Effective Removal of Manure/Mud Balls from Cattle Hides using Thioglycolate Salt Containing Formulations

by

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Abstract

The effective removal of hardened manure/mud balls from cattle hides remains a challenge for the livestock industry. Hardened manure/mud balls must be removed to minimize the risk of microbial meat contamination and hide quality deterioration. To overcome this challenge, we developed thioglycolic acid and its sodium, potassium and ammonium salts containing formulations. In this study, the developed four formulations were compared based on their efficacy in removing adobe type mud/manure balls from the hides in short time. Hide pieces containing hardened manure/mud balls were soaked in the formulations for 5 minutes followed by gentle brushing to remove the debris. The firmly attached mud/manure balls were removed completely from the hide pieces soaked in sodium, potassium and ammonium thioglycolate containing formulations. However, thioglycolic acid containing formulation did not show that much efficacy and the debris remained attached in the hide pieces soaked in water which was used as a control. Moreover, naturally onboard aerobic bacteria count was significantly reduced along with the mud/manure balls removal from hide pieces. After treatment, the hide pieces were processed into crust shoe leather for quality check analysis including mechanical properties, leather surface analysis and general appearance. The crust leathers made from hide pieces treated with the formulation exhibited similar quality to that made from the control. The implementation of this inventive formulations in slaughterhouses or leather industry will drastically reduce the time consuming and labor-intensive operations currently employed to remove firmly attached debris from cattle hide surface.

Introduction

Formation of hard to remove manure/mud balls on cattle hides is a major problem for both the meat and leather industries. Hardened manure balls form usually during winter season when animals in feedlots get wet and muddy. The problem of hard to remove manure formation is aggravated with long hair cattle breeds. The manure and mud accumulate on the hide hair and hardens as the temperature approaches to freezing. With the addition of more manure, the manure becomes exceedingly attached and entangled with the hide hair leading to the formation of hard to remove manure balls.[1-2] The presence of such type of adobe type mud/manure balls is a major concern to the cattle industry for several reasons. First, they could be a conducive environment for microbial growth leading to increased risk of food safety due to carcass contamination. Second, the adobe type mud/manure balls can create holes on hides during the fleshing process in tannery, as a result the value of the hide will be significantly affected. Third, the integrity of the hide can also be affected because of the enzymes released by the bacteria which will ultimately affect the leather quality.[3-5] To avoid these problems it is important to remove the firmly attached external debris from cattle surface prior to slaughter and leather processing.

Currently, hard-dried manure/mud balls are manually raked or mechanically removed from the animals either by washing the animals with water or using electric shaver. These current practices are labor intensive and suboptimal because of the need to use large amount of water under high pressure for hours and the discomfort experienced by the animals as well as the manual handling of animals by workers.[2,6]

There has been effort to develop formulations that can remove hardened manure. Auer et al. reported[7] that hardened manure balls are mainly composed of lignocellulosic material (cellulose, hemicellulose and lignin) and other minor components, like feed proteins, hair, lipids, carbohydrates and minerals. To that effect solutions containing mixtures of enzymes were developed to break down the components of the hardened manure on cattle surfaces. The enzymes utilized include cellulase, xylanase or hemicellulase and laccase. Even though the formulation of enzyme mixtures was reported to aid in removing the hardened manure but in a proof of concept study the enzyme mixtures were not found to be effective when applied in live animals. In another study Ramos et al reported a soaking formulation that can soften hardened manure and facilitate their removal from the hide surface. The soaking formulations containing sodium carbonate and glycerol with or without biodegradable detergent sophorolipid were

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Manuscript received December 14,2021, accepted for publication March 2, 2022.

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applied on hide samples containing hard-dried manure for 2 hrs. The soaking formulation was reported to be effective in softening and subsequent removal of manure from hide surface. It was also reported that the soaking formulation did not negatively affect the quality of the finished leather.[6] Marsico et al also reported the use of sodium percarbonate as an oxidizing agent in combination with sodium hydroxide in removing hardened manure from hide surface. This combination was reported to remove hardened manure in less than 30 minutes by weakening the hair.[8] Recently Navone et al reported[2] manure removing formulations which contained protease enzymes that attack the interaction between the hardened manure and the hair. Protease enzymes weaken the framework of hairs at the point of attachment between the hair and the manure while degrading adhesive protein that may hold the manure structure together. However, these soaking formulations require long time to be effective in terms of industrial application and can only be applied on hides not carcass, therefore, no use in meat processing plant prior to hide removal. Thus, there is still a need to develop cost effective, labor efficient and fast method of removing hardened debris from hide surface without damaging the hide.

In our previous study,[9] we developed a formulation combining potassium thioglycolate with sodium hydroxide. The formulation was effective in removing mud/manure balls from the hide surface in 5 to 8 minutes depending on the level of concentration. The formulation disrupts the disulfide bonds of keratin protein in hair where the mud/manure balls are firmly attached to on cattle surface. An aqueous soluble weakly toxic antimicrobial agent, sodium dichloroisocyanurate dehydrate[10-12] was also added into the formulation to improve its efficacy on limiting microbial population on hide. Both spraying and soaking methods of dispensing the formulation were found equally effective in performance. However, in this study three more formulations have been developed using thioglycolic acid, sodium and ammonium thioglycolate. The efficacy of these formulations has been compared in removing mud/manure balls from the bovine hide. The impact of the formulations on the quality of the finished leather was evaluated by assessing the grain structure and measuring the mechanical properties of leather produced out of the treated hide samples.

Materials and Methods

Fresh bovine hides were collected from the local meat packing company, JBS Packerland (Souderton, PA). The manure containing part of the hide was cut into smaller pieces of approximately equal size (12 in × 12 in) that weighed around 1kg. Potassium thioglycolate solution (40-44%) was purchased from Acros Organics. Thioglycolic acid, sodium thioglycolate and ammonium thioglycolate were purchased from Sigma Aldrich. All the chemicals were of laboratory grade at the time of use.

TABLE I

Composition of manure removing formulations

Formulations	Composition
F-A (control)	Tap water
F-B	7.5% Potassium thioglycolate + 7.5% NaOH
F-C	7.5% Sodium thioglycolate + 7.5% NaOH
F-D	7.5% Ammonium thioglycolate + 7.5% NaOH
F-E	7.5% Thioglycolic acid + 7.5% NaOH

Preparation of Mud/manure Removing Formulation

The formulations were prepared as described in Table I by dissolving the required amount of the chemicals in tap water at room temperature (~22°C) and ~24 h prior to conducting the experiments.

Determination of pH of the Developed Formulations

Three readings have been taken to determine the pH of each formulation at room temperature. A laboratory pH meter, YSI 1200 Laboratory pH Instrument with a range of 0.00 to 14.00 units and ±0.01 units accuracy has been used for the measurement.

Manure/Mud Removing Method

Picture of the hide pieces containing manure/mud balls were taken before soaking them within the formulations. The hide pieces were soaked in 100% (w/w) float of the manure/mud removing formulations for 5 minutes. The soaked hide pieces were spread on a flat surface and gently brushed with a polymer brush to remove the debris from the hide panels. After brushing off the debris, pictures of the hide pieces were taken again, and finally the hide panels were directly used for leather processing.

Microbial Testing

A 10in × 5in surface of hide area were aseptically and independently swabbed with a sterile sponge before and after brushing and placed into a bag with 25 mL of buffered peptone water from a sampling kit for analysis (Nasco Meat and Turkey Carcass Sampling Kit, Salida, California). Sample bags containing the buffered peptone and the swabbed sponges were hand massaged for ~2 min. Samples were subsequently diluted and spread-plated on Tryptic Soy Agar (TSA). The TSA was obtained from Fisher Scientific, Pittsburg, PA. Samples were incubated between 24-48 hours at 37°C and bacterial colonies were enumerated for aerobic bacterial recovery with the lowest detection level at 1 CFU (colony forming unit) per 10in × 5in area. All assays were carried out in triplicate, and the results are presented as the mean standard deviation as described by Steel et al (1997).[13] GraphPad software was used to perform a one-way analysis of variance with a post hoc Turkey's test (GraphPad Prism 7.0, GraphPad Software, Inc., San Diego, CA).

Table II
pH of the Formulations

Sample	R1	R2	R3	Average	St. Dev.
F-A (Tap water)	7.2	7.15	7.23	7.19	± 0.040
F-B	12.3	12.29	12.34	12.31	± 0.026
F-C	12.07	12.08	12.08	12.07	± 0.005
F-D	12.67	12.68	12.74	12.69	± 0.037
F-E	9.56	9.61	9.56	9.57	± 0.028

Tanning and Leather Quality Analysis

Following the manure removing operation, the hide pieces were washed with water individually and put together in one hair removing drum. The dehairing process was carried out in accordance to the established USDA tanning procedure.[14-16] After the dehairing process, all the hide samples were put into a single drum for pickling, tanning, re-tanning steps. The hide samples were processed into crust upper shoe leather and stored in a controlled environment, where the temperature and humidity are maintained at 21°C and 50% (relative humidity) respectively, until the mechanical tests, organoleptic and microscopic analysis were performed.

The impact of the developed manure removing formulations on leather quality was determined by measuring the mechanical properties of the finished crust leather. The mechanical properties which include tensile strength, elongation ("stretchability"), Young's Modulus ("stiffness") and fracture energy ("energy required to open unit area of crack surface") were determined as per the procedures mentioned in previous article.[17-19] Five dumbbell shaped samples were cut from each experimental leather piece according to the protocol in ASTM D2209. Leather samples with an average thickness range of 1.86 to 2.58 mm was observed. An Insight-5 test frame and Testworks-4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used to determine the mechanical properties of the leather samples. The strain rate and the grip distance for this study were set to 24.5 cm/min and 10.16 cm respectively. Subjective tests (break, handle, fullness, and color) on the finished leather products were conducted by an in-house (USDA) leather expert. Crust leather panels produced from the control and formulation treated hides (F-A to F-E) were also analyzed under a stereo microscope (Nikon Digital Microscope SMZ-2T, Melville, NY) to determine if there is any difference in the grain structure of the experimental leather panels comparing to the control. The stereo microscope images were taken according to the published procedures.[16-18]

Results and Discussion

pH of the Developed Formulations

The pHs of the formulations have been listed in Table II. The formulations, F-B, F-C, F-D are basically the mixture of a neutral thioglycolate salt and sodium hydroxide therefore, the pH of these three formulations is found at the high end of ~12 as expected. However, the pH of F-E is found lower because some of sodium hydroxide is consumed to neutralize the thioglycolic acid in the formulation but still remains basic in nature.

Efficacy of Different Manure/Mud Removing Formulation

Figure 1 shows the pictures of the fresh hide pieces before and after the manure removing operation. For comparison purposes the pictures are displayed side by side as before and after treatment for each manure removing formulation. As it can be seen from the pictures, the manure was removed completely from the hide pieces treated with formulations F-B, F-C and F-D. Adobe type mud/manure balls are still firmly attached to the control sample (F-A) and also the manure was barely removed by the formulation F-E. The debris were dislodged from the attachment site after 5 minutes of soaking followed by gentle brushing. The easy removal of the hard-ball manure can be attributed to the effect of the reducing agents on the hairs where the manure is attached. That means the disulfide bond of the keratin which is the main component of the hair is being reduced and the hair is breaking apart.[20-21] It was also observed that some part of the hardened manure ball was getting decomposed and that may be because of the effect of the reducing agent on the protein component of the manure. Out of all formulations it was easier to remove the debris from the hide pieces soaked in sodium and potassium thioglycolate containing solutions than other formulations and that could be because of the differences in the rate of diffusion of the reducing agents into the hair structure as a whole. The rate of diffusion depends on the chemical nature of the reducing agents (Ogawa et al 2008).[22] Thus sodium/potassium thioglycolate solutions should have higher diffusion rate to cleave the disulfide bond in a short period of time compared to the other reducing agents used in other manure removing formulation.

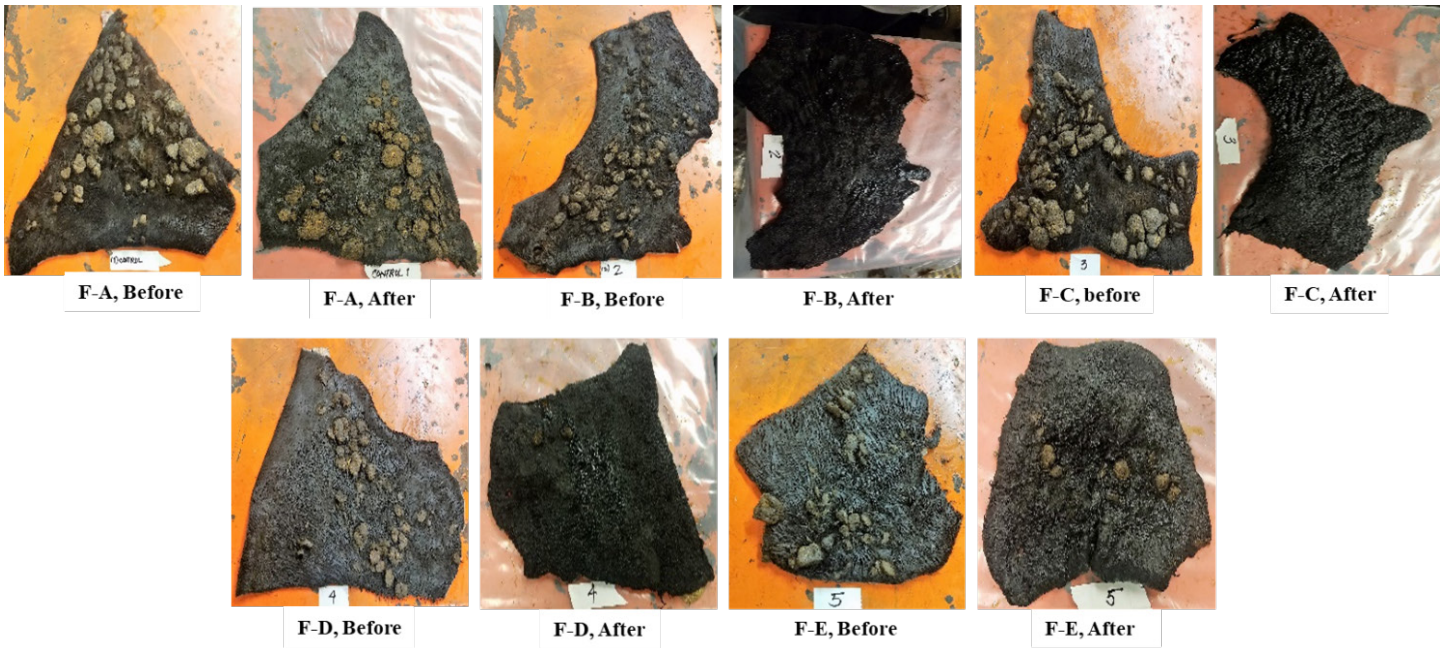

Figure 1. Picture of hide pieces before and after mud/manure balls removing treatment using different formulations. **F-A**, **F-B, F-C**, **F-D**, **F-E** represent formulation composition as described in Table 1.

Aerobic Bacterial Count

Generally, underlying meat surface of the carcass is sterile,[23-27] but it can be contaminated with microorganisms transferred from the hide surface during the hide removal process. Also, excessive bacteria present on raw hide leads to its deterioration quickly. Therefore, hide surface decontamination plays an important role for both meat and hide processing operations. Aerobic bacteria count analysis was carried out to see if the removal of external debris including manure/mud balls helps to reduce the naturally onboard bacteria from the hide surface.

As it is shown in Figure 2, before treatment all the hide pieces carried almost similar number of aerobic bacteria naturally. However, after removing mud/manure balls or debris F-B, F-C and F-D treated hide samples got in reduction of aerobic bacteria by 3.09, 1.87, 4.13 log CFU/50 in^2 respectively. On the contrary, control (F-A) and F-E treated samples got in reduction of only 0.51 and 0.1 log CFU/50 in^2 respectively. This data establishes a co-relation between Figure 1 and 2, where Figure 1 shows F-B, F-C and F-D perform better than the control (F-A) and formulation F-E in removing mud/manure balls from hide surface: less debris result in more reduction in bacteria counts. This experiment overall indicates that by only removing the external debris, a significant reduction of bacteria on hide surface can be achieved as those debris mainly harbor those microorganisms.

Quality Analysis of Crust Leather

Following the USDA standard tanning protocol, the hide pieces were processed into crust upper shoe leather. The impact of the developed manure removing formulations on leather quality was

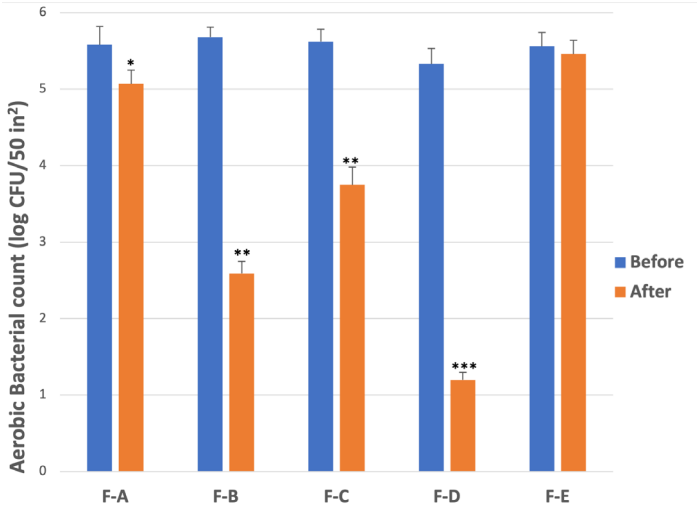

Figure 2. Aerobic bacterial count on hide surface before and after mud/manure removing treatment with different formulations. Asterisks indicate that the differences between the means of various treatments are statistically significant (*P 0.05; **P 0.01; ***P 0.001).

Table III

Mechanical properties of the finished leather product after treating with the manure removing formulations

Sample	Tensile Strength	Elongation	Young's Modulus	Fracture Energy
	(MPa)	(%)	(MPa)	(J/cm^3)
F-A	7.9 ± 0.9	40.1 ± 8.8	12.2 ± 4.9	1.1 ± 0.2
F-B	6.7 ± 2.1	26.0 ± 4.3	23.1 ± 11.6	0.7 ± 0.2
F-C	6.6 ± 1.7	27.8 ± 5.2	20.7 ± 10.9	0.7 ± 0.1
F-D	9.8 ± 0.4	38.1 ± 3.1	15.4 ± 2.5	1.3 ± 0.2
F-E	7.1 ± 2.2	34.6 ± 7.2	13.3 ± 3.4	0.9 ± 0.3

determined by measuring the mechanical properties which include tensile strength, elongation to break, Young's Modulus (stiffness) and fracture energy (the energy required to fracture the leather sample). In addition to measuring the mechanical properties, the grain surface of the finished crust leather was also analyzed. Moreover, the quality of the finished leather was also verified by a subjective test where in-house USDA leather expert assessed the break, handle, fullness, and color of the finished leather product.

Mechanical Properties

Even though the hardened manure is a major source of hide quality deterioration, it is also important that the manure removing formulations do not decrease the performance of the final leather products. As shown in Table III, it is evident that there is some variation in some aspects of the mechanical properties of the finished crust leather obtained from the treated hides with different manure removing formulations. Compared to the control, there is no significant difference observed in the tensile strength among the crust leather products. However, the stretching and stiffness properties of F-B and F-C are different in comparison with the control (F-A) and this is due to the fact that raw hide pieces are collected from the different parts of a bovine hide where natural variation of hide thickness exists. In fact, 1.86 to 2.58 mm range of variation in thickness was found among the dumbbell shaped leather samples (cut from the experimental leather panels) prepared for the mechanical studies. It is very unlikely that only 5 min exposure of formulations with similar pH values (~12) can create such a significant change, rather it is more obvious that, leather products produced from hide pieces of different thicknesses show differences in mechanical properties.

Grain Surface Analysis of Leather Panels

The grain structure of each finished leathers was analyzed under a stereo microscope. The analysis was conducted to see the effect of each formulation on the surface fineness or coarseness of the crust leather. As shown in Figure 3, there is no noticeable difference between the grain structure of leather produced from the hide treated with the manure removing formulations and the control, suggesting that the

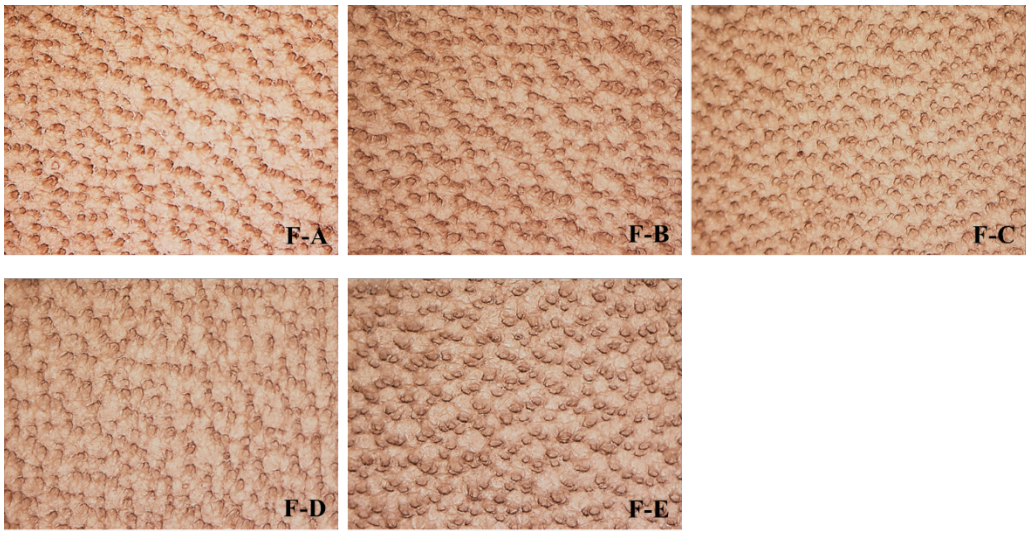

Figure 3. Stereo microscopic images of the crust leather made from hides treated with water (control) and different formulations.

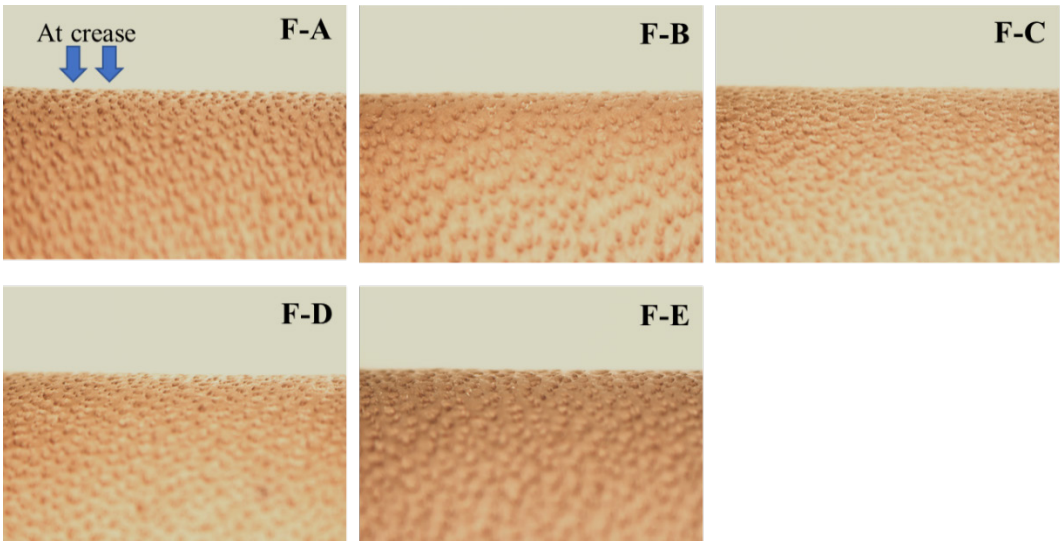

Figure 4. Stereo microscopic images of the finished leathers at the crease.

formulations did not adversely affect the hide pieces in the process of removing hardened manure. To further evaluate the surface feature of the crust leather, stereo microscopic images were captured at the crease upon folding the leather panels (Figure 4). Again, there was no fraying (broken fiber) was observed at the crease on any of the leather pieces indicating no damage on collagen network.

Subjective Test Analysis of Leather Panels

In-house expert on leather assessed the crust leather panels for their fullness, softness, color, grain tightness (break) and general appearance by hand and visual examination (Table IV). The subjective test results were expressed on a scale of 0-5 points with 0 being the worst and 5 being the best outcome. The leathers produced from the control and hide pieces treated with the manure removing formulations displayed similar fullness, grain, handle, color and general appearance. Overall, the subjective test results indicate that the manure removing formulations did not damage the hides as there is no significant difference between the control and the leather produced from treated hides.

Thioglycolic acid, sodium thioglycolate, potassium thioglycolate and ammonium thioglycolate are safe to humans and to the environment because they are commonly used chemicals in permanent wave, hair straightening, hair color preparations and depilatories.[28-30] The use of up to 5% of thioglycolic acid and its salts in cosmetic preparations is generally considered to be safe which is close to the concentrations used in this study. Therefore, the use of these chemicals in mud/manure removing formulation will not present any unreasonable risk to humans and the environment.

Conclusion

To remove firmly attached adobe type mud/manure balls or external debris from the cattle surface is essential but challenging task for the meat and hide industries as it relates to meat safety and byproduct quality. In previous study, we reported the efficacy of potassium thioglycolate based formulation in removing debris from the hide surface. In this study the effectiveness of four formulations containing thioglycolic acid and its potassium, sodium and

Table-IV

Subjective properties for leather samples made after treating with the manure removing formulations

Formulation	Break	Fullness	Handle	Color	Grain	Appearance
F-A	5	3	3	3	3	4
F-B	5	3	3	4	4	3.5
F-C	5	3	3	3	4	3.5
F-D	5	3	3	4	4	3.5
F-E	5	4	4	3	2	4

ammonium salt respectively has been compared. Although, all developed formulations have been found effective in removing debris from hide surface to some extent but sodium and potassium thioglycolate based formulations exhibited better performance than others. Mud/manure balls can be easily removed from the hide panels soaked in those formulations for 5 minutes. The removal of debris also facilitates the reduction of onboard natural bacteria count significantly. From the stereo microscopic analysis and mechanical properties, the developed manure removing formulations do not appear to have any negative effect on the finished crust leather. This study will provide a wide option for meat and hide industries to choose the appropriate mud/manure removing formulation based on cost and the availability of chemicals. Application of the developed formulations in slaughterhouses will minimize the likelihood of meat contamination and avoid the ineffective and labor-intensive work required to remove manure/mud balls from cattle hides.

Acknowledgments

The authors would like to thank Mr. Nicholas Latona and Mr. Joe Lee for their technical assistance. This work was supported by U.S. Department of Agriculture-Agriculture Research Service, National program 306.

Conflict of Interest

The authors declare that there is no conflict of interests on the work published in this paper.

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