exposures from consumption of beef. The range of 50th percentile intakes for exogenously applied BMDL is well within the range of estimated background intakes of naturally occurring endogenous lactoferrin. Therefore, it is unlikely that lactoferrin would become an allergen in those individuals consuming lactoferrin-treated beef.

There is also no compelling evidence that BMDL would have the ability to induce allergic sensitization at the proposed ingestion levels. Studies in Brown Norway rats conducted by the British Industrial Biological Research Association (''BIBRA'') in the U.K. indicate that lactoferrin can induce IgE responses in these rats when injected intraperitoneally (Atkinson and Miller, 1994; Miller et al., 1998). However, this particular Brown Norway rat model is not appropriate for the prediction of human responses to ingestion of lactoferrin or other proteins. First, this model has not been validated. It does not discriminate between allergenic and non-allergenic proteins. No protein ever tested in this model with intraperitoneal injection has been shown to be negative. Therefore, the predictive value of these studies is essentially zero. In further studies with the Brown Norway rat using oral exposures, the ability of lactoferrin to induce an IgE response was much weaker and similar to BSA, another milk protein that is also not a particularly significant milk allergen (Meredith and Atkinson, 2000, 2001), although BSA is a known allergen in beef, a more uncommon allergenic food. This result raises even further questions regarding the initial observations obtained with intraperitoneal injections.

In addition, based on what is currently known regarding the lowest dose that elicits an allergic response in sensitized individuals exposed to several common (and fairly potent) food allergens (see the discussion below regarding peanuts, eggs, and cows' milk), the estimated intakes of BMDL resulting from its intended use as an antimicrobial spray on beef carcasses, as described in this document, are below any of these doses. Furthermore, the likelihood of an allergic response due to exposure to BMDL at these low levels is even more remote when one considers that there are likely to be few (if any) lactoferrin-sensitized individuals in the population. Thus, the possibility that lactoferrin would elicit an allergic reaction in any individuals at the proposed estimated levels of ingestion is unlikely.

The most recent work related to identifying elicitation thresholds for several well-recognized food allergens (i.e., peanuts, eggs, and cows' milk) comes primarily from Hourihane et al. (1997) and Taylor et al. (2002). In Hourihane et al. (1997), investigators used a randomized, double-blind, placebo-controlled study design to challenge 14 human subjects allergic to peanuts (i.e., sensitized) with peanut doses ranging from 10 μg to 50 mg, administered in the form of a commercially available peanut flour. What these investigators found was that even in this group of well-characterized, highly sensitive subjects with established peanut allergies, the ''threshold dose'' of peanut protein varies. More importantly, they observed that as little as 100 μg of peanut

protein could elicit subjective symptoms in some subjects with peanut allergy; 2 mg of peanut protein was the minimum dose needed to produce objective symptoms.

Taylor et al. (2002) reported the results of a roundtable conference between 12 clinical allergists who were invited to share existing data on threshold doses for food allergens in human subjects already sensitized to the allergen. In this paper, the investigators stated: "... sufficient results are available to conclude that the threshold doses for commonly allergenic foods are finite, measurable, and above zero. However, attempting to reach consensus on the threshold doses for peanut, egg, cows' milk, fish, and mustard on the basis of the existing data would probably be premature for a number of reasons." According to the authors, the primary reason for not estimating a threshold dose on the basis of the available data is that the no-observed-adverse-effect level ("NOAEL") was not established for the vast majority of the human subjects involved in these trials. Instead, the "lowest provoking dose" for each individual patient was determined. This dose corresponds to the lowest-observed-adverse-effect level ("LOAEL"), rather than the NOAEL. However, even though threshold doses were not specifically established in this paper due to uncertainties regarding what (if any) safety factors to apply, the tabulated results suggest that the lowest dose that elicits an allergic response in sensitized individuals exposed to peanuts, eggs, and cows' milk (standardized on protein content) appear to be in the low milligram range or higher for most individuals with allergies to these particular foods.

The estimated intakes of BMDL resulting from its intended use on beef carcasses, as shown in Table 3, are 76 μg or less, lower than the lowest dose (i.e., 130 μg) that elicited an observable allergic reaction in any sensitized individual exposed to peanuts, eggs, or cows' milk in the study reported by Taylor et al. (2002), and even less than the lowest dose observed in Hourihane et al. (1997) that elicited an allergic reaction in subjects with known peanut allergies (i.e., 100 μg). Moreover, the likelihood of an allergic response due to exposure to BMDL at these low levels is even more remote when one considers that there are likely to be few (if any) lactoferrin-sensitized individuals in the population.

Therefore, BMDL has not been established as a cows' milk or beef allergen, and there is no reason to believe that it will become an allergen under the proposed conditions of use.

7. Conclusion

Beef carcasses are treated with an antimicrobial spray, one component of which is BMDL (at not more than 2% by weight). The application is at a concentration not to exceed 0.20 ml of formulation per kilogram of beef. The meat from these carcasses is intended for further processing prior to being packaged as a final food product for sale at the retail level. BMDL is applied to beef carcasses simply to facilitate removal of microorganisms adhering to the carcass surface prior to further processing. The applied BMDL is rinsed from the carcass using a lactic acid wash, and it is not intended to have a functional effect in the finished food. Thus, BMDL functions as a processing aid in the preparation of beef products that may also be used as ingredients in other foods.

Beef carcasses naturally contain a background level of lactoferrin that is essentially indistinguishable from the BMDL applied to beef carcasses under the proposed use. The range of estimated intakes of endogenous lactoferrin that result from untreated beef consumption can be compared against the estimated intakes of exogenous BMDL that occur from consumption of beef from lactoferrin-treated carcasses. This comparison shows that the range of 50th percentile intakes of exogenous BMDL is within the range of estimated background exposures to lactoferrin across all population groups.

Some evidence exists to suggest that BMDL is a minor cows' milk allergen. Among individuals with documented cows' milk allergy, fewer than 50% have IgE antibody binding to lactoferrin. However, the fact that: (1) milk-derived and beef-derived lactoferrin are allergenically equivalent, (2) beef allergies are uncommon and no evidence exists whether or not lactoferrin is an allergen in beef, and (3) the proposed use of BMDL on beef carcasses is not expected to significantly increase typical lactoferrin exposures from meat consumption above background, makes the likelihood of an allergic reaction from the use of BMDL as a component (at not more than 2% by weight) an antimicrobial spray on beef carcasses very low. The use of BMDL spray on only beef carcasses (not subprimals or finished cuts) at a concentration not to exceed 0.20 ml of formulation per kg of beef is determined to be safe without the requirement of labeling of food products so treated.

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