Experimental Fleece-Removal with Bioclip® Wool-Harvesting System for Merino-derived Wool Sheep in the US

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***Abstract*—The objective of this trial is to evaluate the efficacy of a biological wool-harvesting system, Bioclip®, as an alternative to the mechanical shearing of wool sheep. Twenty-six 10-month-old ewes were selected for a Bioclip® shearing comparison experiment. Ewes were weighed and stratified by body weight and breed, and then, divided into a control (conventional shearing, n = 10) and Bioclip® treatment group (n = 16). Treatment group animals were each given a 2.5 ml Bioclip® injection formula (7.5 mg/ml epidermal growth factor or EGF) subcutaneously on the inguinal bare skin area, after which a fleece retention net was placed on each animal. Sheep were fed alfalfa hay for 1 week prior to the Bioclip® injection, and 4 weeks post-injection under a semi-sheltered pen, until fleece removal at the 28th day, with wool regrowth monitoring at 5 weeks postharvest. Posttreatment wool regrowth monitoring was conducted and compared for the control and Bioclip® groups at 5 weeks post wool harvesting. There was no difference in the posttreatment body weight, fleece weight, weight gain, fiber diameter, and wool regrowth rate between the control and Bioclip® treatment group. Whereas, fleece staple length and regrowth fiber length measured significantly ($P < 0.01$) longer for Bioclip®-harvested wool than conventionally shorn sheep. This was the first time Bioclip® was used experimentally on US wool sheep and resulted in a simultaneous and complete shedding of fleeces. The results suggest that Bioclip® can improve wool clip quality and animal welfare as well as reduce farm labor intensity.**

***Index Terms*—wool sheep, fleece, Bioclip®, shearing**

I. Introduction

Wool is a dominant product of sheep enterprises and its procurement requires that fleece be harvested by mechanical shearing. Professional shearing is a highly seasonal occupation done only by those with skill and experience. The shortage of experienced shearers has been recognized along with animal welfare issues related to mechanical shearing. Most of the 6 million sheep in the US produce Merino-derived wool (i.e., wool-meat producing flocks) and must be shorn annually. However, a biological shearing procedure developed in Australia may offer an alternative wool-harvesting method that is more humane, less stressful, produces less wool contamination, and is more environmentally friendly. Several chemical and biological de-fleecing reagents and procedures had previously been investigated in wool-growing sheep [1]-[3]. However, none of those reagents were ideal or as practical as a de-fleecing reagent for wool sheep flocks on farms.

A new product, Bioclip®, was shown to be an effective biological agent for use on farms with large-scale commercial wool flocks. This product was developed by Commonwealth Scientific and Industrial Research Organisation (CSIRO) scientists in Australia and licensed to Bioclip® Pty Ltd Australia for on-farm application. Bioclip® is a biological Wool Harvesting System (WHS) that has become an integrated process for the harvesting of wool from Merino and its derived sheep breeds. The Bioclip® is based on a short-chain protein called Epidermal Growth Factor (EGF), which is a natural product of the animal physiology system. In early studies, the fundamental role of EGF in the growth and maintenance of the skin was confirmed by the identification of EGF receptors in cell populations of the epidermis, dermis, and hair follicles [4], [5]. The Bioclip® formulation was based on a series of fundamental studies to determine the effect of EGF on skin, wool follicles, and regrowth wool in sheep [6]-[10].

The application of EGF as a wool-harvesting agent for sheep is being formulated using a protein derived from the bacteria transformed by a synthetic EGF gene [11], which remains in animals for only a short time, with complete clearance from the body and it by-products [12]. As a dose of formulated EGF is injected into fleece-growing sheep, it causes a temporary break in the wool follicle fiber synthesis process and causes the fleece to shed as naturally as in hair sheep breeds. New wool growth commences a few days after injection and emerges at the skin surface level in 2 weeks, and there is sufficient wool cover to protect sheep from sunburn or hypothermia by 28 days post-injection. Therefore, it is recommended to collect bio-clipped fleeces from sheep 4 weeks after EGF administration. The dose rate and formulation of the Bioclip® injection has been designed to maintain EGF in the sheep's physiological system at an effective concentration to act on wool follicles for 16

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hours. Then, EGF is metabolized by the body into its substructure of amino acids and excreted in urine after further breakdown.

The objective of this investigation is to evaluate the efficacy of the Bioclip® application in US Merino or Merino-derived, wool-meat sheep strains as an alternative wool-harvesting system.

II. Materials and Methods

We have evaluated Bioclip® for fleece harvesting effectiveness, wool regrowth, and fleece qualities of harvested wool from Merino or Merino crossbred sheep on the Nevada Agricultural Experiment Station, Reno, Nevada, and Rafter 7 Ranch, Yerington, Nevada, during the spring shearing season. The experimental application of the biological fleece-shedding agent Bioclip® in sheep was approved by the University of Nevada-Reno Institutional Animal Care and Use Committee (IACUC approval #000355).

A. Experimental Animals

Fine wool sheep flocks at Rafter 7 Ranch were registered with the Delaine Merino Breed Registry in the US, whereas, crossbreds were of Merino x Rambouillet sheep. Twenty-six 10-month-old fall-born ewes (Merino = 13, Merino crossbred = 13) at an average body weight of 39 kg were randomly selected for a biological, wool-harvesting comparison experiment. Animals were transferred from a Rafter 7 Ranch grazing pasture to a pen feeding facility at the Nevada Agricultural Experiment Station (Reno) and fed ad lib on alfalfa hay for 7 days prior to the procedure of mechanical shearing or Bioclip® treatment. Ewes were weighed and stratified by body weight and breed/strain, and then divided into a control (conventional shearing, CS = 10) or Bioclip® treatment group (BS = 16). Wool characteristics were not pretested or considered as factors when determining group allocations. Animals were fed alfalfa hay moderately for 4 weeks under a semi-sheltered pen, with constant water supply, until bio-clipping (fleece removal at the 28th day), followed by observation five weeks posttreatment.

B. Feed and Water Supply

Animals were fed in fence line feed troughs ad lib with dry hay harvested from the station farm during the summer. Drinking water was provided by a watering tank using automatic refilling. Hay quality was assessed with conventional feed analysis and determined for in vitro degradability. Weekly subsamples of dry hay bales were prepared for a conventional feed composition analysis and in Vitro Dry Matter Degradability (IVDMD). Organic Matter (OM), ash, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Crude Protein (CP) were measured as 89.2 ± 0.01, 10.8 ± 0.01, 52.3 ± 0.19, 33.7 ± 0.44, and 15.7 ± 0.14, respectively. The in Vitro Dry Matter Degradability (IVDMD) values were determined by incubating dried ground hay samples in tubes with cattle rumen fluid [13], with an average value of 66.3%.

C. Bioclip® Injectable EGF

Bioclip® injection solution (500-ml plastic bottle), a multi-injector, fleece retention nets, and the Bioclip® sheep cradle were supplied by Heiniger Australia Pty Ltd. The Bioclip® formula contains 7.5 mg of EGF in each ml of sterile solution for injection, thus an 8-18-month-old sheep up to a body weight of 50 kg requires a single dose of 2.5 ml per head. Each of the BS animals were injected with 2.5 ml Bioclip® injection formula (7.5 mg/ml EGF) subcutaneously on the inguinal base skin area (inside thigh). Subsequently, a fleece retention net was placed on each sheep using a specially designed netting cradle. The Bioclip® protein injected into the sheep reduces the rate of cell division at the dermal papilla of the wool follicles, which results in a tapering of individual fibers, which ultimately creates a break. Thus, it separates the shedding fleece from the newly emerging fibers. The Bioclip® injection dose delivers the EGF under the skin in a bare area on the inside the sheep's thighs (subcutaneously) using a multidose injector or syringe fitted with an 18 G needle. In addition, an appropriate size of fleece retention net (blue color code for 30-40 kg body weight) was fitted on the sheep after the injection. Several sizes of fleece retention nets are available, based on the animal's body weight. In contrast, CS animals were injected with 2.5 ml of medical saline in the same area of skin inside of the sheep's thighs using a syringe fitted with an 18 G needle but without using fleece retention nets.

D. Shearing, Bio-Clipping and Fleece Collection

The control group of ewes was shorn mechanically by an experienced shearer on the same schedule for bio-harvesting, using a conventional sheep shearing hand piece fitted with a wide cutter and comb. Shearing was scheduled so that Bioclip® shearing was timed at 28 days posttreatment. At the scheduled fleece collection, animals were moved into a clean pen with a raceway where animals were checked to determine their readiness for fleece collection. Fleece handling board was placed at the side of the wool-harvesting pen or raceway. Twelve of 16 Bioclip®-treated animals were fleece-harvested at the Nevada Agricultural Experiment Station (Reno), which were compared to the CS group. The remaining bio-clipped ewes (n = 4) were transported to Rafter 7 Ranch (Yerington) for a biological wool-harvesting demonstration at a producers' field day workshop. Bio-clipped sheep were held in a conventional shearing position and scissors were used to clip open the fleece retention net between the hind legs. Then, the hind legs were pushed back through the hind leg sleeves of the retention nets. Fleece in the retention nets were rolled over the bodies and necks of the sheep. Wool staple samples of the fleeces were collected and labeled to measure fleece characteristics while the fleece net was cut open for skirting on the wool table. Each harvested fleece was weighed, recorded, and assigned a wool class category. Non-fleece wool cover from the head, tail, and limbs was collected and pooled as odds for groups without individual weights.

E. Wool Regrowth Monitoring

Posttreatment Wool Regrowth (WRG) monitoring was conducted to compare the CS (n = 10) and BS (n = 12) groups for the 5-week period. Animals were identified from each of the CS and BS groups, and a small, measured, mid-side square patch (25 cm^2) was hand-clipped on the left flank of each sheep, using a small animal clipper (Oster® clip size 40). After the 5-week regrowth wool was collected, wool fiber length (mm) and weight (mg/cm^2) from the patch area was measured, analyzed, and compared.

F. Measurements and Statistics

Animals were recorded for initial and post-experiment Body Weight (BW), greasy Fleece Weight (FW), and wool characteristics, including Fiber Diameter (FD), Staple Length (SL), and fleece quality. FD was measured according to a standard microscopic method. In addition, the Average Daily weight Gain (ADG) was calculated. Fiber morphology was examined using a projection light microscope (400x) at the base end of the staples of the conventionally and biologically harvested fleece fibers. Posttreatment monitoring data collection was analyzed for one-way ANOVA, and mean values were compared using t-test procedures [14].

III. Results and Discussion

Fitted fleece nets retained the shedding fleeces efficiently, which were placed around the BS sheep body frame without any positions shifting (Fig. 1A) and which had no effect on the animals' movement or comfort. In contrast, the CS sheep appeared to have a loose or open fleece, with dusty staple tips (Fig. 1B).

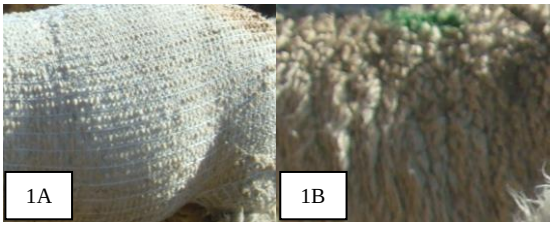

Figure 1. A: Bioclip®-Sheared (BS) sheep fitted in a fleece retention net postinjection; B: Conventionally Sheared (CS) untreated sheep without a fleece retention net. Both BS and CS sheep were managed in a group and fed in the same pen.

Figure 2. A: Bio-clipped, wool-harvested fleece was removed from the retention net from sheep without any physical handling stress, labor intensity, and/or injury to the sheep; (B) conventional sheep shearing required skill and strength to restrain the animal during the shearing process.

Bioclip®-treated fleece removal was easy and fast (Fig. 2A), while sheep shorn by conventional shearing required more than double the time to restrain the sheep and was also more laborious (Fig. 2B).

Bio-clipped fleeces appeared to be visually cleaner and had less hay matter and dirt contamination compared to CS fleeces. While with machine shearing, a skilled shearing worker is required to conduct the process, for the bio-harvested wool collection, no shearing experience is needed to collect fleeces. When using bio-clipped fleece collection, sheep can be held in the conventional shearing position, and scissors can be used to clip open the fleece retention net between the hind legs, after which the hind legs can be pushed back through the hind leg net sleeves. Any fleece in the retention net was rolled over the body and neck of the sheep. There was no difference in fleece shedding responses to Bioclip® by Merino or Merino crossbred ewes. Overall, the Bioclip® injection resulted in a simultaneous and complete shedding of fleeces in all treated animals, while retaining the removed fleeces inside the retention nets. At the collection of bio-clipped fleece, treated sheep had an even and uniform new coat cover of 8-mm wool fibers (Fig. 3A), while the machine-clipped coat had uneven piles (4-18 mm) or second cuts (Fig. 3B).

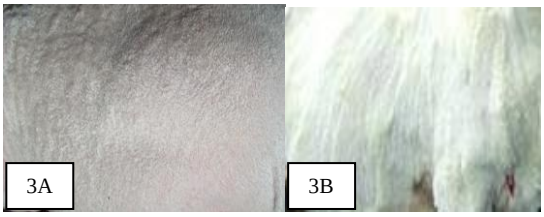

Figure 3. A: Bioclip® wool-harvested sheep's side view, including the flank, belly, shoulder, and leg, which had a new uniform coat cover of 8-mm wool when the shed fleece was collected 28 days after injection; B: Conventional wool- harvested sheep's side view, including the flank, belly, shoulder, and leg, which appeared to have uneven coat piles, second cuts, and sometimes skin injuries.

There was no need for animals to fast or to deprive them of feed and/or water prior to the bio-harvesting operation. However, it was very important to delay at least four weeks between the Bioclip® injection and harvesting to allow the sheep to have sufficient regrowth of wool to protect their body surfaces from sunburn or cold stress. There was no requirement to undertake any forceful plucking off of wool from the extremities, such as the head and/or legs, in this experiment. However, a recent investigation indicated that the level of nutrition and body condition post-Bioclip® injection has a greater effect on biological wool-harvesting efficacy than during the period prior to injection [15]. It was noted that there were small patches of wool staple dropping from the head and breech areas that were not covered in the fleece nets, which should have been trimmed when putting on the fleece retention nets. Biological wool-harvesting procedures have significantly improved the retention of fleece staple length uniformity and fleece purity as well as having exhibited other distinct advantages over mechanically shorn wool, such as not having skin pieces,

second cut wool, medullated fibers, and shearing floor contamination (Fig. 4A, B).

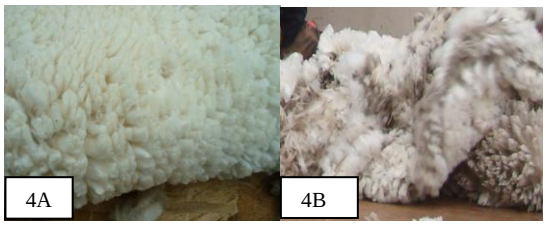

Figure 4. A: Bioclip®-harvested fleece with a uniformity in staple length and free of second cut wool staples, skin pieces, vegetable matter, and floor contamination; B: The main fleece and odds of conventionally shorn fleece tended to mix easily as well as to be more contaminated with dirt and vegetable matter when piled on the floor.

Body weight changes, fleece weight, wool characteristics, and posttreatment wool growth monitoring results are shown in Tables I and II.

TABLE I. BODY WEIGHT (BW), GREASY FLEECE WEIGHT (FW), AND STAPLE LENGTH (SL) OF THE CONVENTIONAL SHEARING (CS) AND BIOCLIP® SHEARING (BS) GROUPS

Group	Initial BW (kg)	Final BW (kg)	FW (kg)	SL (mm)
CS	39.2	43.5	2.83	68.5
BS	38.9	43.2	2.53	84.6
SEM	2.0^{ns}	2.6^{ns}	0.58^{ns}	0.4^{**}

SEM = standard error of means; ns = nonsignificant; * indicates $P < 0.05$; ** indicates $P < 0.01$.

There was no difference in BW, FW, ADG, and fleece wool FD measurements. However, the SL of bio-harvested fleece measured significantly longer than the conventionally shorn fleeces ($P < 0.01$).

TABLE II. AVERAGE DAILY GAIN (ADG), FLEECE FIBER DIAMETER (FD), REGROWTH WOOL WEIGHT (RWG), AND REGROWTH FIBER LENGTH (RGL) OF THE CONVENTIONAL SHEARING (CS) AND BIOCLIP® SHEARING (BS) GROUPS

Group	ADG (g)	Fleece FD	RGW (mg/cm^2)	RGL (mm)
CS	134.4	17.6	32.5	11
BS	133.4	17.0	29.3	13
SEM	56.3^{ns}	0.5^{ns}	0.01^{ns}	0.56^{**}

SEM: standard error of means; ns: non significant; * indicates $P < 0.05$; ** indicates $P < 0.01$.

The posttreatment WRG rate between the control and Bioclip® treatment groups did not differ in 5 weeks. However, the growth weight per unit area was slightly higher for the CS group, which accounted for the difference in the fiber diameters of the groups. In contrast, regrowth fiber length measured in the mid-side patches was significantly ($P < 0.01$) longer for the bio-clipped group than for the conventionally shorn sheep. In the bio-clipped group, there appeared to be a subtle compensatory growth after the EGF treatment delay for fiber synthesis in the skin follicles. Wool staple morphological characteristics were not affected by shearing procedures; however, biologically harvested fibers appeared to end with more smooth and round tips than mechanically shorn fibers. As for fleece characteristics, such as BS, the wool had no second cuts and had short staples, less vegetable matter, and less shearing floor contamination in contrast to the CS group. There was no adverse effect observed on the animals whose fleece was biologically removed. The majority of participants at the biological wool-harvesting demonstration at Rafter 7 Ranch expressed their approval of the procedure and an interest in biological wool-harvesting as an alternative practice. The result of this experiment was in close agreement with earlier studies conducted with Merino wool sheep in Australia [5], [7], [16], [17]. Light microscopy observations of biologically shed fibers also found evidence of the tapering end fibers indicated in earlier studies, in which EGF induced a catagen-like effect in cultured hair follicles during in vitro experiments [17]. The characteristic of tapered shedding fiber root ends was identified by electron transmission microscopic examination in Merino sheep infused with mouse EGF [6], which revealed that shedding fleece by EGF used a similar mechanism of alternatively switching from the anagen to the catagen phase in fiber follicles in hair coat-shedding sheep. The electron microscopic observation indicated that EGF infusion in Merino sheep induced wool follicle apoptosis in all cell types in the proximal region of catagen follicles after 12 hours and up to six days posttreatment [18].

The Bioclip® reagent is a sheep-specific protein product shown to shed wool fleeces in wool sheep effectively and simultaneously without any ill effect on the animal's well-being and wool quality or on human and environmental safety. This experimental result was confirmed with a series of studies conducted to determine EGF's effect on sheep skin and wool follicles [5]-[9], which finally created a possible path to a biological wool-harvesting practice. Those studies showed that the effect of EGF in wool follicles was to inhibit DNA synthesis and also demonstrated that the infusion of EGF into sheep resulted in a "break" and shedding of the fleece. These studies elucidated the mechanism operating at cellular levels that actively divided wool follicles in Merino sheep so that they move into the catagen phase within hours of EGF administration, and the wool fibers taper off as cell division ceases in the follicle, forming a keratogenous zone of the wool follicle bulbs. Subsequently, [12] verified that recovering infused labeled EGF in urine and fecal excretion at 97% (24 hours) and 100% by 48 hours, showed that it was unlikely for sheep to retain any measurable residues in their bodies or by-products. With an adequate EGF dose, sheep had a complete fleece shedding 7 days post-infusion. The advantages of bio-clipping over conventional shearing for textile markets are that bio-clipping provides more uniformity, longer staples, and higher quality wool in the clip [19]. Although it is being accepted as a new, alternative commercial wool-harvesting practice in Australia, this trial of biological wool-harvesting was the first

experimental use of Bioclip® in North America. The result confirms the Bioclip® reagent's efficacy to shed wool fleeces in North American types of Merino and Merino-derived wool sheep. At this stage of application, the Bioclip® procedure seemed especially desirable for shearing lambs, yearlings, wethers, and nonbreeding wool-producing sheep. Using Bioclip® as a de-fleecing tool in hair sheep selection (e.g., Dorpers) and hair-shedding management are also now possible in practice. There are multiple advantages and benefits of Bioclip® WHS, such as enhanced wool qualities and animal welfare as well as reduced disease and shearing injuries. However, the major benefits are for animal welfare by eliminating the physical handling stress; skin injuries; damage to teats, udder, pizzle, ears, and other delicate external organs; and fly strike. Bioclip®-shorn sheep also make it easier for sheep producers to make animal selection and replacement choices, including culling for any undesirable traits, such as coarser fibers, wrinkled skin, or colored wool spots, body conformation, and faulty udders or teats. Lastly, the Bioclip® WHS procedure may turn the traditional, backbreaking sheep shearing job into a highly efficient, biological, wool-harvesting, on-farm profession.

IV. Conclusion

Our experimental results indicate that Bioclip® is effective to induce a simultaneous and complete fleece-shedding in Merino and Merino-derived wool-meat, dual-purpose sheep breeds in US rangeland environments. Therefore, it can be used as a biological, wool-harvesting alternative to the traditional, machine-shearing protocol for the wool sheep industry. Those US wool sheep owners who operate small-scale flocks, where conventional shearing or shearers are at a shortage, might find Bioclip® worth exploring as a biological, wool-harvesting option.

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This project was approved by the University of Nevada-Reno Institutional Animal Care and Use Committee (IACUC approval #000355) and conducted at the Nevada Agricultural Experiment Station, Reno, and Rafter 7 Ranch, Yerington, Nevada.

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