Sheep & Goat Research Journal
Volume 27, 2012 – April

# Effects of Supplemental Dried Distillers Grains or Soybean Hulls on Growth and Internal Parasite Status of Grazing Lambs[1]

*T. L. Felix[2], I. Susin[3], L. M. Shoup[2], A. E. Radunz[2,4], and S. C. Loerch[2,3]*

[1] *Salaries and research support provided by state and federal funds appropriated to the Ohio Agric. Res. and Dev. Center, The Ohio State University.*

[2] *Department of Animal Sciences, The Ohio State University, Wooster 44691*

[3] *Departamento de Zootecnia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo, Brazil*

[4] *Current address: University of Wisconsin, Department of Animal Science, 1675 Observatory Drive, Madison, WI 53706.*

[5] *Corresponding author: loerch.1@osu.edu*

## Summary

The objectives of this study were to determine the effects of supplementation of grazing lambs with dried distillers grains with solubles (DDGS) or soybean hulls (SBH) on growth rate and nematode-parasite status. Over the course of four experiments in consecutive years, 312 lambs were grazed on the same four or six paddocks. Grazing lambs were allotted to one of three supplementation treatments: 1) control, no supplementation (CONT), 2) DDGS, or 3) SBH (Exp. 3 and 4 only). Supplemental DDGS improved ($P < 0.01$) ADG when compared to CONT, and SBH supplemented lambs were intermediate. An analysis comparing CONT vs. DDGS supplementation across all four experiments revealed a reduction in anthelmintic-treatment rate required when DDGS were supplemented (81.2 percent vs. 30.1 percent for CONT and DDGS, respectively; $P < 0.01$). Measures of FAMACHA© score, packed-cell volume (PCV), and fecal-egg count (FEC) were recorded in weeks 3, 5, and 10. An analysis comparing just CONT and DDGS supplementation across all four experiments revealed that DDGS supplementation reduced ($P < 0.01$) FAMACHA score in weeks 3, 5, and 10, but only reduced FEC in week 3 compared to CONT lambs. Supplementation of grazing lambs with DDGS in this study allowed for increased growth, reduced anthelmintic-treatment rate, and reduced risk of becoming anemic as a result of internal parasites.

**Key Words:** Distillers Grains, Grazing, Lambs, Parasite, Soybean Hulls, Supplementation

## Introduction

Grazing weaned lambs on pasture results in slow growth rates and high susceptibility to *Haemonchuscontortus* parasitism (Murphy et al., 1994; McClure et al., 1995; Vanimisetti et al., 2004). Increased resistance of *Haemonchuscontortus* to anthelmintics is also problematic for pasture rearing of weaned lambs (Vanimisetti, et al., 2004). Supplementation of grazing lambs has resulted in increased growth rate (Freer et al., 1988) and may affect resistance or resilience to *Haemonchuscontortus* infection (Shaw et al., 1995; Coop and Kyriazakis, 2001). In their review of the effects of nutrition on nematode parasitism in ruminants, Coop and Kyriazakis (2001) concluded that prevalence and degree of infection have been reduced in grazing lambs fed supplements that provide increased energy, protein, or phosphorus. According to NRC (2000), dried distillers grains with solubles (DDGS) contain 1.50 Mcal of $NE_g$/kg, 29.5 percent protein, and 0.83 percent P. However, research suggests the actual energy value of DDGS is at least 15 percent greater than corn grain when fed at a restricted intake to beef cows (Radunz et al., 2010) or to feedlot cattle (Stock et al., 2000). With increased production of ethanol, DDGS has become competitively priced with other sources of protein and energy; however, little information is available on the effects of DDGS supplementation to grazing lambs on growth rate and nematode parasite status. Soybean hulls may also be a cost-effective strategy to provide fiber-based, supplemental energy to grazing lambs. The objectives of this study were to determine the effects of supplementation of grazing lambs with DDGS or soybean hulls on growth rate and nematode-parasite status.

## Materials and Methods

All animal procedures were approved by the Agricultural Animal Care and Use Committee of The Ohio State University and followed guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998). The experiments conducted were performed in four consecutive years (2007 to 2010).

### Experiment 1

The objectives were to determine effects of DDGS supplementation to grazing lambs on growth rate, efficiency of feed utilization, and nematode-parasite status.

#### *Animals, Sampling and Management*

One week prior to weaning, 62 Dorset × Hampshire cross-bred ewe lambs (24.5 kg ± 0.5 kg) and their dams were allotted to four outcome groups to equalize lamb BW and rearing status (singles or twins). Sheep in each outcome group were placed in separate paddocks with 15 or 16 ewe lambs per paddock and were randomly assigned to one of two experimental treatments: 1) grazed pasture with no supplementation (**CONT**), or 2) grazed pasture with DDGS supplementation. During the 7-d pre-weaning period, dams and their progeny were fed 0.41 kg of DDGS DM per dam as a method to expose lambs to DDGS supplementation prior to weaning. At weaning (average age = 56 d), the four groups of lambs were weighed at 0800, treated with anthelmintics (Prohibit, Agri Laboratories LTD, St. Joseph, Mo.), and randomly allotted to four orchardgrass pasture paddocks (0.65 ha each). Each paddock was equipped with a water tank and water was available ad libitum. A trace-mineral salt block fortified with selenium (Morton Salt, Chicago, Ill.) was also present in each paddock. Initially, DDGS was offered at 0.41 kg DM/lamb and the DDGS supplementation was increased by 81 g DM/lamb every other day until refusal occurred or until supplementation reached 2.5 percent of lamb BW. This amount of supplement would have provided approximately half of expected DM intake for lambs of this size (NRC, 1985). Weight and anemia status of lambs were determined weekly during the 69-d grazing experiment. Weights were measured at 0800 without withholding from feed or water. A single, trained individual determined lamb anemia status by using FAMACHA eye score and blood hematocrit (Kaplan et al., 2004). Fecal samples (5 g) were collected by rectal palpation to determine fecal nematode egg counts (**FEC**; Kaplan et al., 2004). At each weekly weighing, jugular blood samples (8 mL) to determine hematocrit and fecal samples to determine FEC were collected from lambs with a FAMACHA score of ≥ 3 (scale is 1 to 5, with 1 indicating no anemia and 5 indicating severe anemia). Lambs with a FAMACHA score of ≥ 3 were treated with anthelmintic (Cydectin, Fort Dodge Animal Health, Fort Dodge, Iowa). Treated lambs were not re-treated within 21 d unless their FAMACHA score remained the same or increased after anthelmintic treatment. Hematocrit and FEC were determined on all lambs during weeks 3, 7, and 10. On d 0 and 69, forage in each paddock was sampled for analysis of ADF and NDF (using Ankom Technology Method 5 and 6, respectively; Ankom$^{200}$ Fiber Analyzer, Ankom Technology, Fairport, N.Y.), and CP (macro Kjeldahl N x 6.25). Six samples (0.37 $m^2$ each) per paddock were clipped to a height of approximately 1 cm and composited. The DDGS was sampled weekly and composited for analysis of ADF and NDF (using Ankom Technology Method 5 and 6, respectively; Ankom$^{200}$ Fiber Analyzer, Ankom Technology, Fairport, N.Y.), CP (macro Kjeldahl N x 6.25), fat (using ether extract method; Ankom Technology, Fairport, N.Y.),and S (AOAC Method 975.03).

### Experiment 2

The objectives were to determine effects of DDGS supplementation to grazing lambs on growth rate, efficiency of feed utilization, and nematode-parasite status.

#### *Animals, Sampling, and Management*

One week prior to weaning, 62 Dorset × Hampshire cross-bred ewe lambs (26.3 kg ± 0.1 kg) and their dams were allotted to four outcome groups to equalize lamb BW and rearing status (singles or twins). Sheep in each outcome group were placed in separate paddocks with 15 or 16 ewe lambs per paddock and were randomly assigned to one of two experimental treatments: 1) grazed pasture with no supplementation (**CONT**), or 2) grazed pasture with DDGS supplementation. All management and sampling procedures during the 70-d grazing experiment were as described above for Exp. 1 except that anthelmintic treatment was based on anemia measurement, packed-cell volume (**PCV**), in Exp. 2 rather than FAMACHA score, as was done in Exp. 1. This procedural adjustment was made

in Exp. 2 because anthelmintic treatment based on FAMACHA score in Exp. 1 resulted in excessive anthelmintic use (treatment of lambs that did not have PCV below 20). In Exp. 2, if a lamb had a FAMACHA score of greater than 2, a blood sample was collected and blood hematocrit was determined (Kaplan et al., 2004). Lambs were treated with anthelmintic (Cydectin, Fort Dodge Animal Health, Fort Dodge, Iowa) if their blood hematocrit was 20 or less.

## Experiment 3

The objectives were to determine effects of soybean hull (SBH) or DDGS supplementation to grazing lambs on growth rate, efficiency of feed utilization, and nematode parasite status.

### *Animals, Sampling and Management*

One week prior to weaning, 96 Dorset × Hampshire cross-bred lambs (24.9 kg ± 0.4 kg) and their dams were allotted to six outcome groups to equalize lamb sex, BW, and rearing status (singles or twins). Sheep in each outcome group were placed in six separate paddocks (with 16 lambs per paddock) and were randomly assigned to one of three experimental treatments: 1) grazed pasture with no supplementation (**CONT**), 2) grazed pasture with pelleted SBH supplementation, or 3) grazed pasture with DDGS supplementation. During the 7-d pre-weaning period, dams and their progeny were fed 0.41 kg of SBH DM or 0.41 kg of DDGS DM per dam, as a method to expose lambs to SBH or DDGS supplementation prior to weaning and being fed their respective supplement source. At weaning, the six groups of lambs were randomly allotted to six orchardgrass pasture paddocks (0.65 ha each). Initially, SBH or DDGS was offered at 0.41 kg DM/lamb and supplementation was increased by 81 g DM/lamb every other day until refusal occurred or until supplementation reached 2.5 percent of lamb BW. All management, sampling, and anthelmintic-treatment procedures during the 72-d grazing experiment were as described above for Exp. 2.

## Experiment 4

The objectives were to determine effects of SBH fortified with P or DDGS supplementation to grazing lambs on growth rate, efficiency of feed utilization, and nematode-parasite status.

### *Animals, Sampling and Management*

One week prior to weaning, 92 Dorset × Hampshire cross-bred lambs (21.0 kg ± 0.5 kg) and their dams were allotted to six outcome groups to equalize lamb sex, BW, and rearing status (singles or twins). Sheep in each outcome group were placed in six separate paddocks (with 14 to 16 lambs per paddock) and were randomly assigned to one of three experimental treatments: 1) grazed pasture with no supplementation (**CONT**), 2) grazed pasture with pelleted soybean hull supplementation with added P to match the P in DDGS (**SBH+P**), or 3) grazed pasture with DDGS supplementation. The SBH+P supplement was pelleted and consisted of 95.3 percent SBH, 2.7 percent monosodium phosphate, and 2 percent animal-vegetable fat (DM basis). During the 7-d pre-weaning period, dams and their progeny were fed (on a DM basis) 0.41 kg of SBH+P or 0.41 kg of DDGS per dam as a method to expose lambs to SBH+P or DDGS supplementation prior to weaning and being fed their respective supplement source. At weaning, the six groups of lambs were randomly allotted to six orchardgrass pasture paddocks (0.65 ha each). Initially, SBH+P or DDGS was offered at 0.41 kg DM/lamb and supplementation was increased by 81 g DM/lamb every other day until refusal occurred or until supplementa-

**Table 1. Effect of dried distillers grains with solubles (DDGS) supplementation on pasture performance and parasite status of grazing lambs in Exp. 1.**

| Item | CONT[1] | DDGS[2] | SEM | *P-value* |
|---|---|---|---|---|
| No. replicates | 2 | 2 | - | - |
| No. animals | 32 | 30 | - | - |
| BW, kg | | | | |
| Initial | 24.9 | 24.0 | 0.5 | 0.33 |
| Final | 35.0 | 41.3 | 1.4 | 0.09 |
| Days | 69 | 69 | - | - |
| DDGS DMI, g/d | - | 476 | - | - |
| ADG, g/d | 147 | 252 | 23 | 0.08 |
| DDGS efficiency[3] | - | 0.219 | - | - |
| Treated, % [4] | 65.6 | 40.0 | 5.2 | 0.07 |
| No. treatments/lamb treated | 1.5 | 1.6 | 0.2 | 0.61 |
| ADG treated lambs, g/d | 150 | 259 | 32.0 | 0.13 |
| ADG non-treated lambs, g/d | 132 | 249 | 14 | 0.03 |
| FAMACHA© Score[5] | | | | |
| d 21 | 1.8 | 1.7 | 0.1 | 0.54 |
| d 49 | 1.9 | 1.7 | 0.1 | 0.42 |
| d 69 | 1.9 | 1.7 | 0.1 | 0.13 |
| Packed cell volume | | | | |
| d 21 | 36.7 | 36.5 | 0.5 | 0.68 |
| d 49 | 31.5 | 33.3 | 0.9 | 0.30 |
| d 69 | 30.3 | 32.5 | 0.8 | 0.17 |
| Fecal egg count | | | | |
| d 21 | 253 | 71 | 53 | 0.14 |
| d 49 | 1,147 | 1,532 | 352 | 0.52 |
| d 69 | 2,989 | 2,462 | 988 | 0.74 |

[1] CONT = no supplement.
[2] DDGS = supplemented with DDGS (27.4% NDF, 13.3% ADF, 27.2% CP, 12.0% EE, and 1.27% S).
[3] Gain above CONT lambs/g of supplemented feed.
[4] Treated with anthelmintic based on a FAMACHA score greater than 2.
[5] Scale of 1 = darkest to 5 = palest.

**Table 2. Effect of dried distillers grains with solubles (DDGS) supplementation on pasture performance and parasite status of grazing lambs in Exp.2.**

| Item | CONT[1] | DDGS[2] | SEM | *P-value* |
|---|---|---|---|---|
| No. replicates | 2 | 2 | - | - |
| No. animals | 30 | 32 | - | - |
| BW, kg | | | | |
| Initial | 26.3 | 26.3 | 0.1 | 1.00 |
| Final | 33.2 | 41.9 | 0.9 | 0.02 |
| Days | 70 | 70 | - | - |
| DDGS DMI, g/hd/d | - | 531 | - | - |
| ADG, g/d | 100 | 224 | 11 | 0.02 |
| DDGS efficiency[3] | - | 0.235 | - | - |
| Treated, %[4] | 90.2 | 18.7 | 4.8 | 0.01 |
| No. treatments/lamb treated | 1.3 | 1.0 | 0.1 | 0.03 |
| Avg day of first treatment | 27.5 | 35.5 | 4.9 | 0.37 |
| ADG treated lambs, g/d | 98 | 190 | 12 | 0.03 |
| ADG non-treated lambs, g/d | 118 | 227 | 3 | 0.01 |
| FAMACHA© Score[5] | | | | |
| d 21 | 3.5 | 1.9 | 0.3 | 0.07 |
| d 49 | 2.3 | 2.3 | 0.1 | 0.59 |
| d 70 | 1.8 | 1.3 | 0.1 | 0.04 |
| Packed cell volume | | | | |
| d 21 | 20.9 | 30.2 | 1.5 | 0.05 |
| d 49 | 32.6 | 30.5 | 1.0 | 0.27 |
| d 70 | 29.3 | 33.2 | 0.9 | 0.09 |
| Fecal egg count | | | | |
| d 21 | 1,171 | 43 | 225 | 0.07 |
| d 49 | 169 | 274 | 170 | 0.71 |
| d 70 | 3,275 | 342 | 980 | 0.17 |

[1] CONT = no supplement.
[2] DDGS = supplemented with DDGS (27.4% NDF, 13.3% ADF, 27.2% CP, 12.0% EE, and 1.27% S).
[3] Gain above CONT lambs/g of supplemented feed.
[4] Treatment with anthelmintic based on a packed cell volume less than 20.
[5] Scale of 1 = darkest to 5 = palest.

tion reached 2.5 percent of lamb BW. All management, sampling, and anthelmintic-treatment procedures during the 68-d grazing experiment were as described above for Exp. 2.

### Statistical Analysis

Each of these four studies was analyzed as a completely randomized design. Statistical data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, N.C.). The model used for ADG, GF, DMI, weights on and off test, and supplementation treatment was:

$Y_{ijk} = \mu + p_i + S_j + e_{ij}$

Where $Y_{ijk}$ = response variable; = mean; $p_i$ = the random effect of paddock; $S_j$ = the fixed effect of supplementation treatment; and $e_{ij}$ = the experimental error. Paddock was the experimental unit and n = 2 for all four trials. Significance was declared at $P < 0.05$ and trends were discussed at $P < 0.10$. In Exp. 3 and 4, means were separated by PDIFF, FAMACHA, PCV, and FEC were analyzed as repeated measures and the model was:

$Y_{ijk} = \mu + p_i + S_j + W_k + e_{ijk}$

Where $Y_{ijk}$ = response variable; = mean; $p_i$ = the random effect of paddock; $S_j$ = the fixed effect of supplementation treatment; $W_k$= the fixed effect of repeated week of sampling; and $e_{ijk}$ = the experimental error. Paddock was the experimental unit. Significance was declared at $P < 0.05$ and trends were discussed at $P < 0.10$.

An analysis was conducted with the CONT and DDGS data across all four experiments. For this analysis, experiment number was included as a block effect in the models above.

## Results

### Experiment 1

Weekly pasture samples collected during the experiment (mid-July to mid-September) averaged 62.0 percent NDF, 38.2 percent ADF, and 15.6 percent CP. Lambs fed supplemental DDGS consumed 476 g of DDGS DM/d (Table 1). Supplementation tended ($P < 0.09$) to increase ADG and final BW at the end of the 70-d grazing study. Efficiency of DDGS to promote ADG was 0.219 g of gain above the non-supplemented lambs per g of DDGS supplemented. When the decision to treat lambs to control parasite infection was based on a FAMACHA score of greater than 2, there was a trend ($P = 0.07$) for DDGS-supplemented lambs to have a fewer anthelmintic treatments than CONT lambs. Supplemental DDGS did not affect ($P = 0.13$) ADG of lambs treated with anthelmintic compared to CONT lambs. However, for those lambs never treated (FAMACHA score was never greater than 2), DDGS supplementation nearly doubled ADG when compared to CONT lambs ($P = 0.03$). When all lambs were measured on d 21, d 49 and d 69, supplementation with DDGS did not affect ($P \geq 0.13$) FAMACHA scores, PCV, or FEC.

### Experiment 2

Weekly pasture samples collected during the experiment (mid-July to mid-September) averaged 64.7 percent NDF, 41.0 percent ADF, and 13.5 percent CP. Average intake of supplemental DDGS was 531 g of DM/d during the 70-d grazing experiment (Table 2). Supplementation more than doubled ADG and increased ($P = 0.02$) final BW at the end of the 70-d grazing study. Efficiency of DDGS to promote ADG was 0.235 g of gain above the CONT lambs per g of DDGS supplemented. When the decision to treat lambs for nematode parasitism was based on FAMACHA score followed by a confirmed anemia (PCV ≤ 20), supplementation with DDGS greatly reduced ($P = 0.01$) the proportion of lambs requiring treatment with anthelmintic (90.2 percent vs. 18.7 per-

**Table 3. Effect of dried distillers grains with solubles (DDGS) or soybean hull (SBH) supplementation on pasture performance and parasite status of grazing lambs in Exp. 3.**

| Item | CONT[1] | SBH[2] | DDGS[3] | SEM | *P-value* |
|---|---|---|---|---|---|
| No. replicates | 2 | 2 | 2 | - | - |
| No. animals | 32 | 32 | 32 | - | - |
| BW, kg | | | | | |
| Initial | 24.85 | 25.26 | 24.58 | 0.36 | 0.45 |
| Final | 31.79[b] | 38.82[a] | 40.73[a] | 0.73 | 0.01 |
| Days | 72 | 72 | 72 | - | - |
| Supplement DMI, g/hd/d | - | 611 | 631 | 10 | 0.31 |
| ADG, g/d | 95[a] | 188[b] | 224[c] | 7 | 0.01 |
| Supplement efficiency[4] | - | 0.152 | 0.205 | 0.015 | 0.13 |
| Treated, %[5] | 81.3[a] | 31.2[b] | 9.4[b] | 7.4 | 0.01 |
| No. treatments/lamb treated | 1.27[a] | 1.00[b] | 1.00[b] | 0.02 | 0.01 |
| Avg day of first treatment | 42.0 | 42.5 | 55.5 | 5.0 | 0.25 |
| ADG treated lambs, g/d | 95[b] | 177[a] | 200[a] | 15 | 0.03 |
| ADG non-treated lambs, g/d | 86[c] | 195[b] | 227[a] | 1 | 0.01 |
| FAMACHA© Score[6] | | | | | |
| d 22 | 2.1[a] | 1.7[b] | 1.5[c] | 0.04 | 0.01 |
| d 43 | 3.3[a] | 2.1[b] | 1.7[b] | 0.2 | 0.02 |
| d 72 | 2.7[a] | 1.9[b] | 1.6[b] | 0.1 | 0.01 |
| Packed cell volume | | | | | |
| d 22 | 31.5 | 30.5 | 33.2 | 1.4 | 0.49 |
| d 43 | 22.7[b] | 26.9[a] | 29.5[a] | 0.7 | 0.02 |
| d 72 | 28.1 | 29.5 | 31.4 | 1.2 | 0.29 |
| Fecal egg count | | | | | |
| d 22 | 1,616 | 1,675 | 765 | 648 | 0.60 |
| d 43 | 2,743 | 5,173 | 3,185 | 1,221.000 | 0.43 |
| d 72 | 1,070 | 4,232 | 1,567 | 589 | 0.06 |

[1] CONT = no supplement.
[2] SBH = supplemented with SBH (66.4% NDF, 50.6% ADF, 11.2% CP).
[3] DDGS = supplemented with DDGS (24.8% NDF, 11.5% ADF, 26.8% CP, 11.8% EE, and 0.77% S).
[4] Gain above CONT lambs/g of supplemented feed.
[5] Treatment with anthelmintic based on a packed cell volume less than 20.
[6] Scale of 1 = darkest to 5 = palest.
[ab] Means within a row with different superscripts differ *(P<0.05)*.

cent for CONT vs. DDGS-supplemented lambs, respectively). Supplementation with DDGS reduced ($P = 0.03$) the number of anthelmintic treatments per lamb treated and increased ($P \leq 0.03$) the ADG of both treated lambs and those never treated with anthelmintic during the trial. When all lambs were assessed on d 21, supplementation with DDGS tended to reduce FAMACHA score ($P = 0.07$), increased PCV ($P = 0.05$), and tended to reduce FEC ($P = 0.07$) compared to CONT lambs. Supplementation with DDGS did not affect ($P \geq 0.27$) these variables when lambs were sampled on d 49, but did reduce ($P = 0.04$) FAMACHA score and tended to reduce ($P = 0.09$) PCV on d 70.

## Experiment 3

Weekly pasture samples collected during the experiment (mid-July to mid-September) averaged 64.1 percent NDF, 38.9 percent ADF, and 14.9 percent CP. Supplement intake averaged 611 g/d for lambs supplemented with SBH and 631 g/d for lambs supplemented with DDGS (Table 3). Lambs supplemented with DDGS had greater ($P< 0.01$) ADG than CONT lambs while those supplemented with SBH were intermediate. Final BW followed the same trend. Supplementation with SBH or DDGS reduced ($P = 0.01$) the percentage of lambs requiring treatment for internal parasites (31.2 percent for SBH and 9.4 percent for DDGS vs. 81.3 percent for CONT lambs). A similar response was observed for the number of treatments per lamb treated ($P = 0.01$) and the effect of supplementation on the ADG of those lambs that were treated ($P = 0.03$) and those that were not treated ($P = 0.01$). When all lambs were assessed on d 22, DDGS supplementation reduced ($P = 0.01$) average FAMACHA score compared to CONT lambs, while SBH-supplemented lambs were intermediate. Average PCV and FEC on d 22 were not affected ($P \geq 0.49$) by supplementation. On d 43, average FAMACHA score was decreased and PCV were greater ($P = 0.02$) for lambs supplemented with SBH or DDGS compared with CONT lambs.

**Table 4. Effect of dried distillers grains with solubles (DDGS) or P fortified soybean hull (SBH+P) supplementation on pasture performance and parasite status of grazing lambs in Exp.4.**

| Item | CONT[1] | SBH+P[2] | DDGS[3] | SEM | *P-value* |
|---|---|---|---|---|---|
| No. replicates | 2 | 2 | 2 | - | - |
| No. animals | 30 | 32 | 28 | - | - |
| BW, kg | | | | | |
| Initial | 20.8 | 21.0 | 21.3 | 0.5 | 0.73 |
| Final | 27.9[b] | 35.1[a] | 36.7[a] | 0.6 | 0.01 |
| Days | 68 | 68 | 68 | - | - |
| Supplement DMI, g/hd/d | - | 589a | 544b | 10 | 0.05 |
| ADG, g/d | 104[b] | 206[a] | 227[a] | 16 | 0.03 |
| Supplement efficiency[4] | - | 0.173 | 0.225 | 0.032 | 0.37 |
| Treated, %[5] | 87.5 | 71.9 | 52.1 | 13.0 | 0.30 |
| No. treatments/lamb treated | 1.2[a] | 1.1[ab] | 1.0[b] | 0.04 | 0.05 |
| Avg day of first treatment | 33.5 | 33.0 | 34.5 | 1.5 | 0.78 |
| ADG treated lambs, g/d | 104[b] | 197[a] | 236[a] | 10 | 0.01 |
| ADG not treated lambs, g/d | 122 | 229 | 218 | 31 | 0.19 |
| FAMACHA© Score[6] | | | | | |
| d 21 | 2.7 | 2.1 | 2.1 | 0.3 | 0.28 |
| d 42 | 2.7 | 1.7 | 2.2 | 0.4 | 0.26 |
| d 68 | 2.9[a] | 1.5[b] | 1.7[b] | 0.1 | 0.01 |
| Packed cell volume | | | | | |
| d 21 | 27.4 | 29.1 | 29.5 | 1.5 | 0.64 |
| d 42 | 23.3 | 25.5 | 26.7 | 1.1 | 0.22 |
| d 68 | 26.4 | 29.5 | 29.5 | 0.9 | 0.17 |
| Fecal egg count | | | | | |
| d 21 | 1,059 | 2,869 | 652 | 551 | 0.12 |
| d 42 | 3,007 | 4,684 | 3,321 | 718 | 0.35 |
| d 68 | 3,257 | 3,091 | 2,928 | 599 | 0.93 |

[1] CONT = no supplement.
[2] SBH+P = supplemented with P fortified SBH (59.7% NDF, 43.7% ADF, 10.5% CP, 0.91% P).
[3] DDGS = supplemented with DDGS (27.6% NDF, 12.6% ADF, 25.1% CP, 0.73% S, 0.83% P).
[4] Gain above CONT lambs/g of supplemented feed.
[5] Treatment with anthelmintic based on a packed cell volume less than 20.
[6] Scale of 1 = darkest to 5 = palest.
[ab] Means within a row with different superscripts differ *(P<0.05)*.

Likewise, on d 72, supplementation reduced FAMACHA scores ($P = 0.02$) and tended to reduce FEC ($P = 0.06$) for SBH- and DDGS-supplemented lambs compared with the CONT lambs.

### Experiment 4

Weekly pasture samples collected during the experiment (mid-July to mid-September) averaged 62.0 percent NDF, 39.2 percent ADF, and 15.6 percent CP. Lambs consumed 589 g of SBH+P/d and 544 g of DDGS DM /d on average (Table 4). Lambs fed supplemental SBH+P or DDGS had more than double ($P = 0.03$) the ADG of CONT lambs and this response was reflected in increased final BW. Supplemental-feed efficiency was 0.173 for SBH+P and 0.225 for DDGS lambs. Regardless of source, supplementation did not reduce ($P = 0.30$) the percentage of lambs requiring anthelmintic treatment. However, supplementation with DDGS did reduce ($P = 0.05$) the number of anthelmintic treatments per lamb treated compared with the CONT lambs, while the SBH+P lambs were intermediate. For the treated lambs, supplementation increased ($P < 0.01$) ADG compared with CONT lambs. Supplementation did not affect ($P \geq 0.12$) FEC, PCV, or FAMACHA scores, except on d 68 where FAMACHA scores were greater ($P = 0.01$) for CONT lambs than for those supplemented with DDGS or SBH+P.

## Discussion

Lambs grazing orchardgrass pastures had increased ADG and final BW when supplemented with DDGS. Many reports confirm the positive effects on ADG of grain supplementation of grazing lambs (Freer, et al., 1988; Daura and Reid, 1991; Karnezos, 1994). However, we found no reports on efficacy of DDGS supplementation for grazing lambs. In the current study, CONT lambs gained an average of 112g/d while lambs supplemented with DDGS gained an average of 232g/d. Supplementation with SBH or SBH+P also resulted in greater ADG compared to CONT lambs. Efficient use of a protein-energy supplement to increase growth rate is an important eco-

nomic consideration. The efficacy of supplemental DDGS to increase growth above CONT lambs was consistent across experiments and averaged 0.221g of gain above CONT for each g of supplement consumed. In other words, lambs required 4.5g of DDGS for each g of BW gain above the CONT lambs. Efficiency of SBH to increase gains above CONT lambs averaged 0.152 in Exp. 3 and 0.173 in Exp. 4. Trends for decreased ADG and efficiency for SBH vs. DDGS could be due to the difference in energy and/or protein content of these two byproducts. The NRC (2000) indicates DDGS has 1.50 Mcal of $NE_g$/kg, while SBH contain 1.30 Mcal of $NE_g$/kg. The DDGS used in these experiments was 26 percent CP while the SBH contained only 11 percent CP.

For Exp.1, the decision to treat lambs for internal parasites was based on the weekly assessment of FAMACHA score. Based on comparison of the PCV data with individual FAMACHA scores, this procedure resulted in anthelmintic treatment of lambs before they were considered anemic (The Merck Veterinary Manual, 2005). In Exp. 2, 3, and 4, lambs did not receive anthelmintic therapy unless a PCV of ≤ 20 confirmed the anemic status of the lambs. Despite these different criteria for therapy decisions, DDGS supplementation decreased required anthelmintic treatment in three of the four experiments when compared to the CONT lambs. An analysis comparing CONT vs. DDGS supplementation across all four experiments revealed a reduction in anthelmintic treatment rate when DDGS were supplemented (81.2 percent for CONT vs. 30.1 percent for DDGS, $P< 0.01$). The mechanism of action for this response could not be determined in this study. However, DDGS supplementation more than doubled ADG of grazing lambs (232 g of gain/d for DDGS vs. 112 g of gain/d for CONT, $P < 0.01$) and lambs in a more positive-energy balance may have been more resistant or resilient when faced with a parasite challenge. Protein supplementation, and a general increase in energy balance, has been shown to improve ability of lambs to withstand a nematode infection (Coop and Kyriazakis, 1999 and 2001). Supplemental SBH were investigated as a strategy to increase fiber-based energy intake without providing a large amount of supplemental protein. Lambs fed SBH had growth rate and anthelmintic treatment rates that were intermediate between CONT lambs and those supplemented with DDGS. As with DDGS, resilience or resistance to internal-parasite infection was likely related to the increased growth rate of the lambs supplemented with SBH.

Additionally, supplemental P has been shown to reduce parasite infection in grazing lambs (Coop and Holmes, 1996). The NRC (2000) indicates that DDGS contains 0.83 percent P whereas SBH contains only 0.18 percent P. In Exp.4, we investigated SBH+P to determine if added P intake that occurs with DDGS supplementation contributed to the reduced anthelmintic treatment rate observed when lambs were supplemented with DDGS. In Exp. 4, SBH+P resulted in increased ADG but did not affect anthelmintic treatment rate compared to the CONT. Lambs supplemented with SBH+P had numerically decreased ADG and numerically greater anthelmintic-treatment rate compared to DDGS-supplemented lambs but these differences were not significant.

All lambs in these four experiments were assessed for FAMACHA score, FEC, and PCV at the end of weeks 3, 5, and 10 of the grazing period. Data collected from lambs later in the grazing period (especially weeks 5 and 10) would be affected by timing and treatment with anthelmintic. Because, in general, more CONT lambs were treated, the data assessing parasite-infection status at the end of weeks 3, 5, and 10 would be confounded by the response of these lambs to the anthelmintic. Because few lambs were treated before d 21, the confounding effect of anthelmintic treatment would be less at this time point. The average FAMACHA score, FEC, and PCV data provide an indication of the severity of the infection in lambs identified as requiring anthelmintic treatment. Lambs supplemented with DDGS had a decreased anthelmintic treatment rate and fewer treatments per lamb treated (except for Exp. 1). An analysis comparing just CONT and DDGS supplementation across all four experiments revealed that DDGS supplementation reduced ($P < 0.01$) FAMACHA score in weeks 3, 5, and 10, but only reduced FEC in week 3.

## Conclusions

Supplementing grazing lambs with DDGS at 2.5 percent of their BW increased growth, reduced anthelmintic treatment rate, and reduced risk of becoming anemic as a result of internal parasites. Responses to supplemental SBH were intermediate between no supplementation and supplementation with DDGS. These byproducts provide the sheep industry with an economical strategy to increase performance and reduce anthelmintic treatment rate of grazing lambs.

## Literature Cited


AOAC. 1988. Official method 975.03: Metals in plants and petfoods. Atomic absorption spectrophotometric method. In Official Methods of Analysis.13th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Coop, R. L., and P. H. Holmes. 1996. Nutrition and parasite interactions. Int. J. Parasit. 26:951-962.

Coop, R. L., and I. Kyriazakis.2001. Influence of host nutrition on the development and consequences of parasitism in livestock. Trends in Parasit. 17:325-330.

Coop, R. L., and I. Kyriazakis. 1999.Nutrition–parasite interaction. Vet. Parasit. 84:187-204.

Daura, M. T., and R. L. Reid. 1991. Energy and protected protein supplements to lambs on endophyte-infected tall fescue pasture. J. Anim. Sci. 69:358-368.

FASS. 1998. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Champaign, IL.

Freer, M., H. Dove, A. Axelsen, and J. R. Donnelly. 1988. Responses to supplements by weaned lambs when grazing mature pasture or eating hay cut from the same pasture. J. Ag. Sci. 110: 661-667.

Kahn, C.M. (ed.). 2005. The Merck Veterinary Manual, 9th ed., pp. 8-18. Merck Publishing Group, Rahway, NJ.

Kaplan, R., J. Burke, T. Terrill, J. Miller, W. Getz, S. Mobini, E. Valencia, M. J. Williams, L. H. Williamson, M. Larsen, and A. F. Vatta. 2004. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasit.123:105-120.

Karnezos, T. P., A. G. Matches, R. L. Preston, and C. P. Brown. 1994. Corn supplementation of lambs grazing alfalfa. J. Anim. Sci. 72:783-789.

McClure, K. E., M. B. Solomon, N. A. Parrett, and R. W. Van Keuren. 1995. Growth and tissue accretion of lambs fed concentrate in drylot, grazed on alfalfa or ryegrass at weaning, or after backgrounding on ryegrass. J. Anim. Sci. 73:3437-3444.

Murphy, T. A., S. C. Loerch, K. E. McClure, and M. B. Solomon. 1994. Effects of grain or pasture finishing systems on carcass composition and tissue accretion rates of lambs. J. Anim. Sci. 72:3138-3144.

NRC. 1985. Nutrient Requirements of Sheep. 6th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed., 1996. Natl. Acad. Press, Washington, DC.

Radunz, A. E., F. L. Fluharty, M. L. Day, H. N. Zerby, and S. C. Loerch. 2010. Prepartum dietary energy source fed to beef cows: I. Effects on pre- and postpartum cow performance. J. Anim. Sci. 88:2717-2728.

Shaw, K. L., J. V. Nolan, J. J. Lynch, O. R. Coverdale, and H. S. Gill. 1995. Effects of weaning, supplementation and gender on acquired immunity to *Haemonchuscontortus* in lambs. Intern. J. Paras. 25:381-387.

Stock, R. A., J. M. Lewis, T. J. Klopfenstein, and C. T. Milton. 2000. Review of new information on the use of wet and dry milling feed by-products in feedlot diets. J. Anim. Sci. 77:1-12.

Vanimisetti, H. B., S. P. Greiner, A. M. Zajac, and D.R. Notter. 2004. Performance of hair sheep composite breeds: resistance of lambs to *Haemonchuscontortus*. J. Anim. Sci. 82:595-604.

Sheep & Goat Research Journal
Volume 27, 2012 – October

# Performance of Meat Goats Control-Grazed on Winter Annual Grasses

*J-M. Luginbuhl[1,2,3] and J.P. Mueller[1]*

*North Carolina State University, Departments of Crop Science[1], Animal Science[2], Raleigh NC 27695*

*[3] Corresponding author: jean-marie_luginbuhl@ncsu.edu*

## Acknowledgements

The North Carolina Agricultural Research Service and the North Carolina Cooperative Extension Service supported this research. Use of trade names in this publication are simply for the convenience of the reader and does not imply endorsement by North Carolina State University of the products named or criticism of similar ones not mentioned.

The authors wish to thank Amy Conrad for animal care and laboratory analyses, Tim Hall and Tom Millane for their assistance with the field work, Abdul Chaudhry of Halal Meats for the use of his processing facility, and E. B. Shankle, North Carolina Department of Agriculture and Consumer Services, for his assistance with the determination of carcass grades.

## Summary

The performance of yearling replacement does and castrated male goats (*Capra hircus hircus*) controlled-grazed on cereal rye (CR; *Secale cereale* L.), annual ryegrass (RG; *Lolium multiflorum* L.) and triticale (TT; *Triticosecale rimpaui*) was evaluated during a 3-year study. Each year, 54 Boer and Boer-cross goats (avg initial age and BW: 8 mo to 10 mo and 30.4 kg, respectively) were assigned to nine plots (0.19 ha each) each containing six "tester" goats. Additional goats (put and take) were used to control forage growth. Forage species had no effect on ADG; however, castrated males gained more weight than does in year 2 (139 g/d vs. 94 g/d; $P < 0.003$) and during period 2 in year 3 (224 g/d vs. 146 g/d; $P < 0.0004$). Gain per ha was greater for RG than CR and TT (year 1: 514, 311, 293 kg, $P < 0.001$; year 2: 237, 144, 184 kg, $P < 0.004$; year 3: 528, 268, 149 kg, $P < 0.004$). In year 3, pH of ruminal fluid, ruminal ammonia and chilled-carcass yield from castrated males grazing RG, CR and TT was similar (avg: 6.67, 25.7 mg/dL and 51.3 percent, respectively), whereas plasma-urea nitrogen (16.4, 21.9, 24.1 mg/dL; $P < 0.024$), ruminal acetate (62.0, 60.7, 57.7 mM/100mM; $P < 0.017$), propionate (22.0, 25.2, 27.0 mM/100mM; $P < 0.006$) and acetate:propionate (2.83, 2.43, 2.22; $P < 0.017$) differed among forage species. Results indicated that yearling goats achieved satisfactory BW gains when fed only on these forages under controlled, rotational-grazing management, but that RG resulted in significantly greater BW gains per hectare.

**Key Words:** Annual Ryegrass, Cereal Rye, Meat Goat, Performance, Triticale

## Introduction

In the Southeastern United States, meat goats (*Capra hircus hircus*) are becoming increasingly important contributors to the income of many small producers. Meat goats frequently obtain more than 50 percent of their daily ration from browse (Norton, 1984; Ball et al., 2007), but will perform well grazing cultivated pastures if grazing management practices are not in conflict with their grazing behavior. This "generalist" feeding behavior represents a clear advantage in the ability to utilize a variety of landscapes and plant communities. Furthermore, if managed to match goat-nutritional demands, these plant communities, represented by pasture and browse species, can provide an abundant, lost-cost, feed supply supplanting the need for expensive concentrates (Neuman et al., 1995). Nevertheless, few research data are available from the region specifically directed toward intensive grazing of cultivated pastures by goats reared for meat production. Muir (2006a) compared a range of stocking rates with crossbred-Boer doe kids grazed on cultivated, grass-legume winter pastures or examined the performance of crossbred-Boer doe kids grazing wheat (*Triticum aestivum* L.) pastures fertilized at different nitrogen (N) levels (Muir, 2006b). Lema et al. (2007) used continuous grazing and set stocking to evaluate the performance of weaned, crossbred-doe kids on tall fescue (*Schedonorus phoenix* [Scop.] Holub) cv. Kentucky 31 and two species of cereal grains, and Hart et al. (1993) examined the efficiency of high- and low-quality forage by three goat breeds. Our 3-year study was designed to evaluate the performance of replacement does and castrated males allowed to control-graze on cereal rye (*Secale cereale* L.) cv. Elbon (**CR**), annual ryegrass (*Lolium multiflorum* L.) cv. Marshall (**RG**) and triticale (*Triticosecale rimpaui*) cv. Resource Seeds 102 (**TT**).

## Materials and Methods

### Forage Establishment and Management

The 3-year study was conducted at North Carolina State University Field Research Station in Raleigh, N.C., located at approximately 35.8°N latitude and 78.7°W longitude. The climate is temperate, averaging 1,233 mm annual precipitation with annual maximum and minimum temperatures of 20.8°C and 10.1°C, respectively, during the periods of the study (NCSU, 2008-2011). Soils of the study area were Cecil Series (Clayey, Kaolinitic, Thermic, Typic Hapludults) on slopes ranging from 6 percent to 10 percent (USDA, 1970). The experimental area consisted of 1.7 ha divided into nine paddocks of 0.19 ha each. The experimental site, an old stand of tall fescue, was sprayed with glyphosate (Roundup, Monsanto Co., St. Louis, Mo.) approximately two weeks before each planting, and the remaining plant residue clipped a few days later. Forage species were no-till drilled (Marliss Soybean & Grain Drills, Jonesboro, Ark. [year 1 & 2]; Truax Co., Inc., New Hope, Minn. [year 3]) on 2 October (year 1), 26 September (year 2), and 2 October (year 3). In year 2, RG paddocks were replanted on 21 October. Seeding rates corrected for germination averaged 124 kg/ha, 35 kg/ha and 121 kg/ha for CR, RG and TT, respectively. Soil tests indicated the pH, P, and K were in the optimum range for plant growth; however, all forages were fertilized each year in November and February with ammonium nitrate at a rate of 56 kg N/ha.

### Animals and Grazing Management

Each year 54, eight- to ten-month-old, growing, purebred Boer and Boer X Landrace (brush or wood) goats (year 1: four purebred Boer and two halfbred Boer/plot, initial BW 28.2 kg ± 0.6 kg; year 2: six halfbred Boer/plot, initial BW 31.0 kg ± 0.4 kg; year 3: one purebred, four three-quarter and one halfbred Boer/plot, initial BW 32.0 ± 0.4 kg) were stratified by BW, placed into six groups of nine animals with similar BW, assigned randomly to one of nine plots (three field replicates per forage species), and managed using controlled rotational grazing with electronetting (Premier1 Supplies, Washington, Iowa). Each year, the study was initiated based on multiple criteria, including mean-forage-canopy height (8 cm to 15 cm) and estimated-herbage mass (500 kg/ha to 1,000 kg/ha) based on calibrated-disk-meter values (Vartha and Matches, 1977). In year 1, all paddocks were stocked with six goats from start to finish, whereas in year 2 paddocks first were stocked with only three goats (Table 4). In year 3, variable stocking was used in December to January (Period 1) due to environmental conditions unfavorable to forage growth. Grazing resumed with six goats per paddock from the end of February until the termination of the study (Period 2). In the spring, these annual forages shift from vegetative to reproductive growth, in which forage quality declines rapidly and herbage production becomes sensitive to periods of dry weather. Therefore, each year, the study was terminated when forage quality was judged insufficient to support animal performance goals, based on a visual estimate of leaf-to-stem ratio (< 0.5), and quantity (canopy height < 5 cm and < 500 kg/ha), based on calibrated-disk-meter values.

In year 1 all six tester goats were females, whereas in years 2 and 3 two of the six tester goats per plot were castrated males. Goats were born and raised at the Small Ruminant Educational Unit of North Carolina State University in compliance with the North Carolina State University Institutional Animal Care and Use Committee regulations. Each goat was treated for elimination of gastrointestinal parasites (Ivermectin, 0.4 mg/kg BW; Merial, Division of Merck and Co., Rahway, N.J.) at the start of the trial. Goats were moved to a fresh strip of grass three to four times weekly depending on forage availability, and were excluded from the previously grazed strips to promote forage regrowth. Variable-stocking management was used with additional goats (2 to 14 goats/plot) available as put-and-take animals to control forage availability. Goats had free-choice access to water and movable shelters (Polydome, Litchfield, Minn.). Water was provided using underground, polyvinyl chloride (PVC) lines branching off at regular intervals with risers and valves (Kenkove Farm Fence, Blairsville, Penn.). Portable, UV-resistant, water troughs equipped with automatic float valves were tied to the underground water system using garden hoses fitted with quick couplings. This system allowed water troughs to be moved along with the animals with a minimum of effort and provided maximum, controlled-grazing flexibility. Goats were fed

approximately 20 g/goat loose minerals (SSC557-55001; Southern State Cooperative, Inc., Richmond, Va.) three times weekly and were weighed on-site every two to three weeks using an electronic scale (Tru-Test Inc., San Antonio, Texas).

### Forage Measurements and Sampling

During the grazing season, forage measurements were taken at two-week intervals to characterize forage availability (Chamblee and Green, 1995). Height and mass of the forage were quantified during a random walk through each paddock using the average of 15 canopy heights and 15 compressed, bulk heights measurements of the forage (Vartha and Matches, 1977) using a falling-plate disk meter (area = 0.22 $m^2$, weight = 1.1 kg). The compressed, bulk-height readings were used to predict herbage mass based on a regression equation developed from paired sampling of quadrats from three short, three medium, and three tall forage samples. A compressed-height reading was taken from each quadrat, immediately followed by cutting the forage within the nine 0.25 $m^2$-quadrats. The forage was cut within 1 cm of ground level. Samples were then dried in a forced-air oven (BlueM - Lunaire Ltd., Williamsport, Penn.) at 55°C for 48 to 72 h, and weighed to determine herbage mass. Additional forage samples were hand-plucked as an estimate of the forage selected by the goats. Plucked samples were dried at 55°C as previously described, ground in a forage grinder (Wiley Mill, Thomas Scientific, Swedesboro, N.J.) to pass through a 1-mm screen, and stored in sealed, plastic bags until they were analyzed to determine chemical composition and *in vitro* true DM digestibility (IVTDMD).

### Blood and Ruminal Fluid Collection

At the completion of the study in year 3, blood samples were collected by jugular venipuncture from the castrated males using 20-gauge, 2.54-cm needles and 10-mL vacutainer tubes containing $K_2$ EDTA solution as an anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, N.J.). Blood samples were placed on ice for transport to the laboratory, centrifuged at 10,000 rpm for 10 minutes, and the plasma was frozen until analyzed. Ruminal fluid samples were taken from the same animals by stomach tube and ruminal pH was determined immediately using a Cardy Twin pH meter (Spectrum Technologies, Inc., Plainfield, Ill.). Ruminal fluid samples were then placed in a crushed ice and water solution to stop fermentation and then frozen until they were thawed in preparation for analysis. The castrated males then were harvested at a USDA-inspected, commercial facility.

### Chemical Analyses

Hand-plucked forage samples were analyzed for DM and Kjeldahl N according to AOAC (1999). Kjeldahl N was multiplied by 6.25 to estimate crude protein (CP). Neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and 72-percent, sulfuric-acid lignin (ADL) were determined sequentially according to Van Soest et al. (1991), as modified by Komarek et al. (1994), but without the addition of amylase and urea for starch removal. Cellulose was calculated as the difference between ADF and ADL plus ash, and hemicellulose as the difference between NDF and ADF concentrations. Acid-detergent lignin (ADL) was corrected for mineral matter by ashing the ADL residue in a muffle furnace at 400°C. In vitro true DM digestibility was determined using a 48-h incubation period in a batch fermenter (Ankom Technology, Fairport, N.Y.) with steer ruminal inoculum and buffer (Tilley and Terry, 1963) and NDF termination.

Blood plasma samples were thawed and plasma urea N (PUN) was determined colorimetrically by an automated diacetyl-monoxime method (Marsh et al., 1965). Ruminal-fluid samples were thawed and centrifuged at 3,600 rpm for 10 min. Eight milliliters of supernatant were mixed with 2 mL of 25-percent metaphosphoric acid. The mixture was covered, held at room temperature for 30 min, centrifuged again at 3,600 rpm for 10 min, and analyzed for ammonia and VFA. Ruminal VFA concentrations were determined on a Varian 3,800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, Calif.) using a Nikol-fused, silica-capillary column (15 m; 0.53 mm i.d.; 0.5 μm film thickness; Supelco, Bellefonte, Penn.). Ruminal ammonia (RUA) was determined by the colorimetric procedure used for Kjeldahl N (AOAC, 1999).

### Statistical Analyses

Data were subjected to ANOVA for a randomized complete block design with three field replicates (Steel et al., 1997) using the GLM procedure of SAS (2003) for the model Y = μ + replicate + forage species + replicate x forage species + residual. The interaction replicate x forage species was used as error term to test for forage species effects. In years 2 and 3, ADG data were analyzed for sex effects using the model Y = μ + replicate + forage species + sex + forage species x sex + residual. The residual was used as an error term to test for sex and forage species x sex effects. Forage means were examined using pre-planned, orthogonal contrasts (Steel et al., 1997). Average daily gain for each goat was estimated by linear regression of BW as a function of days on study using the REG procedure of SAS (2003).

## Results and Discussion

### Planting

Adverse weather conditions delayed planting 11 d to 17 d beyond the optimum planting dates in all three years (Table 1). In year 1, wet conditions in late summer delayed the building of fences and water lines followed on 6

**Table 1. Planting season monthly cumulative precipitation during the 3-year study and 20-year average (mm), Raleigh, N.C., USA[a].**

| Month | Year 1 | Year 2 | Year 3 | 20-year avg |
|---|---|---|---|---|
| August | 80 | 35 | 83 | 109 |
| September | 330 | 71 | 654 | 106 |
| October | 102 | 73 | 99 | 104 |

[a] NCSU (2008-2011).

September by a hurricane with wind gusts of 129 km/h and more than 211 mm of rain in 6 h to 7 h. Excessively wet conditions in year 3 again delayed planting as 220 mm and 197 mm of rain fell on the experimental site due to a tropical storm on 5 and 6 September and a hurricane on 15 and 16 September, respectively. In year 2, planting was delayed because of excessively dry conditions. In Piedmont of North Carolina, the best dates to plant winter annual grasses are between 25 August and 15 September, with possible planting dates extending from 20 August to 31 October (Green et al., 1995).

## Herbage Quality

Concentrations of NDF, ADF, CELL and ADL in year 1, CP in year 2 and ADF and CELL in year 3 were lower in RG ($P < 0.002$ to $P < 0.04$) compared to CR and TT (Table 2). Differences between CR and TT ($P < 0.012$ to $P < 0.037$) were observed in year 1 for CELL and year 3 for NDF, ADF, HEMI and CELL. The IVTDMD did not differ among forage species, averaging 93.0 percent in year 1, 92.6 percent in year 2, and 94.7 percent in year 3. In addition, CP concentrations ranged from 25.0 percent to 14.1 percent, 26.0 percent to 16.4 percent and 26.6 percent to 12.6 percent for RG, CR and TT, respectively, in year 1, 24.4 percent to 10.7 percent, 27.5 percent to 16.4 percent, and 28.0 percent to 15.9 percent in year 2, and 32.0 percent to 18.9 percent, 32.6 percent to 23.9 percent and 33.4 percent to 24.0 percent in year 3 (data not shown). The lower CP concentrations resulted from stem elongation and head formation resulting in significant decreases in leaf-to-stem ratios at the end of the grazing season. Nevertheless, the chemical composition attested to the high quality of the grazeable forage, the CP concentrations and IVTDMD values observed being well above the nutritional requirements for actively growing or lactating meat goats (NRC, 2007). Comparable CP concentrations and decline in quality as the grazing season or the maturity of the herbage progressed were reported by Muir (2006b) for soft white wheat fertilized twice a year with 56 kg N/ha, as in the present study. Similar results were obtained by Short and Segelquist (1975) for CR cv. Elbon, Edmisten et al. (1998) for CR, wheat, oat (*Avena sativa* L.) and barley (*Hordeum vulgare* L.), and by Coblentz and Walgenbach (2010) for wheat, oat and TT cultivars. The latter authors reported slightly lower average IVTDMD values. Concentrations of CP reported by Lema et al. (2004; 2007) for two CR or several TT cultivars were either similar to or lower than those reported in the present study. Conversely, the NDF and ADF concentrations reported by the same authors were either higher (Lema et al., 2004) than those reported herein, or lower for NDF and higher for ADF (Lema et al., 2007). Finally, in an over-wintering study conducted from late November until mid-April with Angora does, Hart and Sahlu (1995) reported CP and ADF concentrations averaging 17.2 percent and 24.8 percent, respectively, for a pasture consisting of a mixture of ryegrass, cv. Marshall, and wheat.

**Table 2. Chemical composition and *in vitro* true DM digestibility (DM basis, %) of annual ryegrass, cereal rye and triticale grazed as winter annual forages by meat goats, Raleigh, N.C., USA.**

| | | | | | Treatment Contrasts *P-value* | |
|---|---|---|---|---|---|---|
| Item[a] | RG[b] | CR[c] | TT[d] | SE[e] | RG vs CR + TT | CR vs TT |
| Year 1 | | | | | | |
| CP | 18.54 | 20.81 | 19.07 | 0.99 | 0.31 | 0.28 |
| NDF | 42.11 | 43.65 | 45.01 | 0.55 | 0.029 | 0.15 |
| ADF | 20.37 | 21.56 | 22.77 | 0.37 | 0.018 | 0.09 |
| HEMI | 21.73 | 22.10 | 22.25 | 0.21 | 0.16 | 0.63 |
| CELL | 18.94 | 19.46 | 21.26 | 0.29 | 0.016 | 0.012 |
| ADL | 1.50 | 2.28 | 1.75 | 0.14 | 0.04 | 0.055 |
| IVTDMD | 92.21 | 93.60 | 93.17 | 0.43 | 0.09 | 0.52 |
| Year 2 | | | | | | |
| CP | 19.28 | 21.38 | 21.35 | 0.23 | 0.002 | 0.91 |
| NDF | 40.94 | 42.46 | 42.49 | 1.11 | 0.32 | 0.99 |
| ADF | 19.65 | 19.47 | 20.46 | 0.53 | 0.66 | 0.26 |
| HEMI | 21.29 | 22.99 | 22.03 | 0.62 | 0.18 | 0.33 |
| CELL | 17.68 | 17.06 | 18.28 | 0.59 | 0.99 | 0.22 |
| ADL | 1.53 | 1.98 | 1.92 | 0.13 | 0.054 | 0.77 |
| IVTDMD | 92.54 | 92.99 | 92.20 | 0.56 | 0.94 | 0.37 |
| Year 3 | | | | | | |
| CP | 26.73 | 27.58 | 28.59 | 0.44 | 0.066 | 0.18 |
| NDF | 37.78 | 40.63 | 37.87 | 0.57 | 0.10 | 0.026 |
| ADF | 17.99 | 19.22 | 18.50 | 0.17 | 0.012 | 0.037 |
| HEMI | 19.80 | 21.41 | 19.37 | 0.40 | 0.295 | 0.023 |
| CELL | 16.57 | 17.44 | 16.91 | 0.10 | 0.008 | 0.021 |
| ADL | 1.38 | 1.76 | 1.64 | 0.13 | 0.11 | 0.53 |
| IVTDMD | 94.79 | 94.98 | 94.35 | 0.20 | 0.63 | 0.095 |

[a] CP = crude protein; NDF= neutral detergent fiber; ADF = acid detergent fiber; HEMI = hemicellulose; CELL = cellulose; ADL = acid detergent lignin; IVTDMD = *in vitro* true DM digestibility.
[b] RG = ryegrass.
[c] CR = cereal rye.
[d] TT = triticale.
[e] SE = standard error of the mean from the statistical model.

## Biomass Production, Grazing Periods and Goat Grazing Days

Herbage biomass was greater in year 1, followed by year 3 and finally year 2 (Table 3). Although the growing season

**Table 3. The growing-season average and range of available biomass of annual ryegrass, cereal rye and triticale control-grazed with meat goats as winter forages, Raleigh, N.C., USA.**

| Item | RG[a] | Range | CR[b] | Range | TT[c] | Range |
|---|---|---|---|---|---|---|
| | kg DM/ha | | | | | |
| Year 1 | 3,606 | 1,904-4,815 | 3,436 | 2,240-3,919 | 3,639 | 2,352-5,151 |
| Year 2 | 1,491 | 1,069-1,950 | 1,560 | 886-2216 | 1584 | 1,146-1,828 |
| Year 3 | | | | | | |
| Period 1[d] | 273 | 149-342 | 185 | 151-253 | 390 | 276-542 |
| Period 2[e] | 2,741 | 2,592-2,874 | 2,347 | 2,150-2,544 | 1,908 | 388-2,960 |

[a] RG = ryegrass.
[b] CR = cereal rye.
[c] TT = triticale.
[d] RG: 9 Dec to 18 Jan; CR: 9 Dec to 28 Dec; TT: 9 Dec to 28 Dec and 11 Jan to 20 Jan.
[e] 28 Feb until end of grazing (RG: 10 May; CR: 31 Mar; TT: 20 Mar).

was longer in year 2 (Table 4), the amount of biomass produced was half that of year 1 and nearly half that of year 3, due to environmental conditions unfavorable to forage growth. The higher-range values shown occurred toward the end of the grazing season, due to increased proportion of stem material. Similar increases in biomass were reported by Lema et al. (2004) when evaluating several cereal grain cultivars on small plots in Alabama. In addition, forage-availability values for period 1 (December to January) in year 3 were similar to those reported by Muir (2006b) for January. Hart et al. (1995) reported an average-forage availability of 3,478 kg DM/ha for a pasture consisting of a mixture of RG and wheat grazed by Angora does. Production values reported by Muir (2006b) for wheat pastures grazed by Boer X Spanish doe kids encompassed the biomass yields reported in the present study. Finally, many of the forage-DM production values reported by Lema et al. (2004) for two CR and several TT cultivars were lower than those reported in this study. In the present study, estimation of residual-forage biomass immediately after moving goats to a fresh strip of grass ranged from 413 kg/ha to 1270 kg/ha (data not shown), depending on the growth stage of the

**Table 4.Grazing periods and number of grazing days per hectare in yearling meat goats managed on winter annual grasses using controlled, rotational grazing, Raleigh, N.C., USA.**

| Item | Grazing periods | Grazing season, No. days | Tester grazing days[a] No./ha | Put and take grazing days[a] No./ha | Total grazing days[a,b] No./ha |
|---|---|---|---|---|---|
| Year 1[c] | | | | | |
| RG[d] | 28 Feb – 19 May | 83 | 2,505 | 547 | 3,053 |
| CR[e] | 25 Feb – 14 Apr | 48 | 1,474 | 0 | 1,474 |
| TT[f] | 28 Feb – 21 Apr | 52 | 1,621 | 179 | 1,800 |
| Year 2[g] | | | | | |
| RG | 22 Jan – 4 May | 102 | 1,432 | 1,200 | 2,632 |
| CR | 22 Jan – 8 Apr | 76 | 1,200 | 532 | 1,732 |
| TT | 22 Jan – 23 Apr | 91 | 1,437 | 758 | 2,195 |
| Year 3[h] | | | | | |
| RG[i] | 9 Dec – 18 Jan ; 28 Feb – 10 May | 111 | 3,193 | 1,512 | 4,705 |
| CR | 9 Dec – 28 Dec; 28 Feb – 31 Mar | 50 | 1,611 | 200 | 1,811 |
| TT[j] | 9 Dec – 28 Dec; 11 Jan – 20 Jan; 28 Feb – 20 Mar | 48 | 1,263 | 139 | 1,402 |

[a] Grazing day = one animal grazing for one 24 hour period.
[b] Averaged across years 1 to 3: RG vs CR + TT, $P < 0.03$; CR vs TT, $P < 0.09$; SE = 436.
[c] 6 tester female goats/plot.
[d] RG = ryegrass.
[e] CR = cereal rye.
[f] TT = triticale.
[g] 3 tester female goat/plot until 3 Mar, then addition of 1 female and 2 castrated male tester goats/plot.
[h] All forages: 6 tester goats (4 females and 2 castrated males) 9 Dec to 28 Dec and 28 Feb until end of grazing.
[i] RG: 3 tester female goat/plot 28 Dec to 18 Jan.
[j] TT: 3 tester female goats/plot 11 Jan to 20 Jan.

forage and associated climatic conditions.

In year 1, grazing periods ranged from 28 February to 19 May for RG, 25 February to 14 April for CR and 28 February to 21 April for TT with six tester female goats grazed on each plot (Table 4). During year 2, three tester female goats were grazed from 22 January to 3 March on each plot, followed with six tester goats (four females and two castrated males) per plot until 4 May for RG, 8 April for CR, and 23 April for TT. In year 3, grazing started on 9 December with six tester goats (four females and two castrated males) per plot for each forage species. All goats were removed from the pastures on 28 December due to a lack of forage, with the exception of RG, where three tester female goats were left grazing per plot until 18 January. For TT, three tester female goats were grazed again on each plot starting 11 January. Five to seven cm of snow fell on the experimental site on 19 January, and all goats were removed from the plots on 20 January. In addition, 52 cm of snow fell on 24 January and covered the ground for the following 3 d. The combination of grazing TT for 9 d in January and heavy snowfall may have affected the subsequent regrowth of the plots that were last grazed, as evidenced by the range in TT biomass (Table 3) compared to RG and CR. Grazing of the three forage species resumed with six tester goats per plot on 28 February. Grazing ended on 10 May for RG, 31 March for CR, and 20 March for TT. The length of the grazing season in years 1, 2, and 3, respectively, were 83 d, 102 d and 111 d for RG, 48 d, 76 d and 50 d for CR, and 52 d, 91 d and 48 d for TT (Table 4). In addition, goats were grazed more days into spring on RG than CR (year 1 : + 35 d; year 2 : + 26 d; year 3 : + 40 d) or TT (year 1 : + 28 d; year 2 : + 11 d; year 3 : + 51 d), while differences between CR and TT were + 7 d and + 15 d for years 1 and 2, respectively, in favor of TT and -11d for year 3 in favor of CR. Differing from the strategy used in the present study, Muir et al. (2006a, b) used fixed, stocking rates to graze meat-goat kids on cultivated-winter pastures in east central Texas from January to April.

The total number of grazing days per ha (Table 4) averaged over the 3-year study was twice ($P < 0.03$) for RG (avg: 3,463) than for CR (avg: 1,672) or TT (avg: 1,799), with the latter two being similar ($P < 0.09$). The longer grazing season, as well as the greater number of goats grazed on RG paddocks as put-and-take animals to control forage growth, accounted for this difference.

**Table 5. Average daily gain (ADG) and gain per hectare of meat goats managed on annual ryegrass, cereal rye and triticale under controlled, rotational grazing, Raleigh, N.C., USA.**

| | | | | | Treatment Contrasts *P-value* | |
|---|---|---|---|---|---|---|
| **Item** | **RG[a]** | **CR[b]** | **TT[c]** | **SE[d]** | **RG vs CR and TT** | **CR vs TT** |
| Average daily gain, g/d | | | | | | |
| Year 1 | | | | | | |
| Female[e] | 168.3 | 160.6 | 173.0 | 9.4 | 0.90 | 0.40 |
| Year 2 | | | | | | |
| Female[f] | 90.5 | 83.2 | 84.0 | 5.0 | 0.32 | 0.92 |
| Short-term testers[g, §] | | | | | | |
| Female | 91.8 | 135.7 | 91.7 | 9.8 | 0.11 | 0.03 |
| Castrated male | 143.4 | 138.3 | 150.9 | 13.2 | 0.94 | 0.52 |
| Year 3 – Period 1[h] | | | | | | |
| Female | 48.9 | 49.1 | 33.6 | 12.4 | 0.65 | 0.43 |
| Castrated male | 29.7 | 54.4 | 70.3 | 14.4 | 0.14 | 0.48 |
| Year 3 – Period 2[j, ¶] | | | | | | |
| Female | 117.0 | 172.6 | 147.0 | 24.3 | 0.22 | 0.50 |
| Castrated male | 185.9 | 258.4 | 227.5 | 33.9 | 0.24 | 0.56 |
| Gain/hectare, kg[k] | | | | | | |
| Year 1 | 514 | 237 | 311 | 23 | 0.001 | 0.08 |
| Year 2 | 237 | 144 | 184 | 10 | 0.004 | 0.05 |
| Year 3 | 528 | 268 | 149 | 42 | 0.004 | 0.12 |

[a] RG = ryegrass.
[b] CR = cereal rye.
[c] TT = triticale.
[d] SE = standard error of the mean from the statistical model.
[e] 6 tester female goats/plot for duration of grazing season.
[f] 3 tester female goats/plot for duration of grazing season.
[g] 3 tester goats/plot (1 female, 2 castrated males) from 4 Mar until end of grazing.
[§] Sex effect: $P < 0.007$; Forage x sex interaction: $P < 0.04$; $SE = 13.0$.
[h] All forages: 6 tester goats/plot (4 females and 2 castrated males) 9 Dec to 28 Dec; RG: 3 tester female goats/plot 28 Dec to 18 Jan; TT: 3 tester female goats/plot 11 Jan – 20 Jan.
[j] All forages: 6 tester goats/plot (4 females and 2 castrated males) 28 Feb until end of grazing.
[¶] Sex effect: $P < 0.0004$; SE = 28.8.
[k] Gain/ha: ADG x No. grazing days/ha.

## Average Daily Gain and Gain per Hectare

Forage species had no effect on ADG (Table 5) with the exception of CR vs. TT (135.7 g/d vs. 91.7 g/d; $P < 0.03$) in year 2 for the short-term female testers. Castrated males gained more weight than does in year 2 (139 g/d vs. 94 g/d; $P < 0.007$) and during period two of year 3 (224 g/d vs. 146 g/d; $P < 0.0004$). A similar growth rate pattern between castrated males and females was reported by Allan and Holst (1989) in grazed, Australian-bush kids. Differences

in ADG between sexes were similar across forages in year 3, whereas a forage-species-by-sex interaction was observed in year 2 ($P < 0.04$) as CR values were similar in both females and castrates. The low ADG observed for period one in year 3 was expected, due to the short grazing duration and the cold conditions that resulted in slow forage growth. Similarly, the harsh winter conditions encountered during year 2 of the study were reflected in lower ADG.

Gains per hectare (GHA) were greater (Table 5) for RG ($P < 0.001$ to $P < 0.004$) than for CR and TT each year of the study because CR and TT produced less biomass, resulting in fewer grazing-days-per-unit area (Tables 2 and 3). In addition, GHA in year 2 were greater for TT than CR ($P < 0.05$). Finally, GHA were 47 percent, 31 percent and 61 percent greater for RG than for the average of CR and TT in years 1, 2, and 3, respectively.

Hart et al. (1993) reported that growing Alpine, Angora and Nubian kids grazed on high-quality, wheat forage gained 50 g/d, whereas Kiesling et al. (1994) reported ADG ranging from 65 g/d to 141 g/d in growing Angora goats grazing CR. The performance-per-animal and per-unit area reported in the study herein were substantially higher than the values reported by Lema et al. (2007) and Muir (2006a, b). These differences arose from the objectives and approaches adopted. The former authors used continuous grazing at a set stocking rate of 12.5 goats/ha, and the latter authors used continuous grazing at stocking rates ranging from 5 goats/ha to 20 goats/ha, as well as several N-fertilization rates. We used a controlled-grazing approach with frequent moves and variable stocking using put-and-take animals in an attempt to control forage growth and optimize forage quality and animal performance. Physical location, climate, environmental conditions, soil fertility, animal age and genetics are among many factors that also will affect plant and animal response.

### Ruminal pH, ruminal ammonia, plasma urea nitrogen, volatile fatty acids, full and empty live weight and hot and chilled carcass yield of castrated male goats (year 3).

Ruminal pH and RUA of castrated male goats (Table 6) were similar among forage species and averaged 6.67 and 25.7 mg/dL, respectively. These pH values were in the range appropriate for optimal activity of cellulolytic microflora (Church, 1979). Similar pH values (6.64) were reported by Molina Alcaide et al. (2000) with non-pregnant Granadina goats fed alfalfa (*Medicago sativa* L.) hay and with adult Damascus goats (Hadjipanayiotou and Antoniou, 1983) fed either barley or sudex (*Sorghum*, spp) hays of much lower quality (9.8 percent CP and 9.4 percent CP, respectively), or alfalfa hay of similar quality (23.3 percent CP). Conversely, lower pH values (6.4 and 6.2) and RUA concentrations (11.6 mg/dL and 5.6 mg/dL) were reported by Hart and Sahlu (1993) in yearling, Angora does grazing

**Table 6. Ruminal pH, ruminal ammonia, plasma urea nitrogen, ruminal volatile fatty acids, full and empty live weight and hot and chilled carcass yield of castrated meat goats control-grazed on ryegrass, cereal rye and triticale as winter annual forages – determined at end of the study in year 3, Raleigh, N.C., USA.**

| | | | | | Treatment Contrasts *P-value* | |
|---|---|---|---|---|---|---|
| Item | RG[a] | CR[b] | TT[c] | SE[d] | RG vs CR and TT | CR vs TT |
| Ruminal pH | 6.76 | 6.62 | 6.65 | 0.06 | 0.15 | 0.73 |
| Ruminal ammonia, mg/dL | 25.4 | 24.7 | 26.9 | 1.9 | 0.88 | 0.45 |
| Plasma urea nitrogen, mg/dL | 16.4 | 21.9 | 24.1 | 1.5 | 0.024 | 0.37 |
| Volatile fatty acids | | | | | | |
| Acetate (mM/100 mM) | 62.0 | 60.7 | 57.7 | 0.59 | 0.017 | 0.023 |
| Propionate (mM/100 mM) | 22.0 | 25.2 | 27.0 | 0.63 | 0.006 | 0.114 |
| Acetate:Propionate | 2.83 | 2.43 | 2.22 | 0.11 | 0.017 | 0.23 |
| Isobutyrate (mM/100 mM) | 1.68 | 1.47 | 1.57 | 0.14 | 0.41 | 0.65 |
| Butyrate (mM/100 mM) | 11.01 | 9.56 | 10.24 | 0.41 | 0.09 | 0.30 |
| Isovalerate (mM/100 mM) | 2.12 | 2.01 | 2.41 | 0.13 | 0.47 | 0.13 |
| Valerate (mM/100 mM) | 1.20 | 1.01 | 1.09 | 0.05 | 0.06 | 0.30 |
| Full live weight, kg | 36.1 | 36.5 | 36.6 | 1.82 | 0.85 | 0.97 |
| Empty live weight, kg[e] | 35.1 | 35.3 | 34.8 | 1.76 | 0.97 | 0.87 |
| Hot carcass yield, % | 51.3 | 51.2 | 51.6 | 0.74 | 0.92 | 0.75 |
| Chilled carcass yield, % | 50.1 | 49.9 | 50.4 | 0.73 | 0.93 | 0.63 |

[a] RG = ryegrass.
[b] CR = cereal rye.
[c] TT = triticale.
[d] SE = standard error of the mean from the statistical model.
[e] Determined following overnight shrunk in a dry lot without feed or water

either alfalfa or sainfoin (*Onobrychis vicifolia* Scop.), respectively. Hart et al. (1993) recorded lower pH and RUA concentrations in 6-month-old to 8-month-old Alpine, Angora and Nubian kids grazing a high-quality wheat (avg: 6.35 and 9.83 mg/dL, respectively) or a low-quality bermudagrass pasture and fed 0.20 kg/d of a 24-percent CP supplement (avg: 6.25 and 7.17 mg/dL, respectively). In addition, lower RUA concentrations (6.5 mg/dL) were obtained in Angora does fed chopped bermudagrass (*Cynodon dactylon* [L.] Pers.) hay and limit-grazed for 2 hours daily on a wheat/annual-ryegrass pasture (Hart and Sahlu, 1995). Finally, lower pH and or RUA values as reported herein were recorded in crossbred, castrated goats fed orchardgrass hay (*Dactylis glomerata* L.) and soybean (*Glycine max* L.) meal (Moore et al., 2002b), orchardgrass hay and varying amounts of grain (Luginbuhl et al., 1999), or orchardgrass hay and increasing amounts of soybean hulls (Moore et al., 2002a). Lower ruminal pH values due to addition of concentrate feed to forage diets are well documented in goats, sheep and steers (Rumsey et al., 1970; Hadjipanayiotou and Antoniou, 1983).

The RUA values from the present study were higher than most values reported in the literature for goats fed all forage or mixed diets with the exception of the study by Molina Alcaide et al. (2000) in non-pregnant Granadina goats fed alfalfa hay (34.6 mg/dL). Values closest to those reported herein were obtained with castrated, mixed-breed goats from Southern Ethiopia (21.0 mg/dL) fed vetch (*Vicia dasycarpa* Ten.) hay (Woodard and Reed, 1997) and with castrated, crossbred-Boer goats (25.21 mg/dL) fed orchardgrass hay *ad libitum* and corn (*Zea mays* L.) gluten feed at 1 percent BW (Moore et al., 2002b). Johnson et al. (1973) indicated that because wheat-forage protein is highly soluble, it is likely to be readily degraded. According to Contreras-Govea and Albrecht (2006), oat cultivars that are sown in late summer and harvested 77 d later contained high concentrations of both CP and water-soluble carbohydrates. The high values obtained in the present study could be due to the high-CP concentrations of RG, CR and TT (Table 2). In addition, the supply of water-soluble carbohydrate and N in terms of timing and amount may have been less than adequate to provide a ruminal environment favorable to the optimization of microbial growth (Hoover and Stokes, 1991). According to Mehrez et al. (1977), however, 23.5 mg RUA/dL are necessary to maximize the rate of barley DM fermentation, whereas Ørskov (1982) suggested that lower values (20 mg/dL) are adequate for highly fibrous diets.

Castrated male goats grazed on RG had lower PUN (16.4 mg/dL; $P < 0.024$) concentrations than goats grazed on CR or TT (avg: 23 mg/dL). Concentrations of 25.6 mg/dL (Hart et al. 1993) were observed in Alpine, Angora and Nubian goats grazed on high-quality, wheat pasture (19.8 percent CP), whereas Luginbuhl et al. (2009) reported PUN values of 26.2 mg/dL in does suckling kids and grazed on three, tall-fescue cultivars (avg CP: 20.6 percent), Conversely, Lema et al. (2007) reported lower PUN values (avg: 14.7 mg/dL) in crossbred does grazed on CR (18.7 percent CP) or TT (18.3 percent CP). Similarly, Serrato-Corona et al. (2011) obtained PUN values of 17.6 mg/dL in young Alpine goats fed oat hay (8 percent CP). In addition, feeding sericea lespedeza (*Lespedeza cuneata* [Dum.-Cours] G. Don) or alfalfa hay (11.2 percent CP and 18.7 percent CP, respectively) with a 16 percent CP corn/cottonseed-based supplement to bucklings, Turner et al. (2005) recorded PUN values of 10.7 mg/dL and 21.1 mg/dL, respectively. Finally, growing, purebred- and crossbred-Boer bucks fed 71-percent orchardgrass hay and 29-percent concentrate mixture had PUN concentrations of 13.6 mg/dL (Luginbuhl et al., 2000). These results contrast with findings by Wildeus et al. (2007) who reported PUN concentrations of 30 mg/dL or above when feeding orchardgrass and alfalfa-hay-based diets with limited-concentrate supplementation to Spanish, Boer and Boer-cross wethers. As for RUA, differences in dietary protein, protein degradability in the rumen and protein-to-energy ratios may account for the dissimilarity of PUN results between studies. Stevens et al. (1994) reported average PUN concentrations of 17.6 mg/dL in clinically healthy, adult goats from a random sample of 30 farms from a list of clients of the College of Veterinary Medicine at North Carolina State University and Smith and Sherman (1994) indicated that normal PUN values are in the range of 10 mg/dL to 28 mg/dL. In the present study, the lower PUN concentrations found in the castrated goats grazed on RG are surprising given that RUA concentrations were similar across forages. According to Ørskov (1992), PUN and RUA are correlated positively, PUN being a function of ammonia absorption from the rumen and lower tract, and of the efficiency of protein utilization. The same author further indicated that PUN closely reflects dietary N balance from the perspective of the nutritional requirements of the host animal and of the ruminal microorganisms. The higher PUN concentrations observed in goats grazed on CR and TT may indicate a larger degree in excess N consumption relative to energy (Hammond et al., 1994). Additionally, non-protein N compounds can account for as much as one third of the total N in pasture herbage (Maynard et al., 1979).

Ruminal-acetate ($P < 0.017$) and propionate ($P < 0.006$) concentrations (Table 6) were higher and lower, respectively, in goats grazed on RG compared to those grazed on CR and TT, resulting in a greater acetate:propionate ratio ($P < 0.017$). In addition, ruminal-acetate concentrations were higher ($P < 0.023$) in goats grazed on CR than in those grazed on TT. Concentrations of isobutyrate, butyrate, isovalerate and valerate were similar across forage species. The VFA values reported herein were within the range of values typical of ruminants fed all forage diets (Maynard et al., 1979). Similar VFA values were reported by Hart et al. (1993) in Alpine, Angora and Nubian goats grazing high-quality wheat pasture (19.8 percent CP) and by Molina Alcaide et al. (2000) in non-pregnant Granadina goats fed high-quality alfalfa hay (18.1 percent CP). Butyrate values from the present study, however, were twice the values reported by Hart et al. (1993).

Full and empty BW and hot-and-chilled-carcass yield did not differ among forages (Table 6), and all harvested goats graded choice (data not shown). Similar chilled-carcass yields were reported by Johnson et al. (2010) for goats on a grazing- and hay-feeding system sacrificed at BW comparable to those reported herein.

## Conclusions

Results indicated that these winter annual grasses were of excellent quality and exceeded the nutritional requirements of growing replacement stock. Growing goats achieved satisfactory weight gains when fed only on these forages under controlled-rotational-grazing management; however, RG resulted in superior per-hectare-live-weight gains. It seems evident that these winter annual grasses could represent a flexible and valuable component of a perennial-pasture and browse-based feeding system.

## Literature Cited


Allan, C. J., and P. J. Holst. 1989. Comparison of growth and dressing percent between intact male, castrated male and female kids of Australian bush goats. Small Rumin. Res. 2:63-68.

AOAC. 1999. International Official Methods of Analysis. 16th Ed. Association of Official Analytical Chemists, Arlington, VA.

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. Southern Forages. Modern Concepts for Forage Crops Management (4th Ed). International Plant Nutrition Institute. Norcross, GA.

Chamblee, D.S., and J.T. Green, Jr. 1995. Determining the amount of feed available in a pasture. In: D.S. Chamblee and J.T. Green, Jr. (Ed.) Production and Utilization of Pastures and Forages in North Carolina. Technical Bulletin 305: Appendix G, 154-157.

Church, D.C. 1979. Digestive Physiology and Nutrition of Ruminants. Vol. 1: Digestive Physiology. 2nd Ed. O & B Books. Corvallis, OR.

Coblentz, W.K., and R.P. Walgenbach. 2010. Fall growth, nutritive value, and estimation of total digestible nutrients for cereal-grain forages in the north-central United States. J. Anim. Sci. 88:383-399.

Contreras-Govoa, F.E., and K.A. Albrecht. 2006. Forage production and nutritive value of oat in autumn and early summer. Crop Sci. 46:2382-2386.

Edmisten, K.L., J.T. Green, Jr., J.P. Mueller, and J.C. Burns. 1998. Winter annual small grain potential. II. Quantification of nutritive characteristics of four small grain species at six growth stages. Commun. Soil Sci. Plant. Anal. 29:881-899.

Green, J.T. Jr., J.P. Mueller, and D.S. Chamblee . 1995. Planting guide for forage crops in North Carolina. Chapter 11. In: D.S. Chamblee and J.T. Green, Jr. (Ed.) Production and Utilization of Pastures and Forages in North Carolina. pp 57-61. North Carolina Agricultural Research Service Technical Bulletin No. 305.

Hadjipanayiotou, M., and T. Antoniou, 1983. A comparison of rumen fermentation patterns in sheep and goats given a variety of diets. J. Sci. Food Agric. 34:1319-1322.

Hammond, A.C., W.E. Kunkle, P.C. Genho, S.A. Moore, C.E. Crosby, and K.H. Ramsay. 1994. Use of blood urea nitrogen concentrations to determine time and level of protein supplementation in wintering cows. Prof. Anim. Scientist 10:24:31.

Hart, S.P., and T. Sahlu. 1993. Mohair production and body weight gains of yearling Angora goats grazing forages with different tannin levels. Proc. XVII International Grassld Congress. P 575. Palmerston North, New Zealand.

Hart, S.P., and T. Sahlu. 1995. Limit-grazing of cool-season pastures for wintering Angora does. Sheep and Goat Res. J. 11:1-3.

Hart, S.P., T. Sahlu, and J. M. Fernandez. 1993. Efficiency of utilization of high and low quality forage by three goat breeds. Small Rumin. Res. 10: 293-301.

Hoover, W.H., and S.R. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci. 74, 3630-3644.

Johnson, C.R., S.P. Doyle, and R.S. Long. 2010. Effect of feeding system on meat goat growth performance and carcass traits. Sheep Goat Res. J. 25:78:82.

Johnson, R. R., M. McGeehon, E. Williams, and H. C. Young, Jr. 1973. Studies on the nutritive value of wheat pasture. pp 13-15. Oklahoma State Univ. Res. Rep. MP-90. Stillwater, OK.

Kielsing, H.E., C.E. Barnes, T.T. Ross, J. Libbin, M. Ortiz, R.L. Byford, and K.W. Duncan. 1994. Grazing alfalfa and winter annual cereals with Angora goats. Agricultural Experiment Station Res. Rep. 684. New Mexico State University. Las Cruces, NM.

Komarek , A.R., J.B. Robertson, and P.J. Van Soest. 1994. Comparison of the filter bag technique to conventional filtration in the Van Soest Analysis of 21 feeds. In: Proc. Natl. Conference on Forage Quality, Evaluation and Utilization, Lincoln, NE. p 78.

Lema, M., E. Cebert, and V. Sapra. 2004. Evaluation of small grain cultivars for forage in North Alabama. J. Sustain. Agric. 23:133-145.

Lema, M., S. Kebe, R. Opio, C. Fenderson, and N. Adefope. 2007. Evaluation of TRICAL-336 triticale, Maton rye and Kentucky-31 fescue as winter pasture for meat goats. 2007. J. Sustain. Agri. 30:89-104.

Luginbuhl, J-M., J.P. Mueller, and H.M. Glennon. 2009. Performance of suckling kids and their dams control-grazed on three tall fescue cultivars. J. Anim. Sci. 87 (E-Suppl. 3):32.

Luginbuhl, J-M., M.H. Poore, and A.C. Parsons. 1999. Performance of goats fed hay and varying levels of grain. J. Anim. Sci. 77 (Suppl. 1):25.

Luginbuhl, J-M., M.H. Poore, and A.P. Conrad. 2000. Effect of level of whole cottonseed on intake, digestibility, and performance of growing male goats fed hay-based diets. J. Anim. Sci. 78:1677-1683.

Maynard, L.A., J.K. Loosli, HF. Hintz, and R.G. Warner. 1979. Animal Nutrition. 7th Ed. McGraw-Hill Book Co. New York. NY.

Marsh, W.H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct method for the determination of blood urea. Clin. Chem. 11:624-627.

Mehrez, A.Z., E.R. Ørskov, and I. McDonald. 1977. Rates of rumen fermentation in relation to ammonia concentration. Br. J. Ntr. 38:437:443.

Molina Alcaide, E., A.I. Martin García, and Z.J.F. Aguilera. 2000. A comparative study of nutrient digestibility, kinetics of degradation and passage and rumen fermentation pattern in goats and sheep offered good quality diets. Livest. Prod. Sci. 64:215-223.

Moore, J.A., A.T. Maye, M.H. Poore, and J-M. Luginbuhl. 2002a. Effects of increasing level of dietary soybean hulls on ruminal characteristics and serum urea nitrogen of Boer-cross goats. J. Anim. Sci. 80 (Suppl. 1):70.

Moore, J.A., M.H. Poore, and J-M. Luginbuhl. 2002b. By-product feeds for meat goats: effects on digestibility, ruminant environment, and carcass characteristics. J. Anim. Sci. 80:1752-1758.

Muir, J.P. 2006a.Stocking rates on cultivated winter pastures for meat goats. Sheep and Goat Res. J. 21:6-11.

Muir, J.P. 2006b. Weight gains of meat goat kids on wheat (*Triticum aestivum* L.) pastures fertilized at different nitrogen levels. Small Rumin. Res. 66:64-69.

Neuman, D.F., S. Mickey, J.T. Green, Jr., and J.P. Mueller. 1995. Establishment and annual production costs of major forages and silages. Chapter 23. In: D. S. Chamblee and J.T. Green, Jr. (Ed.) Production and Utilization of Pastures and Forages in North Carolina. pp 121-124. North Carolina Agricultural Research Service Technical Bulletin No. 305.

Norton, B.W. 1984. Nutrition of the goat: A review. Proc. Goat Nutr. Res. in the Tropics. pp 75-81. Brisbane, Australia.

NCSU (North Carolina State University). 2008-2011. State Climate Office of NC. CRONOS Datasbase version 2.7.2. http://www.nc-climate.ncsu.edu

NRC (National Research Council). 2007. Nutrient Requirements of Small Ruminants: sheep, goats, cervids, and new world camelids. The National Academies Press. Washington, DC.

Ørskov, R.E. 1992. Protein Nutrition in Ruminants. 2nd Ed. Academic press. New York. NY.

Rumsey, T.S., P A. Putnam, J.Bond, and R.R. Oltjen. 1970. Influence of level and type of diet on ruminal pH and VFA, respiratory rate and EKG patterns of steers. J. Anim. Sci. 31:608-616.

SAS. 2003. SAS/STAT® 9.1.3 Service Pack 4. SAS Inst. Inc., Cary, NC.

Serrato-Corona, J. S., J. I. Sanchez Duarte, M. Murillo Ortiz, and E. Castellanos Perez. 2011. Oat hay digestibility, rumen ammonia nitrogen and BUN in goats supplemented with fermented molasses-poultry litter. J. Anim. Vet. Adv. 10:808-812.

Smith, M.C., and D.M. Sheman. 1994. Goat Medicine. Chapter 12: Urinary System. p 392. Lea & Febiger. Philadelphia.

Short, H.L., and A. Segelquist. 1975. Elbon rye as quality forage to Ozark deer during winter. Agron. J. 67:92-93.

Steel, R.G.D., J. H. Torrie, and D.A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach (3rd Ed.). McGraw-Hill Series in Probability and Statistics, WCB/McGraw-Hill Co., New York.

Stevens, J.B., K.L. Anderson, M.T. Correa, T. Stewart, and W.E. Brazelton, Jr. 1994. Hematologic, blood gas, blood chemistry, and serum mineral values for a sample of clinically healthy adult goats. Vet. Clin. Pathol. 23:19-24.

Tilley, J.M., and R.A. Terry. 1963. A two-stage technique for in vitro digestion of forage crops. J. Br. Grassl. Soc. 18:104–111.

Turner, K.E., S. Wildeus, and J.R. Collins. 2005. Intake, performance, and blood parameters in young goats offered high forage diets of lespedeza or alfalfa hay. Small. Rumin. Res. 59:15-23.

United States Dept. Agric. (USDA) Soil. Cons. Serv., and North Carolina Agric. Exp. Station, 1970. Soil Survey of Wake County North Carolina.U.S. Gov. Printing Office, Washington, DC.

Van Soest, P.J., J.B. Robertson, and B.A.Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vartha, E.W., and A.G. Matches. 1977. Use of a weighted-disk measure as an aid in sampling the herbage yield on tall fescue pastures grazed by cattle. Agron. J. 69:888-890.

Wildeus, S., J-M. Luginbuhl, K.E. Turner, Y.L. Nutall, and J. R. Collins. 2007. Growth and carcass characteristics in goat kids fed grass and alfalfa hay-based diets with limited concentrate supplementation. Sheep Goat Res. J. 22:15-19.

Woodard, A., and J. D. Reed. 1997. Nitrogen metabolism of sheep and goats consuming *Acacia brevispica* and *Sesbenia sesban*. J. Anim. Sci. 75:1130-1139.

Sheep & Goat Research Journal
Volume 27, 2012 – November

# Efficacy Of Garlic Juice, Copper Oxide Wire Particles, And Anthelmintics To Control Gastrointestinal Nematodes In Goats

*A. D. Courter[1], T. K. Hutchens[2], K. Andries[2], J. E. Miller[3], J. E. Tower[1], and M. K. Neary[1,4]*

[1] *Purdue University, West Lafayette, IN*

[2] *Kentucky State University, Frankfort, KY*

[3] *Louisiana State University, Baton Rouge, LA*

[4] *Corresponding author: mneary@purdue.edu; 915 West State Street, West Lafayette, IN 47907-2054*

## Summary

Resistance of gastrointestinal nematodes (GIN) to anthelmintics and a need for nonchemical control of GIN necessitates investigation of alternative control methods. This study examined the efficacy of garlic juice (99.5 percent pure) (G), copper oxide wire particles (COWP), levamisole (L), moxidectin (M), a combination treatment of COWP and G (CG), and no treatment (C) for GIN control in lactating Boer x Kiko does. Treatments were administered at d 0 and the G treatment was repeated every 7 d throughout the 28 d study. Mixed-model procedures for repeated measures were used to evaluate the effect of treatment and date of sampling on fecal-egg counts (FEC), and percent-packed-cell volume (PCV). Larval cultures from fecal samples at d 0 contained *H. contortus*, but *Telodorsagia* and *Trichostrongylus* were the predominant parasites. There was no difference ($P > 0.05$) in FEC or PCV of does due to GIN-control method. The PCV was greater ($P < 0.05$) at d 0 (31.2 percent ± 0.7 percent) when compared to d 7 (29.1 percent ± 0.7 percent), d 14 (28.7 percent ± 1.1 percent), and d 21 (28.8 percent ± 0.8 percent). The PCV at d 28 (23.5 percent ± 0.9 percent) was lower ($P < 0.001$) than all other sampling d. The FEC did not differ ($P > 0.05$) at d 0 (756 eggs/g ± 414 eggs/g), d 7 (1349 eggs/g ± 448 eggs/g), and d 14 (1782 eggs/g ± 436 eggs/g). The FEC at d 21 (2259 eggs/g ± 464 eggs/g) was trending ($P = 0.08$) higher as compared to d 0. The FEC at d 28 (3935 eggs/g ± 449 eggs/g) was greater ($P < 0.05$) than FEC at all other sampling d. *Trichostrongylus* and *Telodorsagia* were the primary GIN species and not *H. contortus* as is often assumed at the research and farm level. These data support determining which GIN species are present in a goat herd at various times of the year and applying an internal-parasite-management protocol accordingly. Treatments used in this study were not effective in controlling any of the GIN species present.

**Key Words:** Goat, Parasitism, Garlic, Copper Wire, Anthelmintic

## Introduction

The small-ruminant industry has relied heavily on the use of conventional anthelmintics to control gastrointestinal nematodes (GIN), resulting in increased levels of parasite resistance and a search for alternate methods of control. Some alternatives to traditional anthelmintics include pasture- and grazing-management techniques, feeding condensed tannins, dosing with copper oxide wire particles (COWP), and garlic.

Some of the pasture- and grazing-management techniques include mixed species grazing (Marley et al., 2006) and pasture rotation (Barger et al. 1994). Min et al. (2004) concluded that sericea lespedeza, a forage high in condensed tannins, effectively reduced fecal-egg production from GIN.

Several authors have reported using copper oxide wire particles (COWP) to control GIN in small ruminants. Burke et al. (2007) researched the effectiveness and safety of COWP in sheep using 0 g , 0.5 g, 0.75 g, 1 g, or 2 g boluses. It was found that administration of a 2 g bolus was effective in preventing a rise in fecal-egg counts (FEC). Similarly, Soli et al. (2010) concluded that a 2 g COWP bolus administered to sheep and goats significantly reduced FEC compared with control 12 days after administration. A later study by Burke et al. (2009) found conflicting results. That experiment involved the administration of a 1 g COWP bolus in 90-d-old kids. Results showed an increase in FEC over time and no decrease due to COWP.

Garlic is thought to have anti-parasitic, immune-boosting, and anti-helminthic properties (Schmidt, 1973; Guarrera, 1999). There have been conflicting results in use of garlic to decrease the parasitic load in small ruminants. Noon et al. (2003) showed reduced levels of *Haemonchus contortus*, when all groups were treated with garlic on the last week of the study. Although the data were not statistically analyzed, some small-ruminant producers look to it to validate their use of garlic for GIN control. In a later experiment, Wang et al. (2008) studied feeding Spanish wethers a hay-based diet for four weeks at maintenance level of intake with or without 2 percent of garlic powder. They reported that continual feeding of garlic powder reduced FEC. It was attributed to cell-mediated immunity. In contrast, O'Brien et al. (2009) evaluated a single administration of 4.54 grams of garlic juice in goats and found that a single dose of garlic juice was not effective in reducing FEC. In Burke et al. (2009), three treatment groups, garlic juice, garlic bulbs, and a water-dosed control were evaluated. Results from their study showed that a one-time dosage of garlic juice tended to reduce the mean FEC compared to the control by d 7, but FEC were similar on d 14 regardless of treatment.

The objective of this experiment was to determine the effectiveness of garlic alone, COWP, COWP and garlic, levamisole, and moxidectin against GIN in Boer x Kiko goats. Fecal egg counts (FEC), percent-packed-cell volume (PCV), and fecal cultures were used as indicators of GIN parasitism.

## Materials and Methods

The Purdue University Animal Care and Use Committee (PACUC) reviewed and approved all experimental procedures used in this study. The research was conducted at the Purdue University Southern Indiana Agriculture Center (SIPAC) located near Dubois, Ind., U.S.A.

Mature (3- to 5-year-old) Boer x Kiko cross does were used in a five-week study (June - July 2010). Does kidded on pasture in May and were lactating throughout the study. Does were placed on the kidding pasture in late April. The kidding pasture was predominately a forage base of Kentucky 31 tall fescue (*Festuca arundinacea*) and had been grazed consistently by goats for the previous five years. After kidding, does and kids were then moved to a pasture containing predominately annual ryegrass (*Lolium multiflorum*) from late May to early June (May 28, to June 4, 2010) which had been grazed by goats the previous two springs. The goats were then returned to the Kentucky 31 tall fescue pasture just prior to the study (June 4, to June 8, 2010). Does were grazed on these two pastures for a total of 50 days, of which 42 of these days were on the fescue pasture. This amount of grazing time should have resulted in ample opportunity for natural infection of the goats by GIN. A summary timeline of events follows: treat goats (June 9, 2010; d 0); graze fescue pasture (d -4 to d 0); graze ryegrass pasture (d -11 to d -5); and graze fescue pasture (d -50 to d -12).

Once the study began, does were housed in an open-sided, concrete-floored barn without access to pasture for the duration of the study. A total mixed ration of 2/3 soyhulls and 1/3 chopped hay was fed at a rate of 1.8 kg per day for each doe. Does had access to clean water and free-choice mineral.

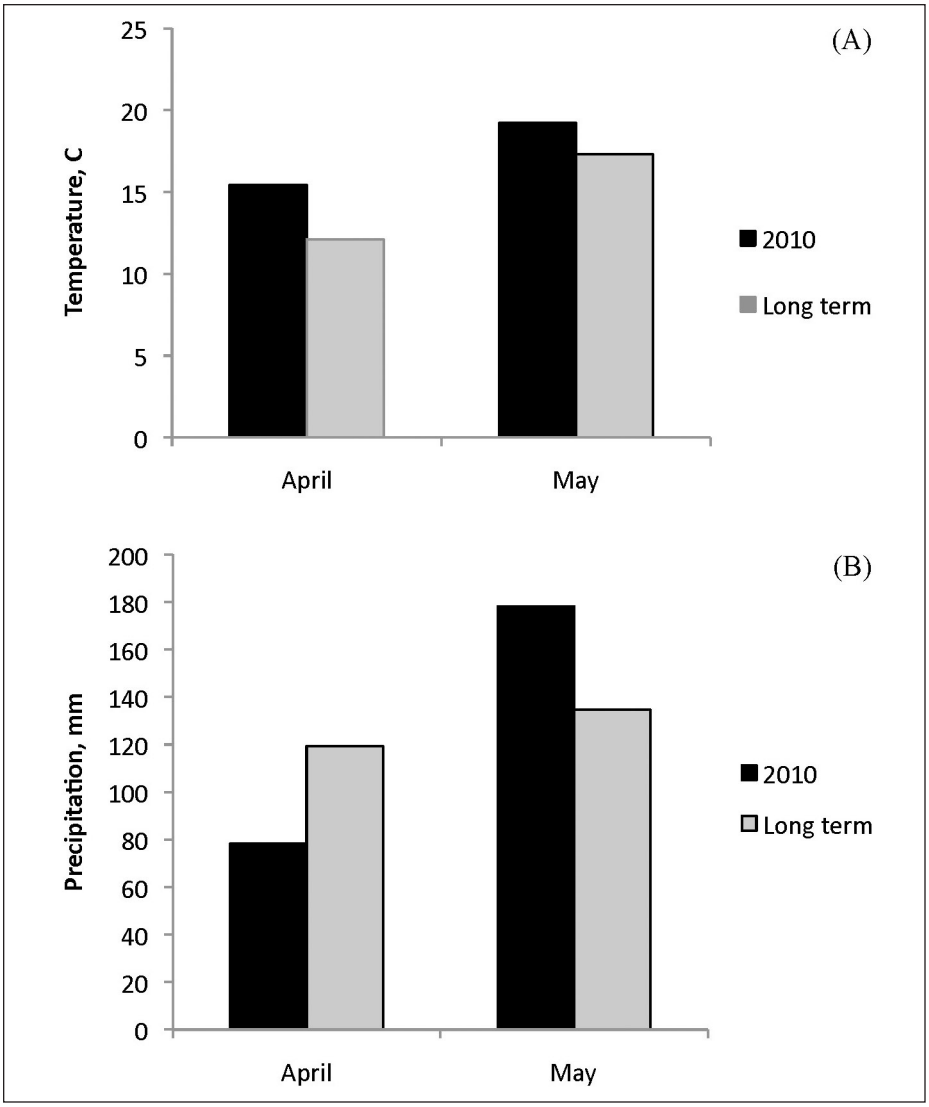
Does (n = 8/treatment) were assigned randomly by age and number of kids to be treated (June 9, 2010) with 12 ml of garlic juice (1:1 dilution with 6 ml 99.3 percent formula Garlic Barrier and 6 ml of water, according to label directions), 2-gram copper oxide wire particles (COWP), levamisole (12 mg/kg orally, moxidectin (0.2 mg/kg injectable), or a combination treatment of garlic juice (1:1 dilution of 99.3 percent formula Garlic Barrier) and COWP (2-gram bolus). Dosages for levamisole and moxidectin were administered in accordance with the American Consortium for Small Ruminant Parasite Control (ACSRPC) dosing guidelines. All treatments were administered once at the beginning of the study on day 0. The garlic-treatment groups were dosed every 7 d in order to further test the decreased FEC results of the garlic-juice-treatment group as reported by Burke et al. (2009).

Blood was collected from the jugular vein weekly to determine packed-cell-volume (PCV). If PCV scores dropped below 18 percent, the animals were removed from the study. Fecal samples were collected per rectum every 7 d for fecal egg count (FEC) analysis by a modified McMaster technique sensitive to 50 eggs per gram (Whitlock, 1948). Extra fecal samples were collected on d 0, d 14, and d 28 for a pooled culture to recover nematode larvae analyzed at Louisiana State University. Larvae were recovered from cultures using the Baermann procedure. On d 0 and d 28 does were weighed and a body condition score (BCS) was determined using a system of 1 to 5, with 1 being emaciated and 5 being obese.

Data were analyzed using repeated measures by the mixed-models procedure of SAS 9.1 (SAS Institute, Inc., Cary, N.C.). The mathematical model used for PCV, FEC, BW, and BCS included treatment, day, and treatment-by-day interaction. FEC were log trans-

**Figure 1. Temperature (A) and precipitation (B) data from the Louisville, Kentucky, National Weather Service at Southern Indiana Purdue Agricultural Center reported as monthly averages.**




formed and statistical inferences were made on transformed data but untransformed means are presented. Differences among means were considered significant when $P < 0.05$.

## Results and Discussion

An Indiana weather collection station, located at SIPAC, reported temperature and precipitation data to a National Weather Service site located in Louisville, Kentucky. Results are reported in Figure 1 for two months prior to the study (April through May), when natural GIN infection occurred. Compared to long term (30 years), temperatures in these months were slightly higher than the long term; and precipitation was slightly lower in April and slightly higher in May.

Doe BWT and BCS are presented in Table 1. There was no ($P > 0.05$) difference between beginning and ending BWT or beginning and ending BCS due to treatment.

Figure 2 illustrates the effect of d of sampling and GIN treatment regimen on FEC. Treatment had no effect ($P > 0.05$) on FEC. Sampling d was significant ($P < 0.05$) for FEC. The FEC did not differ ($P > 0.05$) at d 0 (756 eggs/g ± 414 eggs/g), d 7 (1349 eggs/g ± 448 eggs/g), and d 14 (1782 eggs/g ± 436 eggs/g). The FEC at d 21 (2259 eggs/g ± 464 eggs/g) tended ($P = 0.08$) to be higher as compared to d 0. The FEC at d 28 (3935 eggs/g ± 449 eggs/g) was greater ($P < 0.05$) than FEC at all other sampling days.

The PCV data are presented in Figure 3. There was no difference ($P > 0.05$) in PCV of does due to GIN-control treatment. There was a ($P < 0.05$) d effect on PCV results from the does. The PCV was greater ($P < 0.05$) at d 0 (31.2 percent ± 0.7 percent) when compared to d 7 (29.1 percent ± 0.7 percent), d 14 (28.7 percent ± 1.1 percent), and d 21 (28.8 percent ± 0.8 percent). The PCV at d 28 (23.5 percent ± 0.9 percent) was lower ($P < 0.001$) than all other sampling d.

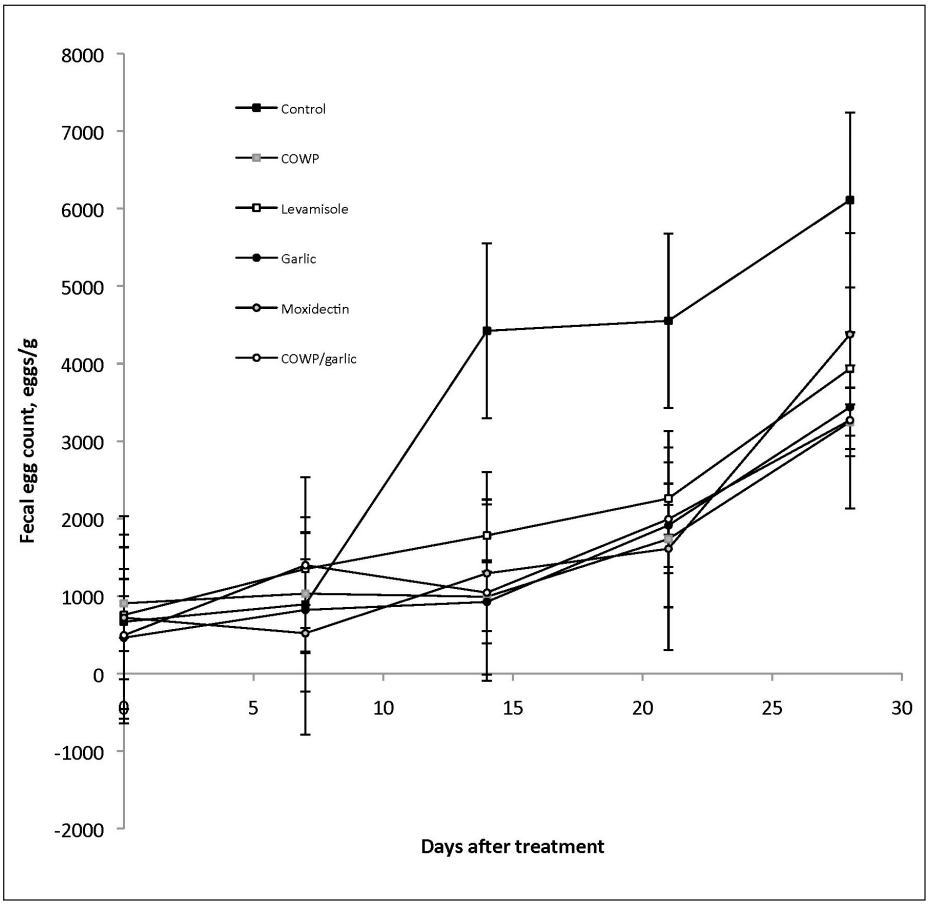
Culture results at d 0 showed that although *H. contortus* (Figure 4) was present, *Telodorsagia* and *Trichostrongylus* were the predominant parasites (Figures

**Table 1. Beginning and ending BWT and BCS for COWP, Control, COWP + Garlic, moxidectin, Garlic, and levamisole.**

| | Beginning | | | | Ending | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | BWT,kg | SEM | BCS[1] | SEM | BWT,kg | SEM | BCS | SEM |
| COWP | 48.2 | 3.8 | 2.3 | 0.22 | 45.7 | 5.3 | 1.9 | 0.31 |
| Control | 47.4 | 3.8 | 2.2 | 0.22 | 52.8 | 5.3 | 2.5 | 0.31 |
| COWP + Garlic | 48.1 | 3.8 | 2.3 | 0.22 | 46.8 | 5.3 | 1.9 | 0.31 |
| Moxidectin | 47.5 | 3.8 | 2.3 | 0.22 | 55.7 | 5.3 | 2.2 | 0.31 |
| Garlic | 47.2 | 3.8 | 2.1 | 0.22 | 49.3 | 5.3 | 1.9 | 0.31 |
| Levamisole | 47.6 | 3.8 | 2.2 | 0.22 | 47.4 | 5.3 | 1.5 | 0.31 |

[1] BCS = 1 to 5; 1 being emaciated and 5 being obese

**Figure 2. Least squared means and standard errors of fecal egg counts (FEC) of ewes treated with nothing (closed square), COWP (shaded square), levamisole (open square), garlic (closed circle), moxidectin (shaded circle), or COWP and garlic (open circle) treated on d 0. There was a significant ($P < 0.0001$) day effect on FEC.**




5 and 6, respectively). At d 28 *H. contortus* larvae was present at levels below 1 percent.

The FEC increased over time across all treatments and was significantly greater on d 28 due to ineffective control of the *Teladorsagia* and *Trichostrongylus* population. The increased numbers of *Teladorsagia* and *Trichostrongylus* relative to *H. contortous* may have been due to the experiment being conducted in the earlier months of summer, with the 50 d infective-grazing period ending in early June. According to O'Connor et al. (2006), optimum temperatures for trichostrongylid parasites from unembryonated egg to L3 stage were 16° C to 30° C for *Telodorsagia*, 22° C to 33° C for *Trichostrongylus*, and 25° C to 37° C for *H. contortus*. The temperature data revealed average temperatures of 15° C in April and 19° C in May at the study site, when the does were grazing and exposed to GIN larvae. These lower environmental temperatures could have resulted in the greater amounts of *Trichostrongylus* and *Telodorsagia* seen in the culture results relative to *H. contortus*. It is clear from the culture results that the predominant egg-laying adults were *Telodorsagia* and *Trichostrongylus*, and not *H. contortus*.

The ineffectiveness of COWP against *Telodorsagia* and *Trichostrongylus* is in congruence with an experiment done by Burke et al. (2006). Their study showed similar results with a sustained-release, multi-trace, copper-containing vitamin that was administered to does six weeks before kidding (Burke et al., 2006). The proportion of *Trichostrongylus* from the culture data increased from 23 percent to 55 percent, while the *H. contortus* population decreased (Burke et al., 2006). Chartier et al. (2000) concurred, stating that *Trichostrongylus* appeared to be less affected by COWP than *H. contortus*.

Although the infections of *Teladorsagia* and *Trichostrongylus* did not decrease, Figure 4 shows that the COWP treatment effectively decreased *H. contortus* 7 d after administration. Burke et al. (2007) used varying dosages of COWP from 0 grams to 2 grams and found that 2 g of COWP was sufficient to reduce *H. contortus*.

Levamisole has been an effective dewormer in this herd in the past. A fecal-egg-count-reduction test used in the herd in 2009 showed 94 percent efficacy of levamisole, while moxidectin had resistance issues at that time. The resistance to levamisole in this study is not fully understood.

Even though the PCV value of 23.5 percent at d 28 was at the lower limits of a normal range for a lactating doe, the reduction from 28.8 percent at d 21 indicated a modest effect of parasitic infection on PCV. It is possible that lactation was a source of variation in doe-PCV values. However, does at d 28 of the study, when the drop in PCV values occurred, were in the last third of lactation (90-d kid weaning age) and past the peak lactation period. Doe BWT and BCS did not significantly decrease from d 0 to d 28 of the study, indicating nutrition was adequate to support lactation demands.

While lactation could have been a factor, PCV reduction from d 21 to d 28 was more likely due to *H. contortus* infection. Although the cultured larvae revealed very low numbers of *H. contortus*, it is possible that the goats became infected with *H. contortus* late in the pre-grazing period due to lower than optimum environmental temperatures for *H. contortus* larvae development. If *H. contortus* was acquired later in the infective grazing period, then the increase in infection was most likely due to maturing larvae not affected by the initial GIN-control treatments. The L4 stage of *H. contortus* does not shed eggs, but does feed on the blood and can cause anemia. Once development of L4 to immature adult *H. contortus* occurs, egg production doesn't initiate for about 14 d, which could explain the lack of *H. contortus* from the culture results. Anthelmintic treatments at the beginning of the study had no effect on the adult egg-laying *Teladorsagia*, *Tri-*

**Figure 3. Packed cell volume (PCV) of ewes treated with nothing (closed square), COWP (shaded square), levamisole (open square), garlic (closed circle), moxidectin (shaded circle), or COWP and garlic (open circle) treated on d 0. There was a ($P < 0.0001$) day effect on PCV.**

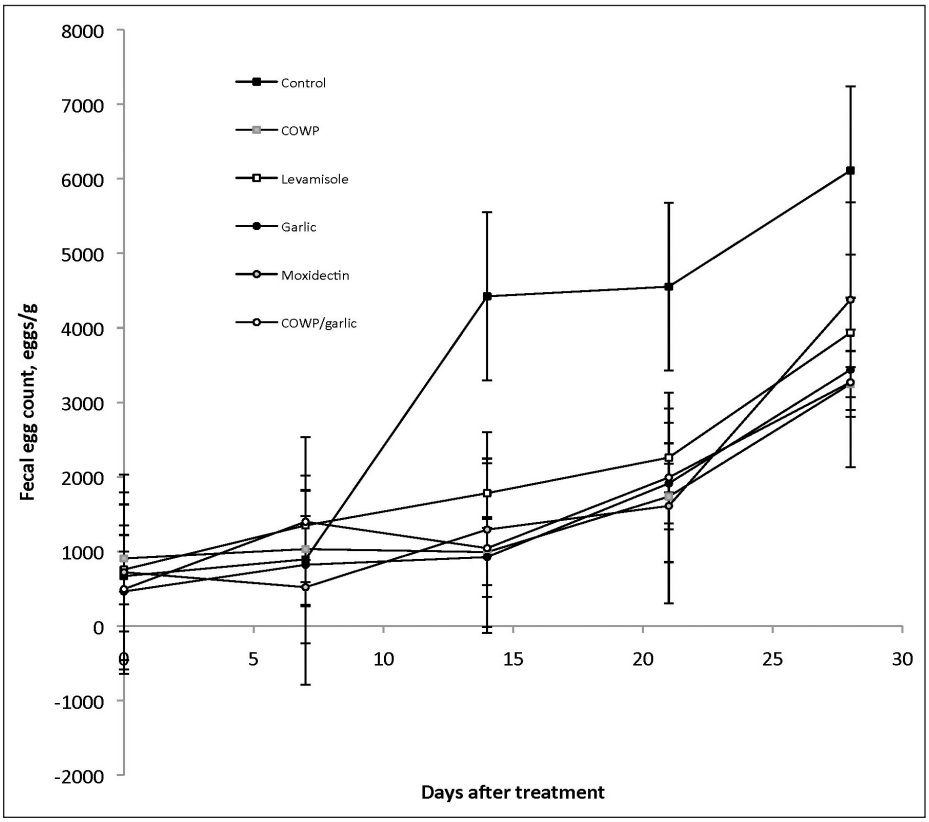
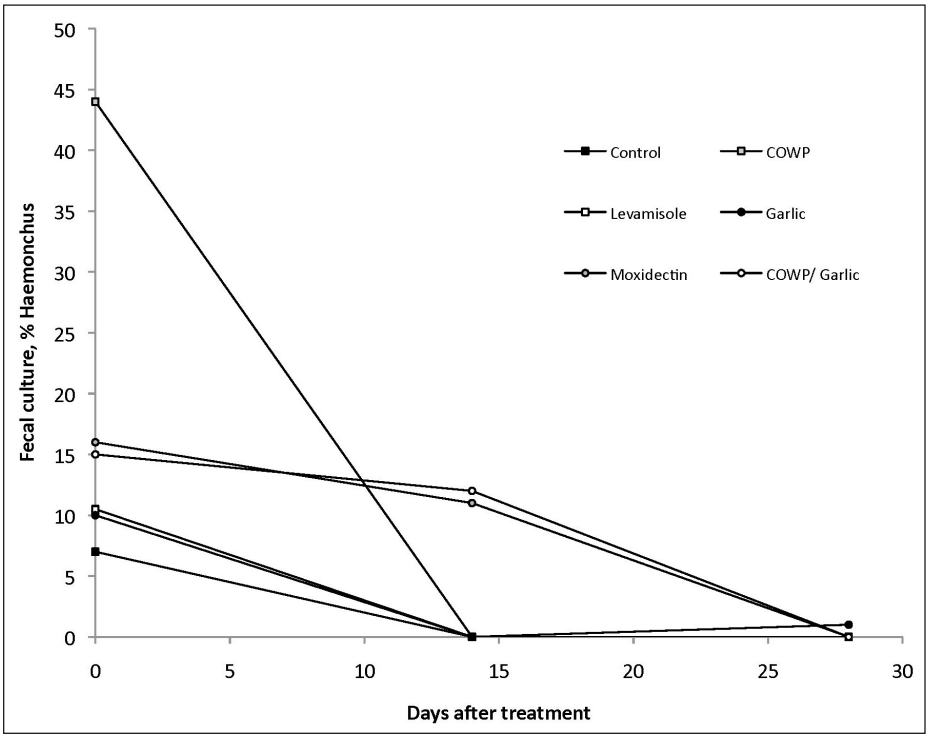



**Figure 4. Fecal culture results with percentages of *Haemonchus Contortus* treated on d 0 with nothing (control; closed square), COWP (shaded square), levamisole (open square), garlic (closed circle), moxidectin (shaded circle), or COWP and garlic (open circle).**




*chostrongylus*, and possibly the immature larval stages of *H. contortus*. The lack of efficacy of treatment, particularly if *H. contortus* larvae were in an immature development stage, could be a function of timing of treatment and not the treatment itself. The garlic-alone treatment was continued every seven d, so timing of treatment would not have been a factor in lack of control of GIN by garlic.

## Conclusions

Garlic juice alone, garlic juice and COWP, moxidectin, and levamisole were not effective in controlling GIN in lactating goats as measured by FEC and PCV. COWP was not effective in controlling *Trichostrongylus* and *Telodorsagia*, but may have been effective against *H. contortus*. The predominant, egg-laying-GIN species in this study were *Teladorsagia* and *Trichostrongylus* and not *H. contortus*. PCV results indicated a *H. contortus* infection, but the culture results did not support this, possibly due to the L4 or non-egg productive stage of *H. contortus*. The fact that in this specific research herd and during the specific time period the study was performed *Trichostrongylus* and *Telodorsagia* were the primary species and not *H. contortus*, has implications at the farm level and at research stations. These data support the recommended practice of determining which GIN species are present in a goat herd at various times of the year and applying an internal-parasite-management protocol accordingly.

## Literature Cited

ACSRPC. 2006. Smart drenching and FAMACHA integrated training for sustainable control of gastrointestinal nematodes in small ruminants. Retrieved from http://www.scsrpc.org/FlashF/pdf/manual.pdf, May, 2010.

Barger, L., K. Siale, D. Banks, and L. Le Jambre. 1994. Rotational grazing for control of gastrointestinal nematodes of goats in a wet tropical environment. *Vet. Parasitol.* 53, 109-116.

Burke, J.M and J.E. Miller. 2006 control of *Haemonechus contortus* in goats with a sustainted-release multi-trace element/vitamin ruminal bolus containing copper, *Vet Parasitol.* 141, 132-137.

**Figure 5. Fecal culture results with percentages of *Telodorsagia* treated on d 0 with nothing (control; closed square), COWP (shaded square), levamisole (open square), garlic (closed circle), moxidectin (shaded circle), or COWP and garlic (open circle).**

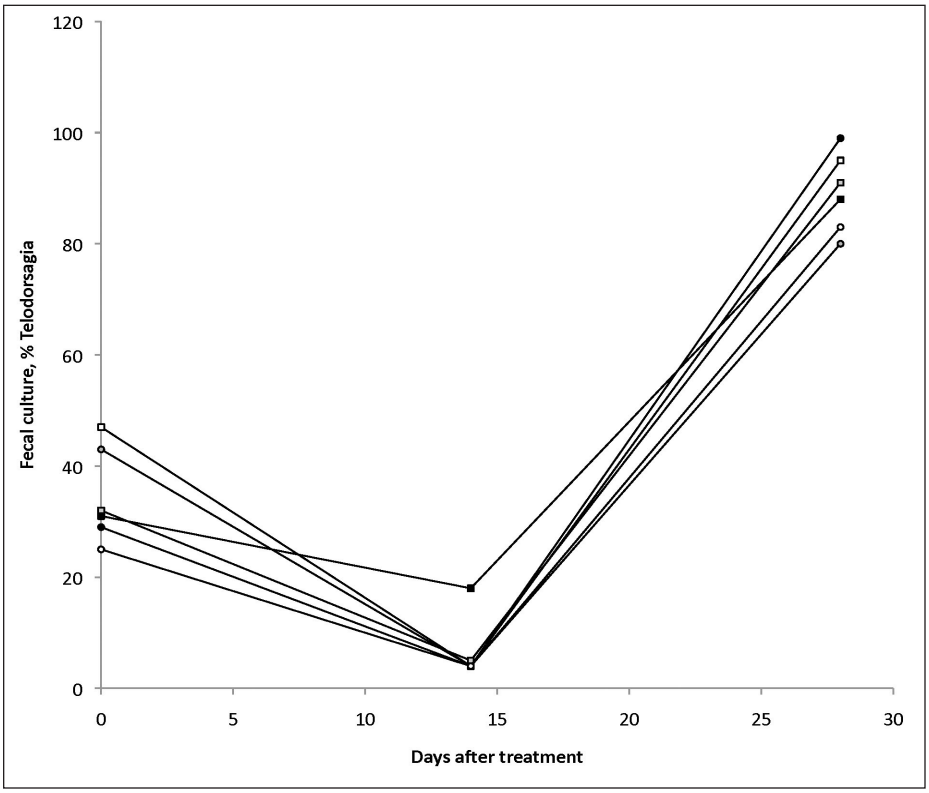
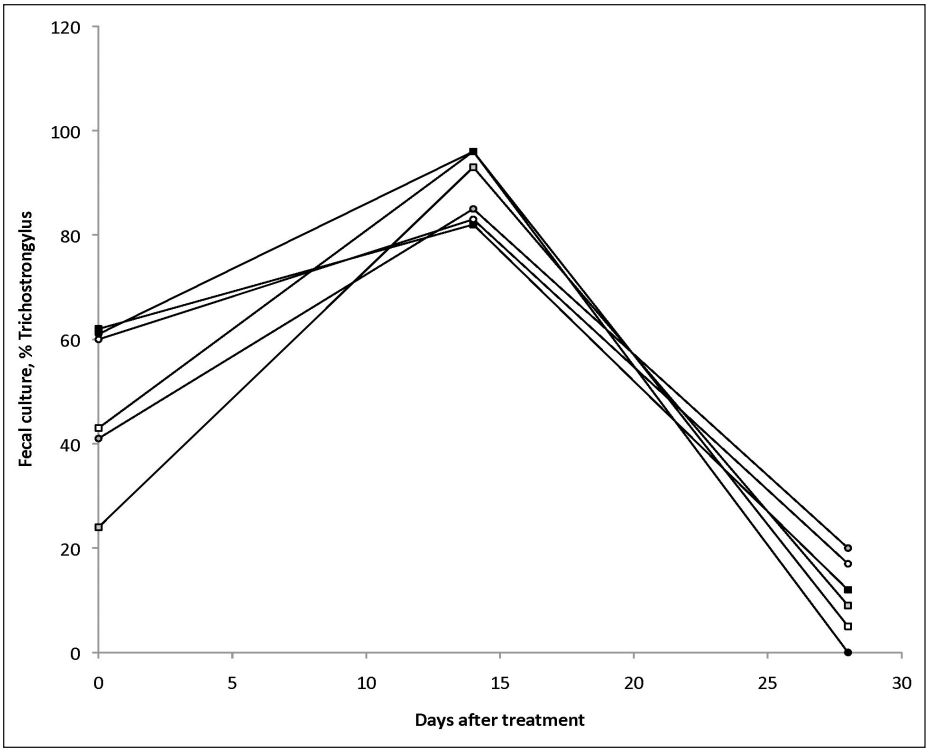



**Figure 6. Fecal culture results with percentages of *Trichostrongylus* treated on d 0 with nothing (control; closed square), COWP (shaded square), levamisole (open square), garlic (closed circle), moxidectin (shaded circle), or COWP and garlic (open circle).**





Burke, J.M., J.E. Miller, and T.H. Terrill, 2009. Use of copper oxide wire particles (COWP) to control gastrointestinal nematodes in stressed lambs or kids. *J. Anim. Sci.* 87: E-Supple. 3.

Burke, J.M., A. Wells, P. Casey, and R.M. Kaplan, 2009. Herbal Dewormer fails to control gastrointestinal nematodes in goats. *Vet. Parasitol.* 160, 168-170

Burke, J.M., A. Wells, P. Casey, and J.E. Miller. 2009. Garlic and Papaya lack control over gastronintestinal nematodes in goats and Lambs. *Vet. Parasitol.* 159: 171-174

Burke, J.M., D. Morrical, and J.E. Miller, 2007. Control of gastrointestinal nematodes with copper oxide wire particles in a flock of lactating Polypay ewes and offspring in Iowa, USA. *Vet Parisitol.* 146, 372-275.

Chartier, C., E. Etter, H. Heste, I. Pors, C. Koch, and B. Dellac. 2000. Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. *Vet. Res. Commun.* 24: 389-399.

Guarrera, P.M. 1999. Traditional anthelmintic, antiparasitic and repellent uses of plants in Central Italy. *J. Ethnopharmacol.* 68:183-192.

Marley, C., M. Fraser, D. Davies, M. Rees, J. Vale, and A. Forbes. 2006. The effect of mixed or sequential grazing of cattle and sheep on the faecal egg counts and growth rates of weaned lambs when treated with anthelmintics. *Vet. Parasitol.* 142: 134-141.

Min, B.R., W. E. Pomroy, S.P. Hart, and T. Sahlu. 2004. The effect of short-term consumption of a forage containing condensed tanning on gastro-intestinal nematode parasite infection in grazing wether goats. *Small Ruminant Res.* 51: 279-283.

Noon, J., T. Settlemire, E. McCain, and M. Bukowski-Thall. 2003. A controlled experiment to measure the effectiveness on lambs of wormers that conform to the new organic standards. Retrieved March 29, 2011, from http://www.garlicbarrier.com/2003_SARE_Report.html

O'Brien D.J., M.C. Gooden, N. C. Whitley, and S. Schoenian. 2009. Use of garlic as a potential natural dewormer in small ruminants. *J. Anim. Sci.* 87: E-Supple. 3.

O' Connor, L.J., S. W. Walkden-Brown, and L. P. Kahn. 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Vet. Parisitol.* 142: 1-15.

Schmidt, G.L., 1973. Garlic. More than just a taste. *Food Technology* New Zealand 8: 15–16.

Soli, F., T.H. Terrill, S.A. Shaik, W.R. Getz, J.E. Miller, M. Vanguru, and J.M. Burke. 2010. Efficacy of copper oxide wire particles against gastrointestinal nematodes in sheep and goats. *Vet. Parisitol.* 168: 93.

Wang, Z. E. Loetz, A.L. Goetsch. S. P. Hart, and T. Sahlu. 2008. The effect of garlic on *Haemonchus contortus* infection in goats. *J. Anim. Sci.* 86, E-Supple. 2.

Whitlock, H.V. 1948. Some modification of the McMaster helminth egg-counting technique apparatus. *J. Counc. Sci. Res.* 21: 177-180.

Sheep & Goat Research Journal
Volume 27, 2012 – December

# Effectiveness of Theobromine and Caffeine Mixtures in Coyote Lure Operative Devices as a Predacide: A Simulated Field Study

*Eric M. Gese[1], Patrick A. Darrow[1], John A. Shivik[1], Bruce A. Kimball[2], John D. Eisemann[3], and Julie K. Young[1]*

*[1] United States Department of Agriculture, Animal and Plant Health Inspection Service,Wildlife Services, National Wildlife Research Center, Logan Field Station, Utah State University, Logan, UT 84322;*

*2United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Monell Chemical Senses Center, Philadelphia, PA 19104;*

*3United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521*

*Corresponding author: Eric Gese, eric.gese@usu.edu; USDA/APHIS/WS/National Wildlife Research Center; Department of Wildland Resources; Utah State University; Logan, UT 84322*

## Acknowledgements

Funding and logistics provided by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center. We thank the assistance of the facility staff for animal care and maintenance of coyotes at the United States Department of Agriculture, National Wildlife Research Center, Predator Research Facility, Millville, Utah; the United States Department of Agriculture Sheep Experiment Station, Dubois, Idaho, for use of their large pens; and L. Clark and M. E. Tobin for reviews of the manuscript.

## Summary

Predators are capable of causing damage to domestic livestock throughout North America. Lethal responses for managing livestock depredations may include the use of sodium cyanide in M-44 devices. Currently, several states have banned the use of M-44s and several other states are forecast to ban these devices. Therefore, additional tools are being sought to expand the repertoire of options available for managing coyote depredations on domestic livestock. We evaluated the use of a theobromine:caffeine mixture delivered within a Coyote Lure Operative Device (CLOD) as an additional predacide for coyotes (*Canis latrans*). Results from six trials involving 38 captive coyotes were ambiguous. Issues related to the attractiveness of the CLOD, palatability of the compound, and absorption of the theobromine:caffeine mixture produced mortality levels below the desired >90-percent-mortality rate deemed adequate for laboratory efficacy study to the United States Environmental Protection Agency (EPA) registration and operational use. While many coyotes died from consumption of the theobromine:caffeine mixture, several coyotes recovered with symptoms of poisoning disappearing within 12 hours in those animals that survived exposure to the toxicant. Several issues related to palatability of the mixture and compound delivery, as well as coyote behavior, sensory abilities, and physiology, indicated the use of a theobromine:caffeine mixture in a CLOD may not be an effective method for managing coyote depredations on domestic livestock.

**Key Words:** Caffeine, *Canis latrans*, CLOD, Coyote, Mortality, Theobromine

## Introduction

Predators cause more than $16 million in damage to sheep producers every year (United States Department of Agriculture 2000). The predator with the largest impact, by far, is the coyote (*Canis latrans*). The United States Department of Agriculture's Wildlife Services (WS) responds to requests to address livestock losses attributed to predation and removes approximately 80,000 coyotes per year to reduce losses of domestic livestock (United States Department of Agriculture 2011). Toxicants are part of an integrated pest management program that may involve both lethal and non-lethal methods to reduce predation on livestock (Knowlton et al. 1999). Sodium cyanide and sodium fluoroacetate (Compound 1080) are the only restricted use pesticides registered with the United States Environmental Protection Agency for use on coyotes. However, several states (California, Colorado, Arizona) have prohibited the use of sodium cyanide and Compound 1080. Public sentiments towards the use of toxicants for managing predators (Arthur 1981, Andelt 1987, Reiter et al. 1999) will likely lead to other states prohibiting the use of these chemicals as well. Such bans severely restrict the ability of ranchers, federal and state agencies, and pest control operators to limit livestock losses and other damage (e.g., disease transmission, irrigation system damage, crop losses, game predation, aircraft hazards, human health and safety) caused by problematic coyotes. As urban wildlife-human conflicts increase in frequency, it is likely that the need for a coyote-control device that is acceptable for use in semi-urban areas will increase. Desirable qualities for such a coyote-control device include being safe to humans and pets, as well as being safe for non-target wildlife species and the environment, and social acceptability. As such, it would be advantageous if the coyote-control compound induced mortality with minimal pre-mortality symptoms and if an antidote or reversal therapy were available for inadvertently exposed commensal dogs.

Criteria for the selection and development of a predacide include effectiveness, taste and odor, speed of action, hazard to humans, antidote/therapy, environmental safety, regulatory concerns, cost, and availability (see Fagerstone et al. 2004 for more details). With respect to a methylxanthine (theobromine:caffeine mixture) coyote-control compound, these criteria were addressed in Fagerstone et al. (2004). Briefly:

**(1) Effectiveness** — Methylxanthines can induce acute toxicity in canids as the propensity for domestic dogs to overdose on methylxanthines via ingestion of chocolate is well documented (Farbman 2001, Gwaltney-Brant 2001, Pittenger, 2002). The most abundant methylxanthines in chocolate are theobromine and caffeine. The toxicity of these methylxanthines to coyotes is summarized in Johnston (2005).

**(2) Taste and odor** — As indicated by articles in the literature, chocolate is consumed readily by canids (Farbman 2001, Gwaltney-Brant 2001, Pittenger, 2002). Additionally, coyotes have readily ingested methylxanthine fortified dog food and lard (Johnston 2005). It appears methylxanthine can be formulated to be palatable to canids.

**(3) Speed of action** — Following ingestion of methylxanthines, coyotes typically exhibit no symptoms for several hours. This lag time offers a margin of safety with respect to non-target pets by providing a window of opportunity for veterinary intervention to reverse the toxicity of accidentally exposed animals. Because symptoms may not be immediately apparent, the delivery system should incorporate a dye marking animals that have consumed the toxic matrix. Furthermore, signage should be used to alert pet owners to potential hazards and to provide the appropriate response to exposure, as indicated by the dye, and before onset of rapid mortality after symptoms are apparent.

**(4) Antidote** — The availability of an antidote or effective medical treatment to reverse the toxic effects of a predacide increases its safety. Given the frequent exposure of dogs to chocolate, veterinary supportive-therapy procedures are well documented (Hornfeldt 1987, Farbman 2001). As there is typically a significant lag time between ingestion and the onset of symptoms, inclusion of a dye in the formulation should facilitate identification and subsequent veterinary intervention of accidentally exposed dogs before the onset of toxicosis.

**(5) Hazard to humans** — All currently registered predacides are toxic to humans. For theobromine, the rat oral $LD_{50}$ is 1,250 mg/kg (U.S. Environmental Protection Agency 2012), and humans are likely more tolerant of caffeine and theobromine. Even though humans are exposed to high amounts of caffeine and theobromine by consuming coffee, tea, cola beverages, and chocolate, there has been no documented human mortality in association with the consumption of these products.

**(6) Environmental safety** — Selectivity of toxicity to the target animal is desirable to minimize accidental poisoning of non-target animals. Methylxanthines appear to be selectively toxic to canids, as reports of accidental poisonings due to the consumption of methylxanthines have mainly been limited to canids.

**(7) Cost and availability** — Pure analytical grade methylxanthines, such as caffeine, theobromine, and theophylline are widely available through chemical supply sources. The delivery device for pest coyotes would likely need to contain approximately 6 g (see reasoning below) of active ingredient.

**(8) Regulatory concerns** – With the exception of 31 compounds considered by the EPA to be of negligible or minimum risk, all pesticides including predacides must be approved for use by the EPA. Acceptance criteria include efficacy, safety and environmental hazards. Methylxanthines, such as theobromine, should display high levels of efficacy and selectivity towards canid predators while being environmentally benign. The EPA's published standard for the laboratory efficacy of rodenticides is 90 percent mortality of the exposed animals (U.S. Environmental Protection Agency, 1991). There are no published standards for the laboratory efficacy of predacides, consequently, our target mortality for these trials was 90 percent.

Johnston (2005) found that caffeine was toxic to coyotes, however the symptoms accompanying toxicosis were suboptimal because caffeine-induced mortality was preceded by severe convulsions and seizures. Theobromine was less toxic to coyotes, but symptoms, such as convulsions and seizures were mild to non-existent. Oral methylxanthine (5:1 theobromine:caffeine) administration to coyotes appeared to represent the optimal mixture of theobromine (minimal

undesirable symptoms) and caffeine (potency) (Johnston 2005). However the toxicity of this mixture dictates that about 6 g of theobromine:caffeine would be required for an effective coyote predacide. This may limit the number of potential delivery devices available for this mixture. The Coyote Lure Operative Device (CLOD; Marsh et al. 1982, Berentsen et al. 2006a, b) can deliver a total volume of formulation containing 6 g of toxicant. It should be noted that Johnston (2005) obtained lethal doses in coyotes using oral gavage, or using a CLOD in a pan, which allowed coyotes to consume the entire contents of the mixture, including spillage of the compound. Therefore, use of the CLOD under simulated field conditions is more representative of an actual management action.

As development and required EPA registration of new toxicants typically takes 5 years to 10 years, it behooves the wildlife-management community to proactively develop a new coyote-control compound that is efficacious, cost effective, induces mortality with minimal undesirable pre-mortality symptoms, and possesses registration potential with the EPA. For this reason, we evaluated the potential of a theobromine:caffeine mixture delivered via the CLOD to induce mortality in coyotes under simulated-field conditions. The main objectives of the study were: (1) to determine the number of coyotes that will be attracted to, chew on, and consume contents of CLODs containing a theobromine:caffeine mixture, and (2) to estimate what proportion of coyotes that chew on CLODs ingest a lethal dose of the theobromine:caffeine mixture and the time interval between consumption and mortality.

## Materials and Methods

Six different trials using a mixture of theobromine and caffeine as the predacide were designed and conducted. These trials were conducted sequentially with modifications to the compound or bait mixture made from information acquired from the previous trial. The research was conducted using captive coyotes in large pens at either the USDA Sheep Experiment Station near Dubois, Idaho (Trial 1), or the USDA/NWRC Predator Research Facility in Millville, Utah (Trials 2-6). In each pen enclosure, shade structures and natural bedding were provided. Food was provided daily, while water was provided ad libitum. This study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the National Wildlife Research Center.

Johnston (2005) demonstrated that, when a coyote received a theobromine:caffeine mixture via oral gavage, or in a pan placed in a kennel, the result was usually death of the animal. However, for application to a field setting that would mimic a management scenario, it was necessary to test the same dosage of the compound by delivering the compound in a CLOD and determine whether an appropriate lethal dose could be administered. In the current 6 trials, the CLOD consisted of a 60 ml plastic bottle and a stake that affixed the CLOD to the ground (Figure 1). A 5-part theobromine: 1-part caffeine mixture was combined with a meat matrix (water with either canned, wet dog food or hamburger), and corn syrup and placed in a CLOD. All ingredients were combined and mixed in a blender until homogeneous. The mixture of corn syrup, meat matrix, and active ingredients were then transferred to the 60 ml CLOD. In the mixture, a maximum dose of 19.6 g of active components (16.3 g of theobromine and 3.3 g of caffeine) was in each CLOD. However, doses were variable as the coyotes were self-administering the test compound. CLODs were prepared at the USDA/NWRC chemistry labs.

### Trial 1

The first trial utilized a CLOD containing 25 percent active ingredient (16.3 g theobromine: 3.3 g caffeine) in a granular form, 37.5 percent dog food, and 37.5 percent corn syrup (to enhance palatability). This trial was conducted in a 65-ha enclosure with four CLODs placed within the enclosure. The coyotes were prebaited with CLODs containing dog food and corn syrup for 1 week before the toxic CLODs were placed in the pen. These CLODs also had an attractant-infused, wax coating on them.

### Trial 2

Following the results from Trial 1, there was a desire to have a marker in the CLOD to inform pet owners in the event of an accidental dosing. Therefore, Rhodamine B was added (0.04 percent wet weight) to the same mixture as described for Trial 1. The use of Rhodamine B would act as a marker (Evans and Griffith 1973, Marsh et al. 1982) by making the animals lips turn red upon exposure signaling to a pet owner that their animal had ingested something unusual, and thereby allowing a pet owner to get the pet to a veterinarian for treatment. Corn syrup was applied liberally to the outside of the CLOD to encourage the coyote to lick and chew the CLOD. Trial 2 was conducted in a 6-ha pen with one CLOD placed in the pen.

**Figure 1. Coyote Lure Operative Device containing the theobromine-caffeine mixture that has been chewed on by a coyote (photo courtesy of P. Darrow).**

### Trial 3

Following the lower efficacy found in Trial 2, there was concern the Rhodamine B may have limited the absorption of the compound, as well as concern that using commercial dog food would be a registration issue. Therefore, the dog food was replaced with hamburger that had been cooked in a microwave as the meat matrix, and the Rhodamine B was removed. Additionally, the amount of active ingredient was reduced to 8.15 g theobromine: 1.65 g caffeine (5:1 mixture) in granular form to determine if this lower dosage would increase palatability, yet remain effective. Corn syrup was applied liberally to the CLOD to encour-

age the coyote to lick and chew the CLOD. Trial 3 was conducted in a 6-ha pen with one CLOD placed in the pen.

### Trial 4

Data from Trial 3 indicated that the lower dosage of active ingredient resulted in lower efficacy. Therefore, Trial 4 employed double (16.3 g theobromine: 3.3 g caffeine in granular form) the dosage of Trial 3. The bait formula consisted of 21 percent (wet weight) theobromine, 4 percent caffeine, ground beef (12.2 percent wet weight), water (24.4 percent wet weight), corn starch (1.8% percent wet weight), and corn syrup (36.6 percent wet weight). Corn syrup was applied liberally to the CLOD to encourage the coyote to lick and chew the CLOD. Trial 4 was conducted in a 6-ha pen with one CLOD placed in the pen.

### Trial 5

The previous trials (1-4) were still lower than the desired level of mortality (90 percent) for EPA registration and some coyotes would not consume the contents of the CLOD. Therefore the methylxanthine mixture was micro-encapsulated (50 percent) with a proprietary lipid coating (Maxx Performance, Inc., Chester, N.Y.) in an attempt to increase palatability, and therefore increase consumption of the compound. Because micro-encapsulation decreased the volume of the active ingredient that could be mixed homogenously in the CLOD, the CLOD contained a maximum dosage of 10.4 g theobromine: 2.1 g caffeine. The meat component of the mixture was removed to maximize the amount of theobromine and caffeine that could be placed in the CLOD. Corn syrup was applied liberally to the CLOD to encourage the coyote to lick and chew the CLOD. Trial 5 was conducted in a 0.1-ha pen with one CLOD placed in the pen.

### Trial 6

Due to the lower mortality demonstrated in Trial 5, there was concern that micro-encapsulation of the active ingredients had lowered absorption in the gut. Therefore, for Trial 6 a spherical form of the active ingredients was used to increase consumption without compromising absorption. A wetted-methylxanthine mixture was subjected to extrusion spheronization resulting in uniform particles of approximately 1 mm diameter. The use of a spherical form of the compound would, in theory, limit solubility in the mouth by reducing the surface area of the compounds. The CLOD was prepared with the active ingredients in a 21:4 (wet weight) mixture of 16.3 g theobromine: 3.3 g caffeine in spherical form mixed with a corn syrup and meat (ground beef) matrix. Corn syrup was applied liberally to the CLOD to encourage the coyote to lick and chew the CLOD. Trial 6 was conducted in a 0.1-ha pen with one CLOD placed in the pen.

For each trial, a single coyote was placed in a large enclosure and allowed to acclimate to the pen for 48 hrs to 72 hrs. After the acclimation period, a CLOD containing the theobromine: caffeine mixture was placed in the pen. The coyote was observed remotely with a spotting scope, thermal imager, or remote-controlled camera. Motion-activated Internet Protocol (IP) cameras were used to monitor the CLOD. When the coyote approached the CLOD, the camera would take a picture and send a text message to the observer's phone. The observer could then view the pictures online to note when the coyote approached and consumed the CLOD. In Trials 1 through 4, the coyotes were fitted with VHF radio-collars to facilitate locating the coyote in the larger pens by the observer. Observations recorded included the time the animal approached and chewed on the CLOD, the estimated amount of the CLOD consumed, and the time of death. If the animal consumed a part, or all, of the CLOD, the behavior of the animal was observed to determine the symptoms of toxicosis. Animals consuming a part, or all, of the compound were observed for 24 hr post-consumption, or until mortality occurred. Coyotes were observed for 5 days after placement of the CLOD in the pen. As this was a simulated field test, after placing coyotes in the study pen, human-coyote interactions (including coyote monitoring before the toxicant is consumed) were minimized to reduce disturbance and allow the animal to approach and consume the contents of the CLOD.

### Study Design and Statistical Analyses

The purpose of the study was to determine what proportion of coyotes interacted with the CLODS, and of those animals, what proportion succumbed to the toxicant in an acceptable manner. Thus, the experimental design was observational and statistical analyses were limited to descriptive statistics (i.e., proportions) and their associated measures of variability (range). The EPA's published standard for the laboratory efficacy of rodenticides is 90 percent mortality of the exposed animals (U.S. Environmental Protection Agency, 1991). There are no published standards for the laboratory efficacy of predacides, consequently, our target mortality for these trials was 90 percent.

## Results

Trial 1 exposed 11 coyotes to the CLODs, of which 9 animals consumed some of the compound resulting in seven mortalities (Table 1), giving an overall mortality of 64 percent. While this 64-percent mortality was less than the desired 90-percent mortality, the resultant deaths of seven animals indicated that the CLOD could deliver a lethal dose of the compound, and thus the addition of a marker appeared justified to reduce the risk to non-target pets. With the addition of Rhodamine B to the compound, the results from Trial 2 indicated there might be an issue of lower absorption or palatability with the additional marker, as overall mortality was only 20 percent in Trial 2. Therefore the marker was not added in subsequent trials. In addition, the use of a commercial dog food as a bait matrix may prevent subsequent registration with the EPA, thus the matrix was changed to ground beef for subsequent trials.

With the marker no longer added to the compound, and the bait matrix consisting of ground beef, results from Trial 3 showed 100 percent of the coyotes consumed part or all of the CLOD, but overall mortality (60 percent) was still less than the desired 90 percent threshold (Table 1). Results from Trial 3 indicated that the combination of hamburger and lower methyxanthine concentration produced 100-percent consumption. However, the new dosage was sub-lethal. Therefore in Trial 4, the dosage of the active ingredient was doubled. Trial 4 showed 100 percent of the coyotes fed on some or all of the CLOD, but overall mortality was 60 percent. Poor palatability was assumed to impact consumption. For Trial 5, the active ingredient was micro-

**Table 1. Results of 6 trials involving coyotes being exposed to CLODs containing a theobromine:caffeine mixture.**

| Trial | #test subjects | # (%) of test subjects consuming part or all of the CLOD | (%) of test subjects that # died | Mean time [range] to death (hrs) |
|---|---|---|---|---|
| 1 | 11 | 9 ( 82%) | 7 (64%) | 6.0 [2.0 - 12.0] |
| 2 | 5 | 4 ( 80%) | 1 (20%) | 4.0 |
| 3 | 5 | 5 (100%) | 3 (60%) | 2.2 [2.0 - 2.5] |
| 4 | 5 | 5 (100%) | 3 (60%) | 3.5 [2.0 - 4.5] |
| 5 | 6 | 4 ( 67%) | 1 (17%) | 4.0 |
| 6 | 6 | 6 (100%) | 4 (67%) | 12.5 [2.0 - 24.0] |

encapsulated with a lipid coating in an attempt to increase consumption by blocking the interaction of the bitter methylxanthines and oral taste receptors. Results from Trial 5 indicated that micro-encapsulation did not increase consumption, nor did it increase mortality as overall mortality in Trial 5 was only 17 percent. It was concluded the micro-encapsulation likely reduced absorption in the gut. The lack of meat in the mixture may have increased the motility of the compound through the gut (Kunze and Furness 1999, Olsson and Holmgren 2001), thereby lowering the absorption of the compound. Much of the issue in lower mortality was limited consumption and/or absorption of the theobromine:caffeine mixture by the coyotes. Therefore, for Trial 6 we used the spherical form of the compound in an attempt to increase consumption while not compromising absorption. Results from Trial 6 showed higher overall mortality of 67 percent, but still below the 90 percent level. In addition, the spherical form resulted in the longest average time to death, thus indicating that solubility of the methylxanthines was lower in the oral cavity and in the gut.

Of 18 coyotes in which the time was observed from consumption of the CLOD to death, the time to death varied among the trials and compounds (Figure 2). Few coyotes (28 percent) died in <2 hours, with most of the mortalities taking >2 hours (72 percent).

Coyotes consuming enough of the CLOD content to show signs of toxicosis often showed initial signs of increased excitation and sensitivity to sounds or other stimuli. As part of the increased excitation, coyotes would spend a greater amount of time running around their pen. Coyotes also seemed to experience hypersalivation, as well as polydipsia, as they would increase their consumption of water. A few coyotes were seen vomiting within a few hours of eating the compound. As toxicosis progressed, the coyote would lose coordination and would no longer be able to walk or stand. The coyotes would then lie in a lateral recumbent position with legs, head, and tail outstretched, muscles rigid and respiration elevated. Some coyotes would pedal infrequently with their feet. Though most coyotes died after assuming the laterally recumbent position, one coyote did make a recovery after lying on the ground for several hours.

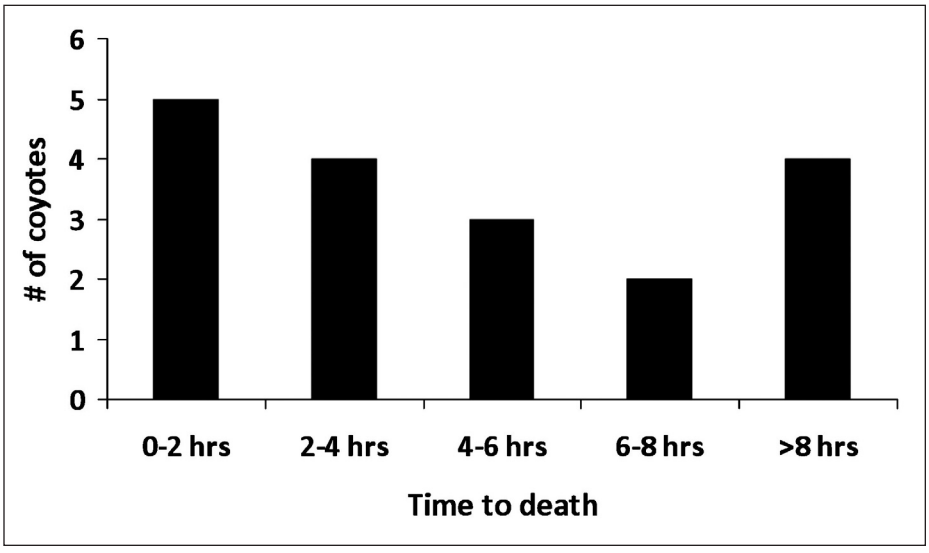
## Conclusions

None of the trials resulted in the 90-percent mortality desired for EPA registration of the theobromine:caffeine mixture as a predacide for coyotes. Reasons for the below-par efficacy are many. In the early trials, many coyotes would not chew on the CLOD, suggesting an issue of attractiveness of the CLOD to precipitate chewing by the coyotes. Subsequent trials incorporated corn syrup to make the CLOD more attractive to consumption. Generally, coyotes desire compounds that contain sugar (Marsh et al. 1982, Mason and McConnell 1997). Another problem was spillage of the mixture from the CLOD after the coyote had bitten the plastic container, thereby preventing complete consumption.

The theobromine:caffeine mixture appeared to have low palatability as the coyotes would often cease consumption once they chewed on the CLOD. Many times the coyotes would bite the CLOD, cease consumption, and the compound would then leak out and spill onto the ground. Therefore, palatability of the compound was in question during the earlier trials, with subsequent trials using either the micro-encapsulated or spherical form of the compound in an attempt to increase palatability. However, these forms appeared to affect absorption of the compound and hence lower morbidity following consumption of the theobromine:caffeine mixture.

Observations of coyotes that survived consuming the contents of the CLOD also indicated other issues in whether the theobromine:caffeine mixture could provide a lethal dosage to the coyote. Some animals would drink copi-

**Figure 2. Frequency of time to death for coyotes exposed to the theobromine:caffeine mixture contained in a Coyote Lure Operative Device.**



ous amounts of water after showing signs of toxicosis and they would survive. Fluid uptake may increase excretion and prevent reabsorption through the urinary bladder, and administering fluids to domestic dogs accidentally ingesting chocolate is recommended as treatment (Farbman 2001). Also, some animals regurgitated the compound and subsequently survived. Coyotes may have recognized the onset of symptoms and induced regurgitation. Because the pH level of the animal stomach contents could greatly influence absorption and uptake of the theobromine:caffeine mixture, insufficient amounts of the mixture may have been absorbed before regurgitation began.

While the time to death is not a standard used for registration, the coyote's time to death in these trials was lengthy and may not be acceptable to the general public (Andelt 1987). Surveys of the general public have repeatedly shown the use of toxicants to be the least favored method for managing predators (Arthur 1981, Andelt 1987, Reiter et al. 1999). However, this long-time period is desirable for it allows for treatment of domestic dogs that may be accidentally dosed. Use of some form of marker that does not interfere with either palatability or absorption of the mixture is desirable to alert pet owners in the event of an accidental dosing and the need for subsequent veterinary treatment of their dog.

It is realized that many of these variables (i.e., access to water, amount of food in the stomach, coyote physiology, and a coyote's ability to regurgitate the compound) are all beyond the control of wildlife managers, particularly in a field setting. However, the use of the theobromine:caffeine mixture in a CLOD, as administered in this experiment, may not be an effective toxicant for managing coyote depredation events. At a minimum, future research needs to be performed that will identify coyote sensory sensitivities, as they appear more than capable of detecting the theobromine:caffeine mixture. The sensory sensitivity of coyotes to bitter-tasting compounds has received limited research (e.g., Mason and McConnell 1997). Equally important is whether the CLOD is the proper delivery device for administering a lethal dose of a toxicant, particularly for coyotes, which are extremely wary of novel objects (Windberg and Knowlton 1990, Windberg 1996, Harris and Knowlton 2001, Séquin et al. 2003).

## Literature Cited


Andelt, W. F. 1987. Coyote predation. Pages 128-140 in M. Novak, J. A. Baker, M. E. Obbard, and B. Malloch. Wild furbearer management and conservation in North America. Ontario Ministry of Natural Resources, Toronto, Ontario, Canada.

Arthur, L. M. 1981. Coyote control: the public response. J. Range Manage. 34:14-15.

Berentsen, A. R., R. H. Schmidt, and R. M. Timm. 2006a. Repeated exposure of coyotes to the Coyote Lure Operative Device. Wildl. Soc. Bull. 34:809-814.

Berentsen, A. R., J. J. Johnston, R. E. Mauldin, and R. H. Schmidt. 2006b. Using the CLOD to deliver pentachlorobenzene to coyotes (*Canis latrans*). Vert. Pest Conf. 22:277-281.

Evans, J., and R. E. Griffith. 1973. A fluorescent tracer and marker for animal studies. J. Wildl. Manage. 37:73-81.

Fagerstone, K. A., J. J. Johnston, and P. J. Savarie. 2004. Predacides for canid management. Sheep Goat Res. J. 19:76-79.

Farbman, D. 2001. Death by chocolate? Methylxanthine toxicosis. Vet. Tech. 3: 146-147.

Gwaltney-Brant, S. 2001. Chocolate intoxication. Vet. Med. 2:108-111.

Harris, C. E., and F. F. Knowlton. 2001. Differential responses of coyotes to novel stimuli in familiar and unfamiliar settings. Can. J. Zool. 79:2005-2013.

Hornfeldt, C. S. 1987. Chocolate toxicity in dogs. Mod. Vet. Practice 68:552-554.

Johnston, J. J. 2005. Evaluation of cocoa- and coffee-derived methylxanthines as toxicants for the control of pest coyotes. J. Agric. Food Chem. 53:4069-4075.

Kunze, W. A. A., and J. B. Furness. 1999. The enteric nervous system and regulation of intestinal motility. Ann. Rev. Physio. 61:117-142.

Knowlton, F. F., E. M. Gese, and M. M. Jaeger. 1999. Coyote depredation control: an interface between biology and management. J. Range Manage. 52:398-412.

Marsh, R. E., W. E. Howard, S. M. McKenna, B. Butler, and D. A. Barnum. 1982. A new system for delivery of predacides or other active ingredients for coyote management. Vert. Pest Conf. 10:229-233.

Mason, J. R., and J. E. McConnell. 1997. Hedonic responsiveness of coyotes to 15 aqueous taste solutions. J. Wildl. Res. 2:21-24.

Olsson, C., and S. Holmgren. 2001. The control of gut motility. Comp. Biochem. Physio. Part A 128:481-503.

Pittenger, S. 2002. Chocolate (methylxanthine) intoxication. Harris County Vet. Med. Assoc. Newsletter. February 2002. 2 pp.

Reiter, D. K., M. W. Brunson, and R. H. Schmidt. 1999. Public attitudes toward wildlife damage management and policy. Wildl. Soc. Bull. 27:746-758.

Séquin, E. S., M. M. Jaeger, P. F. Brussard, and R. H. Barrett. 2003. Wariness of coyotes to camera traps relative to social status and territory boundaries. Can. J. Zool. 81:21015-2025.

USDA. 2000. Sheep and goats predator loss. National Agricultural Statistics Service, Agricultural Statistics Board, Washington, D.C.

USDA. 2011. Wildlife Services' 2011 program data reports. Washington, D.C. <www.aphis.usda.gov/wildlife_damage/prog_data/2011_prog_data/content/wp_c_index.shtml>

USEPA. 1991. Standard Norway rat/roof rat anticoagulant dry bait laboratory test method. OPP Sesignation:1.203. USEPA, Washington D.C., 4 pp.

USEPA. 2012. Reregistration eligibility decision. Washington, D.C. http://www.epa.gov/pesticides/reregistration/status.htm

Windberg, L. A. 1996. Coyote responses to visual and olfactory stimuli related to familiarity with an area. Can. J. Zool. 74:2248-2254.

Windberg, L. A., and F. F. Knowlton. 1990. Relative vulnerability of coyotes to some capture procedures. Wildl. Soc. Bull. 18:282-290.

Sheep & Goat Research Journal
Volume 27, 2012 – December

# Post-Natal Skin Follicle Development in The Raieni Cashmere Goat

*M. Asadi Fozi*[1, 2]

[1] *Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran*
*Corresponding author: email: masadifo@une.edu.au*

## Acknowledgements

The author is grateful to Dr. David Notter, Department of Animal and Poultry Science, Virginia Polytechnic Institute and State University, for providing valuable comments.

## Summary

One hundred eighty (180) skin samples were taken from the mid-right side of 30 Raieni Cashmere goat male and female kids at 1 mo, 1.5 mo, 2 mo, 2.5 mo, 3 mo and 3.5 mo of age. Numbers of primary and secondary skin follicles were determined for each sample. Numbers of secondary follicles increased until 3 mo of age, but numbers of primary follicles did not change after birth of the kids. The skin-follicle traits were not significantly ($P > 0.05$) affected by sex, birth type and age of dam.

**Key Words:** Skin-Follicle Traits, Cashmere Goats, Raieni Cashmere Goats

## Introduction

Animal fibers, such as cashmere, wool and angora grow from small skin structures known as follicles (Carter and Hardy, 1947; Ryder and Stephenson, 1968). Sheep and goats have two different types of skin follicles, primary and secondary follicles, with the latter of major importance to fine wool or cashmere production.

Cashmere goats are said to be "double-coated" with an inner and an outer coat. The inner coat, which is economically important, is formed by fine fiber (cashmere) that is produced by secondary follicles. Primary follicles produce the outer coat or guard hair. Accordingly, the cashmere yield depends upon the ratio of the total number of secondary follicles to the number of primary follicles (Sumner and Bigham, 1993). In Raieni Cashmere goats, secondary (cashmere) and primary (guard hair) fiber diameter is in a range of 12 microns to 22 microns and 35 microns to 55 microns, respectively (Emami Mibodi et al., 1991).

The value of the cashmere product depends on the fiber diameter (Emami Mibodi et al., 1991). Finer cashmere receives higher prices. A favorable genetic correlation has been found between secondary-follicle density and fiber diameter in Merino sheep (-0.65 to -0.68) (Jackson et al., 1975; Mortimer and Atkins, 1993; Purvis and Swan, 1997; Barton et al., 2001 and Asadi Fozi

et al., 2007). Asadi Fozi et al. (2007) indicated that additional response to selection for reduced fiber diameter could be achieved using skin-follicle number, which is moderately heritable, as an additional selection criterion. The same authors reported that the skin traits could potentially be used as an early selection criterion in sheep-breeding programs to improve fleece quality and quantity, whereas usually there is limited information available on wool traits at an early mating age of 7 mo to 8 mo. Although routine measuring of skin-follicle density is not currently practical due to cost, Hill et al. (1997) reported a high genetic correlation between skin-biopsy weight, which is not difficult to measure, and skin-follicle density (-0.74). Therefore skin-follicle number could potentially be predicted from skin-biopsy weight and skin-surface area. The effectiveness of this option depends on the heritability of predicted skin-follicle number and its correlations with objective traits.

Previous studies showed that the initiation and development of the secondary follicles may be affected by environmental factors, such as nutrition, during the time that the follicles are initiated (Doney and Smith, 1964; Millar 1986; Ryder and Stephenson, 1968; Sun et al., 1992). To estimate the number of secondary follicles accurately at an early age for selection purposes and to improve their initiation and development using environmental factors, the time (animal age) when development of the secondary follicle population is completed should be studied.

In sheep and goats, primary follicles are formed first in the fetus, and secondary follicles are formed later (Ryder and Stephenson, 1968). In Australian Cashmere goats, all primary follicles were initiated at birth but secondary follicles continued to develop until 3.5 mo after birth, when skin samples were taken at birth and 2 mo, 3.5 mo and 10 mo after birth (Parry et al., 1992). Asadi Fozi and Pousti (2000) reported that primary and secondary skin-follicle numbers measured at 3 mo and 1 yr, 2 yr and 3 yr of age of Raieni Cashmere goats were not significantly different.

With a population of 2.5 million, the Raieni Cashmere goat is one of the most famous cashmere breeds, raised mostly in the Kerman province of Iran. The breed is used to produce meat, cashmere and milk. These products make significant contributions to the regional agricultural economy (Mohebbi Nejad et al., 2010). The aim of this study was to investigate the skin follicle development more precisely after birth until 3.5 mo of age in Raieni Cashmere goats, and to study effects of sex, birth type and age of dam on the skin-follicle traits.

## Materials and Methods

Skin samples (n = 180) were taken from the mid-right side of 30 Raieni Cashmere goat kids at 1 mo, 1.5 mo, 2 mo, 2.5 mo, 3 mo and 3.5 mo of age, and live weights were determined at each sampling time. The animals were selected randomly from a research flock of Raieni Cashmere goats (n = 30) in Kerman, Iran. Skin-follicle density was defined as the number of skin follicles per $mm^2$ of biopsied skin and was measured using histological techniques described by Carter (1943) and Carter and Clarke (1957).

Based on previous studies, primary follicles can be distinguished from secondary follicles by their accessory structures, such as sweat glands, sebaceous glands and erector pili muscle (Ryder and Stephenson, 1968). Accordingly, primary follicle density (PFD, primary follicles/$mm^2$) and secondary follicle density (SFD, secondary follicles/$mm^2$) were measured.

An inverse relationship between follicle density and body weight in goats has been reported, and the use of follicle densities in studies on growing animals is likely to have limited value (Parry et al., 1992). However, the follicle-number index can be used to remove the effect of surface area on follicle density. The index was estimated as the product of the skin-follicle density and skin-surface area (Parry et al., 1992). Skin-surface area can be approximated using 0.09 x (Body weight) $^{0.67}$ (Freer et al., 1997). Therefore the secondary follicle number index (SFNI) was calculated as the product of SFD and approximate skin surface area (ASSA) and primary follicle number index (PFNI) was the product of PFD and ASSA.

### Statistical Analysis

Dam age (with 5 levels), birth type (single and twin), kid sex (male and female) and kid age (6 levels) were the fixed effects tested for their significance. As the data were repeated measures, with records on the same animals at the six different ages, the animals' permanent environmental effect was included

**Figure 1. Transverse section of skin follicles in Raieni Cashmere goat (400X).**

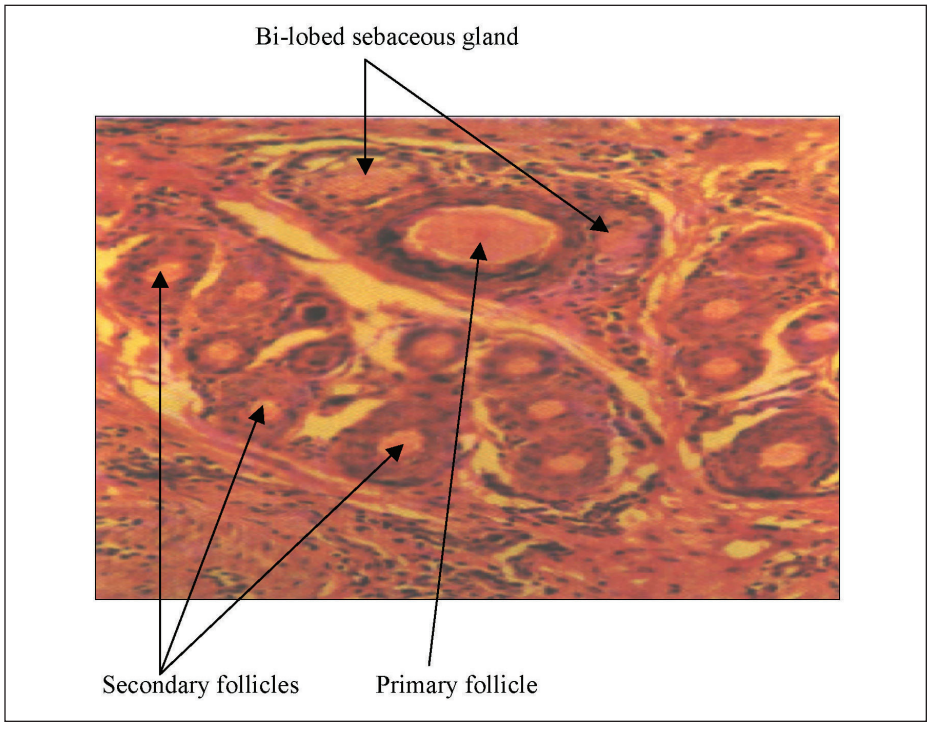
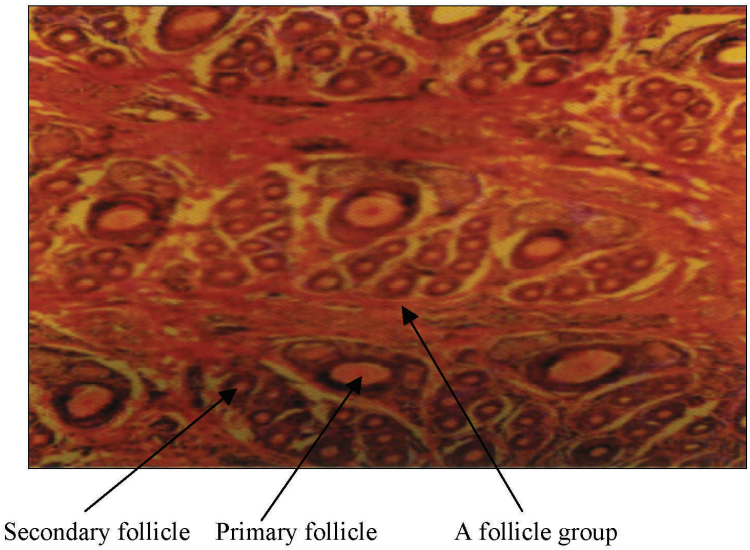


**Figure 2. Transverse section of skin follicles and follicle groups in Raieni Cashmere goat (125X).**




as a random effect and used to test fixed effects of dam age, birth type, sex and age. The data were analysed using a generalized linear model (Gilmour et al., 2006). The complete model was as follows:

$$y_{ijklmn} = \mu + \alpha_i + \beta_j + \sigma_k + \gamma_l + \theta_m + e_{ijklmn}$$

where $y_{ijklmn}$ is an individual measurement of the skin-follicle traits, including SDF, PFD, SFNI and PFNI and live weights; $\mu$ is the population mean; $\alpha_i$ is the $i^{th}$ age; $\beta_j$ is the $j^{th}$ sex; $\sigma_k$ is the $k^{th}$ birth type; $\gamma_l$ is the $l^{th}$ age of dam; $\theta_m$ is the $m^{th}$ animal permanent environmental effect and $e_{ijklmn}$ is the residual.

## Results and Discussion

Based on transverse sections of the skin from Raieni Cashmere goats, two different types of skin follicles, primary and secondary, were found. Primary follicles were larger than secondary follicles. They were associated with a sweat gland, and often with bi-lobed, sebaceous glands and an erector pili muscle. Secondary follicles were much more numerous than primary follicles and associated only with a mono-lobed, sebaceous gland (Figure 1). The skin follicles were arranged within skin-follicle groups. Primary follicles were arranged in rows in trio groups and secondary follicles were lying to one side of the primaries (Figure 2). These results were similar to those reported by Ryder and Stephenson (1968), Parry et al. (1992) and Yongjun et al. (1994).

**Table 1. Least square means and standard errors (in parentheses) of fixed effects for PFD (Primary follicles/mm$^2$), SFD (Secondary follicles/mm$^2$), S/P (Ratio of SFD to PFD), PFNI (PFD x ASSA) SFNI (SFD x ASSA) and live weight (LW).**

| Effects | PFD | SFD | PFNI | SFNI | LW |
|---|---|---|---|---|---|
| Age (mo): | | | | | |
| 1.0 | 4.57 (0.07)$^a$ | 45 (1.05)$^a$ | 16.3 (1.05)$^a$ | 162 (1.05)$^a$ | 6.2$^a$ |
| 1.5 | 3.67 (0.07)$^b$ | 43 (1.05)$^a$ | 16.2 (1.05)$^a$ | 171 (1.05)$^b$ | 7.5$^b$ |
| 2.0 | 3.41 (0.07)$^c$ | 43 (1.05)$^a$ | 16.2 (1.05)$^a$ | 191 (1.06)$^c$ | 8.5$^c$ |
| 2.5 | 3.38 (0.07)$^{cd}$ | 44 (1.05)$^a$ | 16.2 (1.05)$^a$ | 209 (1.06)$^d$ | 9.2$^{cd}$ |
| 3.0 | 3.28 (0.07)$^d$ | 44 (1.06)$^a$ | 16.8 (1.06)$^a$ | 228 (1.06)$^e$ | 10.0$^d$ |
| 3.5 | 2.48 (0.07)$^e$ | 34 (1.06)$^b$ | 16.3 (1.06)$^a$ | 225 (1.06)$^e$ | 14.5$^e$ |

Ages with different superscripts$^{(a,b,c,d,e)}$ within each column are significantly different.

### Secondary Follicle Development

No significant ($P > 0.05$) differences were found for secondary follicle density (SFD), when animal age increased from 1 mo to 3 mo of age. Changes in the skin-follicle density depend on changes in skin follicle numbers and skin surface area. The skin surface area of the animals increased with age in accord with the observed increases in live weights (Table 1, Figure 3 and Figure 4). No significant changes in SFD occurred from 1 mo to 3 mo of age despite the skin observed increase in surface area, demonstrating that the total number of secondary follicles increased from birth until 3 mo of age.

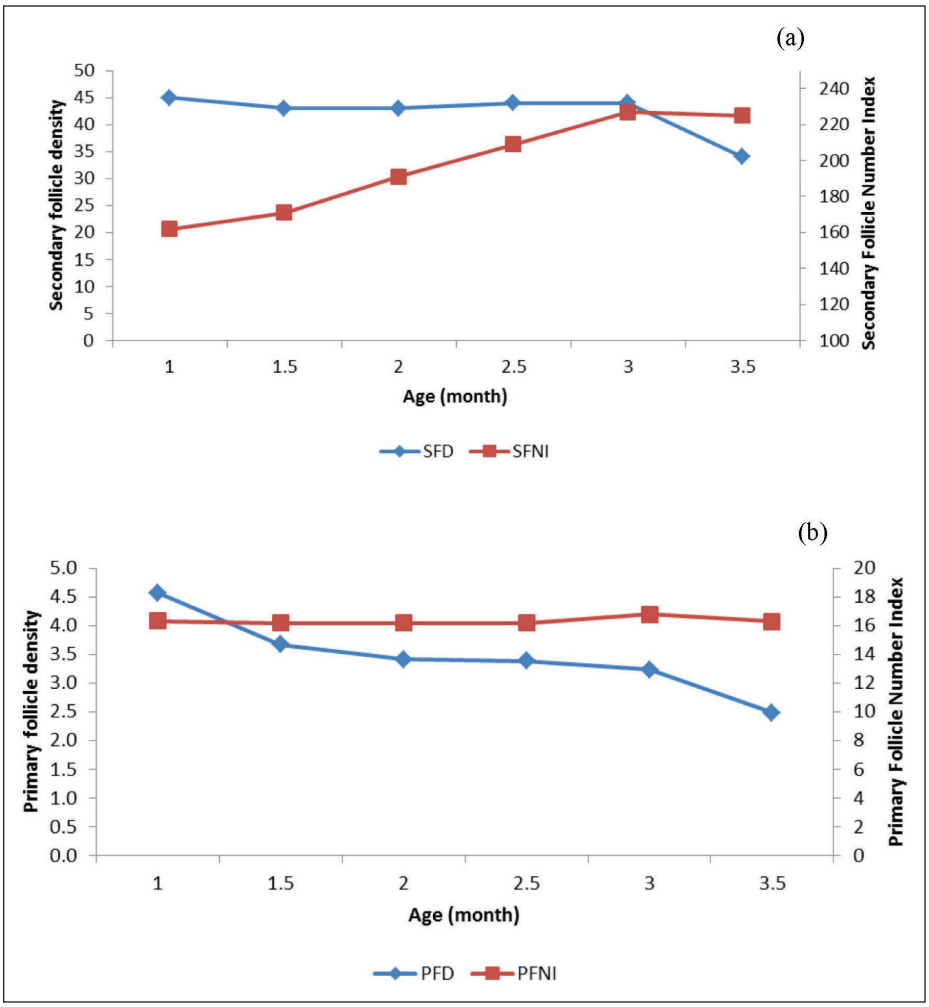
The SFD decreased significantly between 3 mo and 3.5 mo of age, indicating that the secondary follicle population was no longer increasing after 3 mo of age, even though the skin surface-area of the animals continued to increase due to increases in their body weight (Table 1, Figure 3 and Figure 4). Parry et al. (1992) found that SFD decreased after 3.5 mo of age in Australian cashmere kids.

The secondary follicle number index (SFNI), which is an index of total secondary follicle number, increased from birth until 3 mo of age, but did not change significantly between 3 mo and 3.5 mo of age (Table 1, Figure 2 and Figure 3). In other words, post-natal initiation and development of the secondary follicles continued until 3 mo of age. Based on the results derived from SFD and SFNI, it can be concluded that the secondary follicle population is completed at 3 mo of age in Raieni Cashmere goats.

### Primary Follicle Development

Primary follicle density (PFD) decreased significantly after birth as age increased and body weight increased significantly (Table 1, Figure 3 and Figure 4). Thus the primary follicle number did not change after birth, even though the skin surface area was increased. In addition, the primary follicle number index (PFNI) did not change after birth (Table 1, Figure 3 and Figure 4). These results show that all primary follicles had been

**Figure 3. Density of secondary (SFD, a) and primary (PFD, b) follicles and number index of secondary (SFNI, a) and primary (PFNI, b) follicles measured at six different ages of Raieni Cashmere goats.**




initiated when the Raieni Cashmere goat kids are born. This is in agreement with results found by Parry et al. (1992).

In the current study the effects of sex, birth type and age of dam on the skin follicle traits and live weights were investigated. None of the traits were significantly ($P > 0.05$) affected by these factors. Parry et al. (1992) showed that male Australian cashmere kids have more secondary and primary follicles than the female kids at both 3.5 mo and 10 mo of age. Also, they found significant differences between single and twin kids; singles had more secondary follicles than twins at 2 mo, 3.5 mo and 10 mo of age.

## Conclusions

In Raieni Cashmere goats secondary and primary skin follicles, respectively, produce cashmere and guard hair. The results of this study showed that the secondary follicles continued to develop until 3 mo of age. To increase the secondary follicle numbers, the environmental factors (those having effects on the skin-follicle initiation, such as nutrition) should be optimized while the follicles are being initiated i. e., until 3 mo after birth.

The number of secondary follicles can be accurately measured at 3 mo of age and can potentially be used as an early selection criterion to facilitate early age mating in Cashmere goat programs when limited information is available on the fleece traits.

## Literature Cited


Asadi Fozi, M., J. H. J. Van der Werf, and A. A. Swan. 2007. Inclusion of skin follicle traits in selection indices in breeding programs improves genetic gain in Australian fine-wool Merinos. Aust. J Agric. Res., 58: 921–927.

Asadi Fozi, M., and I. Pousti. 2000. Skin follicle characteristics of Raieni Cashmere goats. J. Fac. Vet. Med. Univ. of Tehran, 55: 42-49.

Barton, S. A., I. W. Purvis, and H. G. Brewer. 2001. Are wool follicle characteristics associated with wool quality and production in hogget and adult sheep? Proc. AAABG., 14: 289-292.

Carter, H. B., and M. H. Hardy. 1947. Studies in the biology of the skin and fleece of sheep. 4. The hair follicle group and its topographical variations in the skin of the Merino foetus. Bull. Coun. Sci. Ind. Res., Melbourne, No. 215.

Carter, H. B., and W. H. Clarke. 1957. The hair follicle group and skin follicle population of Australian Merino sheep. Aust. J. Agric. Res., 8: 91-108.

Carter, H. B. 1943. Studies in the biology of the skin and fleece of sheep. I. The development and general histology of the follicle group in the skin of the Merino. Bull. Coun. Sci. Ind. Res., Melbourne, No. 164.

Doney, J. M., and W. F. Smith. 1964. Modification of fleece development in Blackface sheep by variation in pre- and post – natal nutrition, Anim. Prod., 6: 155-167.

Emami Mibodi, M. A., F. Eftekhar Sahroodi, and A. Nikkhah.1991. Estimation of genetic parameters of some economic traits in Raieni Cashmere goats. M. Sc Thesis, Ferdosi University of Mashhad, Mashhad, Iran.

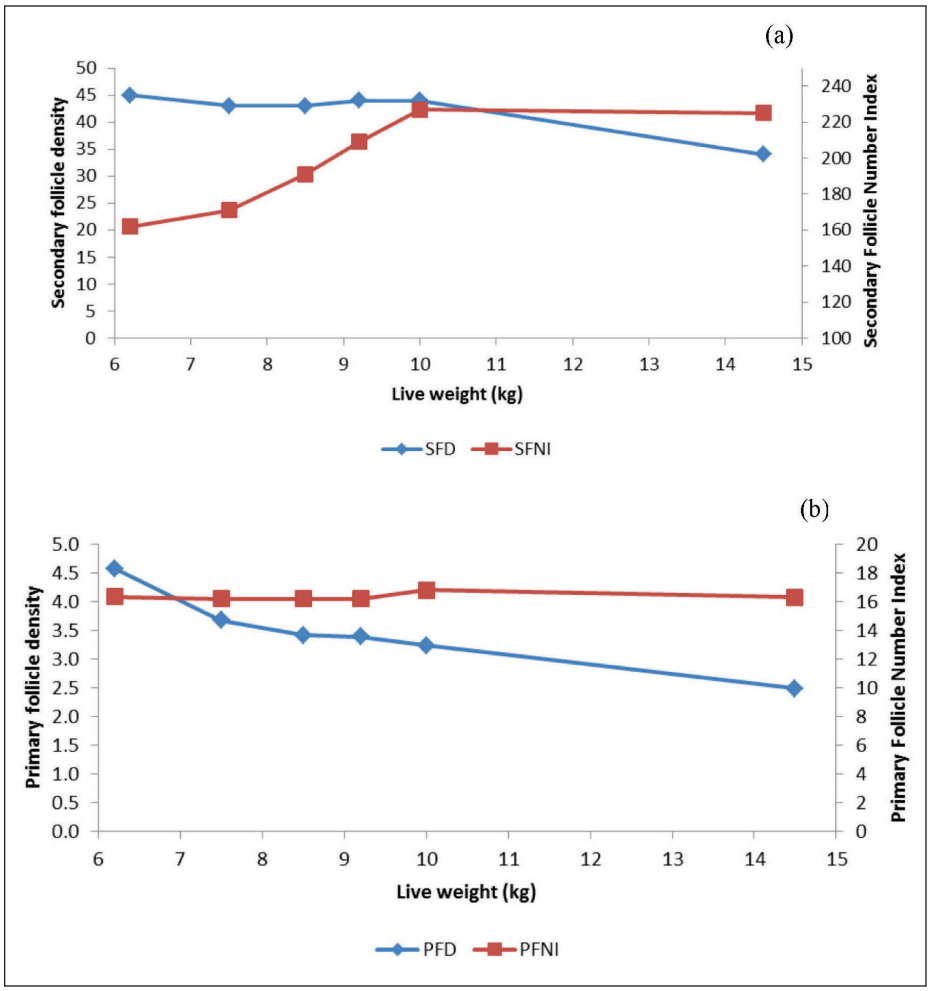
Freer, M., A. D. Moore, and J. R. Donnelly. 1997. GRAZPLAN decision support systems for Australian grazing enterprises-II. The animal biology model for feed intake, production and reproduction and the GrazFeed DSS. Agr. Syst., 54: 77-126.

Gilmour A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, R. Thompson. 2006. ASReml user guide. Release 2.0. (VSN International Ltd.: Hemel Hempstead, UK)

Hill, J. A., P. L. Hynd, R. W. Ponzoni, R. J. Grimson, K. S. Jaensch, R. V. Kenyon, and N. M. Penno. 1997.

**Figure 4. Density of secondary (SFD, a) and primary (PFD, b) follicles and number index of secondary (SFNI, a) and primary (PFNI, b) follicles versus the live weights measured at six different ages of Raieni Cashmere goats.**





Skin and follicle characters I. Heritabilities and correlations among them. Proc. AAABG., 12: 520-523.

Jackson, N., T. Nay, and H. N. Turner. 1975. Response to selection in Australian Merino sheep.VII. Phenotypic and genetic parameters for some wool follicle characteristics and their correlation with wool body traits. Aust. J. Agric. Res., 26: 937-57.

Millar, P. 1986. The performance of cashmere goats. Anim. Breed. Abst., 54: 181-199.

Mohebbi Nejad, E., M. Asadi Fozi, and M. Mohamad Abadi. 2010. Genetic analyses for fleece weight in Raieni Cashmere goats using multivariate model. M. Sc Thesis, Bahonar University of Kerman, Kerman, Iran.

Mortimer, S. I., and K. D. Atkins. 1993. Genetic evaluation traits between and within flocks of Merino sheep. II. Component traits of the hogget fleece. Aust. J. Agric. Res., 44: 1523-1539.

Parry, A. L., B. W. Norton, and B. J. Restall. 1992. Skin follicle development in the Australian cashmere goat. Aust. J. Agric. Res., 43: 857-870.

Purvis, I. W., and A. A. Swan. 1997. Can follicle density be used to enhance the rate of genetic improvement in Merino flocks? Proc. AAABG., 12: 512-515.

Ryder, M. L., and S. K. Stephenson. 1968. Wool Growth, London, Academic Press, 202-247.

Sumner, R. M. W. and Bigham, M. L. (1993) Biology of fiber growth and possible genetic and non-genetic means of influencing fiber growth in sheep and goats-a review. Livest. Prod. Sci., 33: 1-29.

Sun, Y. X., A. Michel, G. A. Wickham, and S. N. McCutcheon. 1992. Wool follicle development, wool growth and body growth in lambs treated from birth with recombinantly derived bovine somatotropin. Anim. Prod., 55: 73-78.

Yongjun, L. 1994 Study on development of skin follicle in the Liaoning Cashmere goat. J. Jilin. Agri. Univ., 13:118.

Sheep & Goat Research Journal
Volume 27, 2012; Special Research Symposium Papers

# Research Symposium Utilization of Genomic Information for the Sheep Industry

## Introduction

*Larry R. Miller*

*Retired from the Cooperative State Research, Education, and Extension Service, USDA*
*Centreville, Maryland 21617, email larryrmiller@atlanticbb.net*

During the ASI Annual Convention in Scottsdale, Ariz., January 26, 2012, a research symposium ***Utilization of Genomic Information for the Sheep Industry*** was co-sponsored by the American Sheep Industry Association (ASI) and the American Sheep and Goat Center (ASGC). The Symposium Program Planning Committee consisted of Paul Rodgers, ASI; Will R. Getz, Fort Valley State University; and Larry R. Miller, ASGC, who also served as Moderator. The symposium was somewhat different from previous ASI research symposia, in that it more comprehensively focused on a single topic, involved speakers from different perspectives and engaged participants in more in-depth discussion.

Especially in the past two decades, volumes of new genomic and genetic information have been generated by means of new research approaches, techniques, and tools. This information created a challenge to harness, interpret, and utilize the wealth of new genomic/genetic information by drawing upon disciplines, such as biochemistry, genetics, statistics, computer science, animal breeding, and several other sciences associated with the biology of the animal.

The speakers addressed the symposium topic from the following points of view, reflecting their different expertise and experiences: *Genomic Information Available for Use by the Sheep Industry*, Noelle E. Cockett, Utah State University; *Application of Genomic Information for Improvement of Quantitative Traits*, David R. Notter, Virginia Tech University; *Utilization and Potential of Estimates of Genetic Value from an Industry Perspective*, David L. Thomas, University of Wisconsin-Madison; *Utilization from a Producer Perspective*, Chase T. Hibbard, sheep producer, Helena, Mont.; and *Genetic Selection Specifically Utilized for Evaluating the Introduction of Outside Breeds and Measuring Their Potential*, John Helle, sheep producer, Dillon, Mont.

Sheep & Goat Research Journal
Volume 27, 2012; Special Research Symposium Papers

# Genomic Information Available for Use by the Sheep Industry

*Noelle E. Cockett[1,2] and Chunhua Wu[1]*

[1]*Utah State University, 4900 Old Main Hill, Logan UT 84322-4900*
[2]*Corresponding author: phone 435-797-2201; fax 435-797-3268; email noelle.cockett@usu.edu*

## Summary

Sheep contribute significantly to food and fiber production across the world through locally and globally distributed meat, milk, and wool markets. In addition, sheep are used in biomedical research as a model organism for heart, lung, and musculoskeletal diseases. A better understanding of the genetic makeup of sheep will lead to improvements in the efficiency of meat, milk, and wool production and contribute to a better understanding of health and disease issues in humans. While several genetic regions associated with economically and biologically important traits in sheep have been identified, the number of known causative mutations is relatively small. However, the development and application of a high-density ovine SNP array and the availability of the whole genome assembly for sheep will undoubtedly lead to more discoveries. It should be noted that the application of genetic markers for selection of quantitative traits in sheep is still in the distant future.

**Key Words**: Sheep, Genomics, Genome-wide Association Study, SNPs

## Introduction

During the past twenty years, several traits in sheep have been mapped to specific regions in the sheep genome using a genetic-linkage analysis, which involves tracing the segregation of marker alleles through pedigrees of animals with known phenotypes. These linkage-analysis studies usually result in very large genetic regions, which contain hundreds of genes, being associated with one or more traits. To dissect these large intervals and ultimately narrow down the region of interest, additional experiments are required. The experimental steps are usually directed towards a higher resolution of the location of the mutation in the genetic region, followed by DNA sequencing of animals differing in performance for the trait of interest. While some causative mutations have been identified utilizing these steps, other genomic regions of suggested importance have not been explored. The cost of these experiences escalates with the complexity of the trait, requiring increasing numbers of animals that are characterized for the trait of interest.

Since the early 1990s, linkage analysis has been used to identify genomic regions that contain causative mutations for several binary traits, including the Booroola fecundity gene (Montgomery et al. 1993), callipyge (Cockett et al. 1994), Spider Lamb Syndrome (Cockett et al. 1999) and muscularity in Texel sheep (Clop et al. 2006). These regions were sequenced in animals within the study populations to eventually determine the causative mutation for the trait. In contrast, only a few mutations that are causative for quantitative traits in sheep have been identified to date. As discussed in a companion paper (Notter, SGRJ, Research Symposium 2012), quantitative traits are more challenging than single gene traits because a large number of genes are involved and the manifestation of the trait is dependent on the animal's environment. In addition, thousands of animals within large pedigrees are needed in the linkage analyses of quantitative traits and many researchers do not have access to those animal numbers. Almost all of the causative mutations for quantitative traits discovered to date have had a major influence on the trait, such as the myostatin (GDF8) gene affecting muscle depth in Charollais sheep (Hadjipavlou et al. 2008) and the calpain and calpastatin genes involved in meat tenderness (Knight et al. 2012). Mutations with minor effects on quantitative traits have been much more elusive, most likely because of the difficulty in detecting differences, given the number of animals and genetic makeup of the study populations. In addition, genetic regions associated with a quantitative trait have differed between studies when different breeds or genetic lines are used in the analyses. For example, recent publications that report genetic regions influencing parasite resistance in sheep (Dominik et al. 2010; Marshall et al. 2009; Matika et al. 2011; Silva et al. 2012) report a total of 10 highly significant QTL on six autosomal chromosomes, with only one genomic region that was identified in more than one study (Dominik et al. 2010; Silva et al. 2012).

## Genome-wide association studies (GWAS)

While it is possible to localize the genetic region associated with a trait using a genetic-linkage analysis, an alternative approach developed in the last few years has been used extensively by sheep researchers. The genome-wide association study (GWAS) is a method that tests for differences in the frequencies of alleles or genotypes between groups of animals or people who are distinctly different for a trait, disease status, production value, etc. No pedigrees are required for GWAS; instead, associations between genetic marker alleles or genotypes and the trait of interest are analyzed in large samples of unrelated individuals. Assuming that the trait allele of interest has descended from one or a few ancestors (so that the "ancestral" segment of DNA contains the trait allele because of linkage disequilibrium), a GWAS analysis will reveal influential genetic regions.

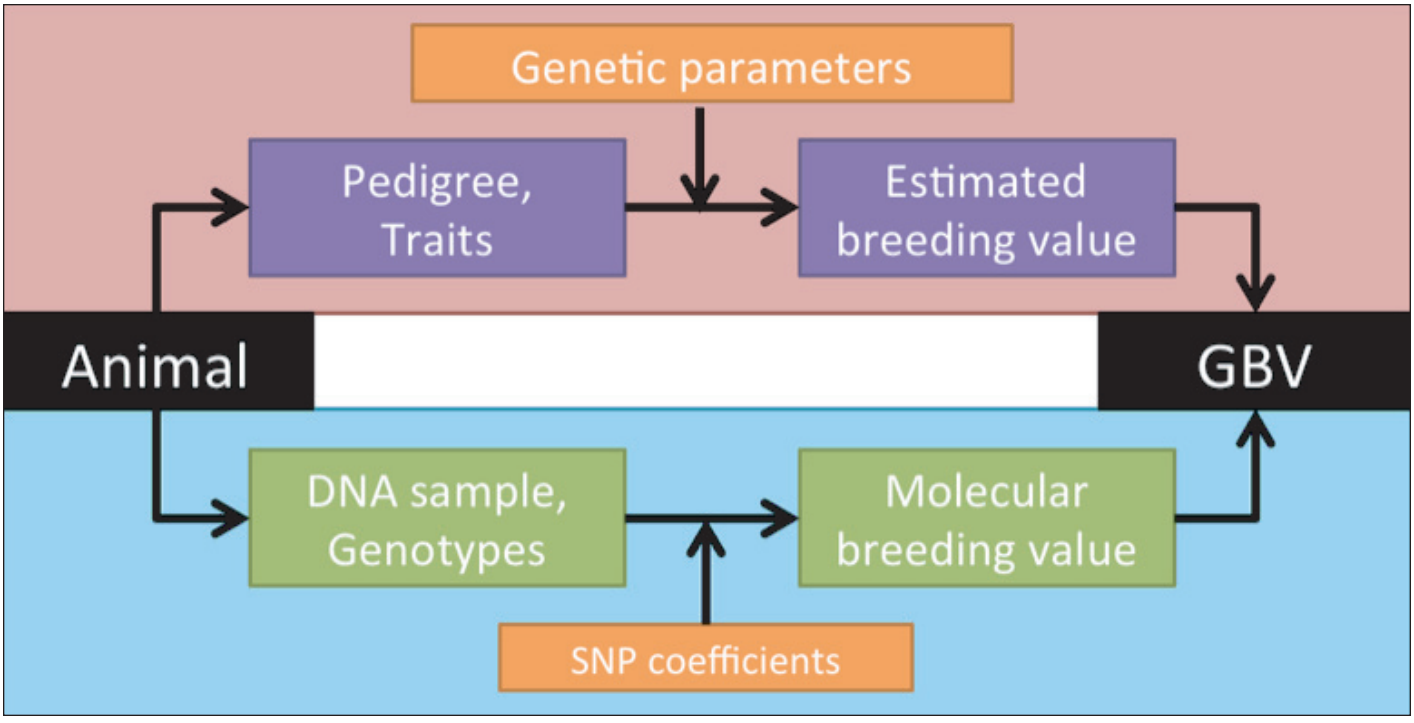
The detection of genetic regions containing the trait allele using the GWAS approach is possible because of "selective sweeps", which are regions that have allele frequency differences because of historical selection for or against the trait (Sabeti et al. 2006). An important aspect of the selective-sweeps analysis is to include genetic-marker genotypes for several closely related species (such as the thin horn Ovis dalli for sheep) in order to determine the ancestral allele of the genetic markers (called SNPs). If multiple breeds are included in the study, the data also can be used to explore the degree of diversity between and within breeds, and make more informed choices about how to best manage and conserve genetic resources. Genetic markers identified in a genome-wide, association analysis must be verified in additional populations, as described by Notter (2012). Verification is necessary because of the likelihood of spurious associations when testing large numbers of SNPs for significance, such as the recently released ovine SNP50 BeadChip, which contains more than 50,000 SNPs. In addition to verification of significant associations, the significance values are adjusted for multiple comparisons when the genotypes from high-density, SNP chips, such as the ovine BeadChip, are analyzed.

Discovery of the causative mutation requires additional steps (Ron and Weller, 2007), such as the physiological manifestation of the trait in transgenic animals that carry the mutation. However, production of transgenic animals for confirmation of the mutation is expensive. Also, appropriate control of the transgene does not always occur, so absence of the trait in the resulting animals does not necessarily mean the mutation is not causative, only that the transgenic animals do not display the trait.

Genetic markers that have been verified can then be incorporated into the calculation of molecular breeding values (MBV) for individual animals. The MBV can be combined with estimated breeding values (EBV) into what is referred to as a genetic breeding value (Figure 1). This approach, which combines the underlying genetics of a trait with pedigree information and the animal's own trait measurement, will lead to more informed genetic-selection decisions.

Some progress has been made in identifying genetic markers for disease traits using a GWAS approach. A recent publication (Heaton et al. 2012) reports a risk factor of 2.75 for the incidence of ovine progressive pneumonia (OPP) in sheep carrying at least one of three mutations in the TMEM154 gene, located on chromosome 17 (OAR17) in sheep. These investigators collected genotypes from the Ovine SNP50 BeadChip from 69 animals that had serum OPPV antibodies (considered a reliable measure of OPPV infection) and 69 con-

**Figure 1. Information that is used to produce a genetic breeding value (GBV). (John McEwan, personal communication)**



trol animals matched for breed composition, exposure, and environmental factors (sex and age), and all animals were from a research flock maintained at the USDA, ARS Meat Animal Research Center in Clay Center, Neb. Analysis of the data associated a specific gene (TMEM154) with OPP infection. Heaton and his colleagues pointed out that the matched control design using older sheep (5 years old to 9 years old) was "key in reducing variation in the management conditions, environment, breed composition, and pathogen exposure." They said that "the use of older sheep increased the chances that sufficient natural exposure had occurred so that a high proportion of susceptible individuals could become infected". They also noted that "the inclusion of different breed compositions increased the likelihood that the association observed in the 69 matched pairs was not limited to one breed".

While no form of the TMEM154 gene is known to be resistant to OPP-virus infection, the ability to identify animals with an increased risk of infection should lead to improved management in sheep flocks. This same approach for designated management of animals with specific genetic markers could be applied to production and carcass traits. Some success has occurred in using genetic-marker panels to assign beef cattle to specific feeding regimens in feedlots in order to reach a certain carcass endpoint (B. Woodward, Merial Limited, personal communication).

## Genomic Resources in Sheep

The availability of a high-density, SNP array specific for the sheep has dramatically impacted the search for important genomic regions using the GWAS analysis. The Illumina Ovine SNP50 BeadChip *(http://www.illumina.com/documents//products/datasheets/datasheet_ovinesnp50.pdf)* was developed by the International Sheep Genomics Consortium and released to the public in January, 2009. Researchers can now obtain genotypes for over 50,000 SNPs for hundreds of animals in a single analysis.

A large, international-sequencing effort was undertaken to generate the information needed for development of the SNP50 chip. The first source of sequence data (9.7 Gbp) was generated from six sheep breeds (Romney, Texel, Merino, Dorset, Rambouillet and Suffolk) using funding from the International Science Linkage Program (Australia) and Ovita (New Zealand). The second source of sequence data used for SNP mining (3 Gbp) was generated from a pool of DNA comprised of 60 genetically divergent animals. These sequences were compared to identify single nucleotide differences (i.e. SNPs) that differed in at least 5 percent of the sequenced animals.

To date, at least 10,000 sheep have been genotyped with the SNP50 chip, and analyses of the genotypes are ongoing in a myriad of research projects across the world. For example, SNP genotypes of 3064 sheep from 64 breeds have been combined with genotypic data from seven species of wild sheep and nine outgroup species as part of the world-wide ovine HapMap project *(http://www.sheephapmap.org/)*. Results from the HapMap analysis indicate that domestic breeds diverged from their wild ancestors about 11,000 years ago, and modern breeds started to differentiate around 200 years ago (Kijas et al. 2009). Although not unexpected, the data also indicated that American breeds are most closely related to European and Middle Eastern breeds rather than to Asian or African breeds.

Another important genomic resource that is now available for sheep is the whole-genome-reference assembly (Dalrymple et al. 2007). The assembly is a compilation of all known information about the sheep genome into a searchable database that is publicly available on the internet on a site maintained by CSIRO Livestock *(http://www.livestockgenomics.csiro.au/sheep/oar2.0.php)*. The assembly contains a myriad of details, including the location of genes and genetic markers from the linkage (Maddox et al. 2001), radiation hybrid (Goldammer et al. 2009; Wu et al. 2008, 2009) and physical (Goldammer et al. 2009) maps, and the location of SNPs from the Illumina chips. Improvement and additions to the assembly are discussed and approved continually by the ISGC, whereas updates are released publicly about every 12 months. The currently available version (Oarv2.0) was released in February, 2011, and an updated version (Oarv3.) is scheduled for release in Summer, 2012 (Y. Jiang, CSIRO Livestock Industries, personal communication). Full annotation of the genes within the genome (Oar v3.0) will be done by NCBI (B. Dalrymple, CSIRO Livestock Industries, personal communication).

A key component of the genome assembly is the reference genome sequence (Jiang et al. 2011), which is the linear order of DNA nucleotides found in the sheep genome. The assembled reference sequence contains approximately 2,710,000,000 of ordered nucleotides assigned to specific chromosomes and covers approximately 92 percent of the ovine genome. Sequence data for the whole-genome-reference sequence were generated at two sequencing facilities (Beijing Genomics Institute and the Roslin Institute) from DNA of a Texel ewe and a Texel ram, respectively. The first step in assembling the reference sequence involved de novo assembly of 75X reads from the Texel ewe into contigs, scaffolds and super-scaffolds. Once that was completed, sequences from both animals were used for gap filling. Information from the sheep linkage, radiation hybrid and physical maps has been used to refine the assembly of the reference sequence. In order to define the expressed portion of the genome, mRNA-seq was performed on seven tissue samples (heart, liver, ovary, kidney, brain, lung, and white fat) of the Texel ewe. This information is being used for annotation of genes within the ovine genome. In addition to the reference assembly, about 5 million SNPs were identified in separate analyses of the male and female Texel sequences.

The whole-genome assembly for sheep will significantly accelerate searches for genetic regions and genes influencing phenotypes in sheep. The assembly also will provide a backbone for the interpretation of low-pass sequences of individual animals expected within the next few years. Without a reference assembly, identification of differences among animals in genome organization, including rearrangements and duplications, will be difficult to interpret.

## Considerations for Genomic Research in Sheep

The availability of a high-density, SNP chip for sheep has revolutionized researchers' ability to locate genetic

regions influencing economically traits. The number of animals needed for identifying a mutation responsible for a single-gene trait has been reduced from more than 100 (with linkage analyses) to 5 to 13 affected animals along with a similar number of controls. Hundreds of animals are still needed for identifying regions containing quantitative trait loci (QTL) but the time to test markers across a population is reduced from years to just a few months. The cost-per-genetic marker is reduced greatly with the SNP chip, but given the number of animals that are needed for QTL discovery, the cost of a QTL search may still be prohibitive, particularly for lowly heritable traits because more animals are needed in the study. A genotype-imputation approach (Hayes et al. 2011) can reduce the cost because a small select group of animals is genotyped with the more expensive high-density chip (50,000 SNPs), while the bulk of the animals are genotyped with a cheaper lower density chip (> 5,000 SNPs). The accuracy of imputation is dependent on the amount of genetic diversity in a breed, with lower-genetic diversity resulting in more accurate imputation. After the identification of significant SNPs, the DNA of key individuals could be sequenced and compared to key ancestors within the breed to reveal interesting mutations associated with the analyzed trait.

The volume of data generated within the high-density chip (50,000 genotypes per animal) can be an issue, requiring increased computer capacity and new analysis methods. However, the density of markers that are tested on the high-density, SNP chip means that the genetic regions identified through GWAS are typically of higher resolution than those identified in linkage studies. This higher resolution helps limit the size of the region in which one needs to search for the causative gene or mutation, leading to a quicker turn-around in the identification and characterization of genomic regions. However, the discovery of genetic markers suitable for genetic selection of quantitative traits is still elusive. At this point in time, there are only a few sheep populations worldwide with appropriate numbers (in the thousands) and appropriate trait measurements. In addition, the genetic markers identified in these populations must be tested in other populations to determine the utility of the markers across genetic lines.

## Literature Cited


Clop, A., F. Marcq, H. Takeda, D. Pirottin, X. Tordoir, B. Bibe, J. Bouix, F. Caiment, J.M. Elsen, F. Eychenne, C. Larzul, E. Laville, F. Meish, D. Milenkovic, J. Tobin, C. Charlier, M. Georges (2006). A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat Genet 38: 813-8.

Cockett, N.E., S.P. Jackson, T.L. Shay, D. Nielsen, M.R. Steele, R.D. Green, M. Georges (1994). Chromosomal localization of the callipyge gene in sheep (Ovis aries) using bovine DNA markers. Proc Natl Acad Sci USA 91: 3019-23.

Cockett, N.E., T.L. Shay, J.E. Beever, D. Nielsen, J. Albretsen, M. Georges, K. Peterson, A. Stephens, W. Vernon, O. Timofeevskaia, S. South, J. Mork, A. Maciulis, T.D. Bunch (1999). Localization of the locus causing Spider Lamb Syndrome to the distal end of ovine Chromosome 6. Mamm Genome 10: 35-8.

Dalrymple, B.P., E.F. Kirkness, M. Nefedov, S. McWilliam, A. Ratnakumar, W. Barris, S. Zhao, J. Shetty, J.F. Maddox, M. O'Grady, F. Nicholas, A.M. Crawford, T. Smith, P. de Jong, J. McEwan, H. Oddy, N.E. Cockett (2007). Constructing the virtual sheep genome. Genome Research 8:R152 (doi:10.1186/gb-2007-8-7-r152).

Dominik, S. , P.W. Hunt, J. McNally, A. Murrell, A. Hall, I.W. Purvis(2010). Detection of quantitative trait loci for internal parasite resistance in sheep. I. Linkage analysis in a Romney x Merino sheep backcross population. Parasitology 137:1275-82.

Goldammer, T., R.M. Brunner, A. Rebl, C.H. Wu, K. Nomura, T. Hadfield, C. Gill, D. Dalrymple, J.E. Womack, N.E. Cockett (2009). A high-resolution radiation hybrid map of sheep chromosome X and comparison with human and cattle. Cytogenet. Genome Res. 125: 40-5.

Goldammer, T., R.M. Brunner, A. Rebl, C.H Wu, K. Nomura, T. Hadfield, C. Gill, D. Dalrymple, J.E. Womack, N.E. Cockett (2009). Cytogenetic anchoring of radiation hybrid and virtual maps of sheep chromosome X and comparison of X chromosomes in sheep, cattle and human. Chromosome Res. 17: 497-506.

Goldammer, T., G.P. Di Meo, G. Luhken, C. Drogemuller, C.H. Wu, J. Kijas, B.P. Dalrymple, F.W. Nicholas, J.F. Maddox, L. Iannuzzi, N.E. Cockett (2009). Molecular cytogenetics and gene mapping in sheep (Ovis aries, 2n=54). Cytogenet. Cell. Genomics 126: 63-76.

Hadjipavlou G., O. Matika, A. Clop, S.C. Bishop (2008). Two single nucleotide polymorphisms in the myostatin (GDF8) gene have significant association with muscle depth in commercial Charollais sheep. Anim Genet 39: 346-53.

Hayes, B.J., P.J. Bowman, H.D. Daetwyler, J.W. Kijas, J.H.J. va der Werf (2011). Accuracy of genotype imputation in sheep breeds. Anim Genet 43: 72-80.

Heaton, M.P., M.L. Clawson, C.G.B. Chitko-Mckown, K.A. Leymaster, T.P. Smith, G.P. Harhay, S.N. White, L.M. Hermann-Hoesing, M.R. Mousel, G.S. Lewis, T.S. Kalbfieisch, J.E. Keen, W.W. Laegreid (2012). Reduced lentivirus susceptibility in sheep with TMEM154 mutations. PLoS Genet 8(1): e1002467.

Jiang, Y. (2011). Sequencing and assembly of the sheep genome reference sequence. Proc., Plant and Animal Genomes XIX Conference, San Diego, CA. W138.

Kijas, J.W., D. Townley, B.P. Dalrymple, M.P. Heaton, J.P. Maddox, A. McGrath, P. Wilson, R.G. Ingersoll, R. McCulloch, S. McWilliam, et al. (2009). A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PLoS ONE 4: e4668.

Knight, M.I., H.D. Daetwyler, B.J. Hayes, M.J. Hayden, A.J. Ball, D.W. Pethick, M.B. McDonagh (2012). Discovery and trait association of single nucleotide polymorphisms from gene regions of influence on meat tenderness and long-chain omega-3 fatty acid content in Australian lamb. Anim Prod 52: 591-600.

Maddox. J.F., K.P. Davies, A.M. Crawford, D.J. Hulme, D. Vaiman, E.P. Cribiu, B.A. Freking, K.J. Beh, N.E. Cockett, N. Kang, C.D. Riffkin, R. Drinkwater, S.S. Moore , K. Dodds, J.M. Lumsden, D. Adelson, H. Birkin, J.E. Broom, J. Buitkamp, L. Cambridge, W.T. Cushwa, E. Gerard, S. Galloway, B. Harrison, R. Hawken, S. Hiendleder, H. Henry, J. Medrano, K. Paterson, S.H. Phua, L. Schibler, R.T. Stone, B. van Hest (2001). An enhanced linkage map of the sheep genome comprising more than 1000 loci Genome Research 11: 1275-89.

Marshall, K., J.F. Maddox, S.H. Lee, Y. Zhang, L. Kahn, J.U. Graswer, C. Gondro, S.W. Walkden-Brown, J.H.J. van der Werf (2011). Genetic mapping of quantitative trait loci for resistance to Haemonchus contortus in sheep. Anim Genet 40: 262-72.

Matika, O., R. Pong-Wong, J.A. Woolliams, S.C. Bishop (2011). Confirmation of two quantitative trait loci regions for nematode resistance in commercial British terminal sire breeds. Animal 5: 1149-56.

Meuwissen, T.H., B.J. Hayes, M.E. Goddard (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819-29.

Montgomery, G.W., E.A. Lord, J.M. Penty, K.G. Dodds, T.E. Broad, L. Cambridge, S.L. Sunden, R.T. Stone, A.M. Crawford (1993). The Booroola fecundity (FecB) gene maps to sheep chromosome 6. Genomics 22: 148-53.

Notter, D.R. (2012). Application of genomic information for improvement of quantitative traits. Sheep and Goat Res J 27: 7-12.

Ron, M., J.I. Weller (2007). From QTL to QTN identifiicaton in livestock – winning by points rather than knock-out: a review. Anim Genetics 38:429-39.

Sabeti, P.C., S.F. Schaffner, B. Fry, J. Lohmueller, P. Varilly, O. Shamovsky, A. Palma, T.S. Mikkelsen, D. Altshuler, E.S. Lander (2006). Positive natural selection in the human lineage. Science 312:1614-20.

Silva, M.V., T.S. Sonstegard, O. Hanotte, J.M. Mugambi, J.F. Garcia, S. Nagda, J.P. Gibson, F.A. Iraqui, A.E. McClintock, S.J. Kemp, P.J. Boettcher, M. Malek, C.P. Van Tassell, R.L. Baker (2012). Identification of quantitative trait loci affecting resistance to gastrointestinal parasites in a double backcross population of Red Maasai and Dorper sheep. Anim Genet 43: 63-71.

Wu C.H., K. Nomura, T. Goldammer, T.L. Hadfield, J.E. Womack, N.E. Cockett (2007). An ovine whole-genome radiation hybrid panel used to construct an RH map of ovine chromosome 9. Anim Genet 38: 534-6.

Wu, C.H., K. Nomura, T. Goldammer, T.L. Hadfield, B.P. Dalrymple, S. McWilliam, J.L. Maddox, J.E. Womack, N.E. Cockett (2008). A high-resolution comparative radiation hybrid map of ovine chromosomal regions that are homologous to human chromosome 6 (HSA6) Anim Genet 39: 459-67.

Wu C.H., K. Nomura, T. Goldammer, T. Hadfield, B.P. Dalrymple, S. McWilliam, J.L. Maddox, J.E. Womack, N.E. Cockett (2009). A radiation hybrid comparative map of ovine chromosome 1 aligned to the virtual sheep genome Anim Genet 40: 435-55.

Sheep & Goat Research Journal
Volume 27, 2012; Special Research Symposium Papers

# Application of Genomic Information for Improvement of Quantitative Traits

*David R. Notter*[1]

*Department of Animal and Poultry Science, Virginia Tech, Blacksburg 24061*
*[1]Corresponding author: phone 540-231-5135; fax 540-231-3010; email drnotter@vt.edu*

## Summary

Use of genomic information in livestock breeding can allow direct assessment of genetic merit of potential breeding animals. Genomic information is particularly valuable for managing the expression of simply inherited genetic defects and for using genes with large effects on prolificacy, disease resistance, and muscularity. Use of genomic information to support genetic improvement in quantitative traits has, however, progressed more slowly. The hypothesis, that a few genes of relatively large effect control these traits, has been shown to be incorrect, and the classic hypothesis involving control by many genes with small individual effects appears to be correct. High-density SNP (single-nucleotide polymorphism) arrays allow assessment of differences among individuals at 50,000 or more sites along the genome and can provide genomic breeding values (GBVs) based on observed relationships between these SNPs and performance. However, performance records on large numbers of animals are required to determine the relationships between genomic information and performance that are necessary for effective use of GBVs to select future descendants of these founder animals. To date, correlations between GBVs and performance-based Estimated Breeding Values (EBVs) in progeny-tested sires have been positive and significant, but well below the desired 1.0, and careful attention is required to optimize resources devoted to collection of phenotypic data versus those devoted to collection of genomic information. However, the U.S. sheep industry needs a baseline capacity to collect and utilize genomic information, particularly on progeny-tested rams, and this review will describe options to develop this baseline capacity.

**Key Words:** Sheep, Genomic improvement, Quantitative traits, Selection

## Introduction

The expanding availability of genomic tools in livestock species provides opportunities to utilize genome-based strategies to improve animal performance. Use of these tools to identify, monitor, and limit the spread of simply inherited genetic defects and to identify and appropriately utilize genes of major effect, such as the Booroola fecundity gene and various mutations associated with enhanced muscularity is already common. Rapid identification of the mutation causing the curly-calf syndrome in Angus cattle (Van Eenennaam, 2009) is a particularly compelling example of the power of molecular techniques to identify mutations of major effect.

Use of genomic approaches to identify risk factors for human diseases is an explosive area of research, and these same tools are being applied in animal medicine. The genetic basis of susceptibility to scrapie has long been recognized and genome-based diagnostics tests of susceptibility are the centerpiece of most programs to control this disease (Smit et al., 2002; Baylis and Goldman, 2004). Recent studies of the genetic basis of susceptibility to Ovine Progressive Pneumonia (OPP) (Heaton et al., 2012) demonstrate the power and sophistication of these techniques.

Against this background, there are similar levels of enthusiasm for development and use of genomic strategies to improve quantitative traits, such as growth rate, wool production and quality, reproductive and maternal performance, and carcass yield and quality that are fundamental contributors to profitability in the sheep industry. Despite the presence of a few genes of major effect on litter size and muscle development, control of these traits mainly reflects the actions and interactions of many genes with moderate or small effects, thereby requiring additional levels of sophistication and creativity for their detection and use in breeding programs. This review considers the potential use of genomic strategies to improve these quantitative traits.

## The Genetic Model for Quantitative Traits

The classic model for genetic control of quantitative traits is generally referred to as the "infinitesimal model". It assumes that many genes, each with small effects, control these traits. In its literal formulation, the infinitesimal model assumes that each trait is controlled by a near-infinite number of genes, each with a vanishingly small individual effect but cumulatively accounting for the heritable genetic differences observed among individuals. Superior individuals have larger numbers

of favorable genetic variants than inferior individuals and are anticipated to pass larger numbers of favorable variants to their progeny, with resulting correspondence ("heritability") between performance of parent and progeny. This "black box" model of genetic control of quantitative traits is the basis for derivation of Estimated Breeding Values (EBVs) in genetic evaluation programs, such as the U.S. National Sheep Improvement Program (NSIP), and, despite its lack of specificity regarding genetic mechanisms underlying animal performance, has been a powerful tool for genetic improvement.

The ability to sequence the DNA of individual animals provides potential to associate genetic variants at specific sites within the DNA with observable differences among animals. This potential has been realized for many simply inherited conditions such as animal color, scrapie susceptibility, the Spider syndrome, and several others, resulting in simple, commercially available, diagnostic tests. Extension of this approach to quantitative traits is conceptually straightforward, but operationally challenging. The sheep genome contains approximately three billion base pairs and approximately 30,000 structural genes that define the molecular structure of the enzymes, hormones, regulatory and structural proteins, and transcription factors associated with variation among individuals. These structural genes are distributed across 26 ovine chromosomes. Each is surrounded by a variety of associated regulatory DNA sequences that permit interactions with other genes and with the environment to control the expression level of the gene. These regulatory sequences may themselves differ among individuals in ways that create individual differences in extent and timing of gene expression. A substantial proportion of the genome does not have a clearly defined role in creation of genetic differences among individuals. These seemingly nonfunctional sequences for a long period of time were derided as "junk DNA", but their role is being continuously reassessed. For example, DNA regulation involves the binding of various regulatory molecules to the DNA, a process that can be modified by the physical orientation of the DNA molecules. If genetic variation in these "nonfunctional" DNA sequences affects the physical orientation of the DNA molecules, they may also affect gene regulation and function.

## Detection of Genetic Markers for Quantitative Traits

If a quantitative trait (e.g., the diameter of the wool fiber) is controlled by 50 genes (an arbitrary, and likely conservative, assumption), then we might anticipate that genetic differences in DNA sequences at those 50 sites (or their associated regulatory regions) are directly associated with heritable differences among individuals in fiber diameter. Identification of the differences in DNA sequences at these sites associated with differences in performance would thus permit development of a DNA-based diagnostic test of genetic merit for fiber diameter. The challenge is how to identify these sites and the variants within them that are associated with differences in performance.

The first step is to identify sites along the genome that are polymorphic in the population of interest. A polymorphic site is one at which the DNA sequence differs in a detectable manner among individuals and can therefore potentially serve as a genetic marker. In most populations, there are millions of polymorphic sites, but only a few are anticipated to be associated with differences in performance. Detection of informative associations between these polymorphisms and quantitative traits allows identification of regions in the DNA that impact the quantitative trait. From this point, more rigorous and detailed analysis of additional polymorphisms within these regions can be used to search for causal mutations associated with differences in performance. This approach is particularly relevant for single genes of large effects and was used to identify the causal mutation involved in the Spider syndrome in Suffolk sheep (Cockett et al, 1999).

A common experimental design to detect genomic regions of interest for quantitative traits involves the crossing of highly divergent sheep types (e.g., prolific Finnsheep and other less-prolific breeds, or parasite-resistant Gulf Coast Native and parasite-susceptible Suffolk sheep). The resulting $F_1$ (first-cross) lambs always carry one chromosome from each parent. However, when $F_1$ lambs are mated together to produce the $F_2$ generation or backcrossed to one of the parent breeds, chromosome segregation in offspring results in individuals with either one, two, or no copies of each of the ancestral genes from the two founder types. From an available catalog of genetic markers, a subset of informative markers can be identified as those that appear **only** in one form, in one breed and exclusively in a different form in the other breed. These breed-specific markers thus identify regions of the DNA with regard to their breed of origin. Screening of performance levels in segregating offspring permits identification of associations between performance and genetic markers derived from the parent breeds. The goal is to find genetic markers from the Finnsheep parent that are associated with more "Finn-like" performance (i.e., larger litters) in crossbred progeny in which these genes and markers are segregating.

Several implicit assumptions in these studies may limit their utility in practical breeding programs. Chief among these is the anticipation that a relatively small number of high-impact regions will emerge as exerting control over the trait(s) of interest. In most cases, the initial results of these marker studies will identify relatively broad regions of control, but if the number of regions is small, then further study (usually involving more advanced generations of mating or detailed study of individual families) is warranted to "drill down" to identify the presumptive causal mutations that underlay observed-breed differences.

Unfortunately, this approach has enjoyed only limited success for most quantitative traits. Several studies have been conducted to attempt to identify genes that influence resistance to GI helminth parasites (Marshall et al., 2009; Silva et al., 2011). A few consistently impactful regions of the genome have been identified, but the common result has been to detect relatively large numbers of potentially important regions and to find different regions of potential interest in different studies. With regard to parasite resistance, the current view is that this trait is behaving as a classical, quantitative trait, with genetic control associated with many genes, most with small effects, and with different genes in different populations. Similar conclusions have been reached for other quantitative traits in a wide range of studies. However, some useful regions involved in tenderness and marbling have been identified in beef cattle (Davis et al.,

2008; McClure et al., 2012) and, more recently, in sheep (Knight et al., 2012).

## Generation of Molecular Breeding Values from Genome-wide Association Studies

Development of high-density single-nucleotide polymorphism (SNP) arrays allows characterization of individual animals for very large numbers of genetic markers. SNP arrays containing 50 thousand to 60 thousand individual SNP have been used in cattle, sheep, swine, chickens, and horses, and arrays with up to 750,000 SNP are being used in cattle. Even larger arrays of 2 million to 3 million SNP are available for humans. Thus we now have the capacity to characterize individual animals at 50,000 or more locations spread across the genome. This tool can be used to detect qualitative trait loci (QTLs) using techniques described in the previous section, but also allows for other approaches for estimation of breeding values (BV) for quantitative traits.

Focus has changed from emphasis on detection and characterization of a small number of high-impact regions to the prediction of genomic breeding values (GBV) derived from the cumulative effects of all, or at least many, of the SNPs. In many ways this approach represents a return to the infinitesimal model, with its assumption of many causal genes, each with small effects. Differences in performance associated with individual SNPs are expected to be small, but, when cumulated across the entire SNP array, are anticipated to give a useful predictor of the animals' overall genetic merit for the trait(s) in question.

Just as EBVs derived from performance records in conventional genetic evaluations are "black-box" calculations with regard to genetic mechanisms, so too are the genomic BVs largely also "black boxes" with regard to the functional characteristics of causal genes associated with individual SNP. However, just as the black-box EBVs derived from performance records have led to significant and substantial improvements in genetic merit, so may these GBVs contribute valuable information for use in genetic evaluation, and with the important additional potential to occasionally uncover individual genes of major, or at least relatively large, effect. Thus SNP markers associated with DGAT, a gene in cattle that has a relatively large effect on milk fat content, contribute strongly to GBVs for fat production in dairy cattle (Grisart et al., 2004) but with substantial additional contributions from many other SNP with smaller individual effects.

Likewise focus has shifted from emphasis on searching for functional mutations of large effect in breeds or families with extreme phenotypes to use of SNP-based approaches within livestock breeds. This approach emphasizes the detection and utilization of potentially large numbers of genomic variants responsible for the heritable genetic variation within each breed. Implicit in this approach is the recognition that those genomic variants, or at least the SNPs associated with them, often will not be the same in different breeds.

Genomic breeding values are not derived from clearly delineated functional characteristics of individual genes, but are instead derived from statistically determined associations between SNP variants and reported animal performance. They thus require both SNP information on candidates for selection and extensive performance records on the genotyped individuals. These performance records provide the basis for estimation of associations between SNP and genetic merit and are absolutely critical to derivation of GBVs. However, once these relationships are derived and validated, future SNP information on descendants may be used to derive GBVs prior to, or in some cases without recourse to, collection of performance data.

## Utilization of Genomic Breeding Values

Generation of GBVs from SNP arrays is possible only if detailed performance records for the traits of interest are available for many genotyped individuals. This "training population" is used to determine the relationships between SNPs and genetic merit that will be used to predict genetic merit in future descendants of the training population. For example, Van Raden et al. (2009) used 3,576 genotyped, progeny-tested Holstein bulls to establish GBVs for milk production in dairy cattle. The genotyping of proven sires has emerged as the strategy of choice in dairy and beef cattle, with EBVs from these sires providing the necessary phenotypic information. Most authors suggest that an adequate training population of progeny-tested sires would require a few thousand sires with accuracy levels for their EBVs approaching 0.9. In beef cattle, the American Angus Association utilized an initial training population of over 3,000 bulls (McClure et al., 2010), and the U.S. Meat Animal Research Center developed a DNA repository representing over 2,000 bulls of several breeds to use as a multi-breed training population (Thallman, 2011).

When progeny-tested sires are used as the training population, the number of individuals required for the training population is influenced by the accuracies of the EBVs. Widespread use of AI and a longer history of performance recording in cattle relative to sheep provide greater access to high-accuracy, proven sires for use in training. Accuracies of EBVs in proven sires with many progeny approach 1.0 and thereby minimize the number of individuals required in the training population. At lower accuracies for EBV of 0.6 to 0.7 that are typical of progeny-tested NSIP rams, correspondingly larger numbers of sires are required to achieve acceptable accuracies of resulting GBVs, with requisite numbers of sires in the range of 5,000 to 7,500 (Hayes and Goddard, 2010). Use of individual-animal records to develop training populations is correspondingly even more daunting, with requisite number of animals with genotypes and detailed phenotypes ranging from 10,000 to 15,000 for a heritability of 0.4 up to around 25,000 at a heritability of 0.2 (Hayes and Goddard, 2010).

The exact numbers of individuals required to generate an informative training population is still the subject of debate and continuing research, but the above considerations indicate that:

- EBVs for progeny-tested sires are likely to be the most efficient phenotypes for use in developing GBVs in cattle and sheep;
- Use of sires with high-accuracy EBVs is strongly advantageous, but the number of sires required within a breed is still likely to exceed 1,000;
- Use of lower-accuracy sires is associated with a corresponding requirement for larger numbers of sires, usually at least a few thousand; and
- Use of individual-animal records (as opposed to EBVs) requires very large numbers of individuals and is probably not feasible for most ruminant species.

To put this situation in perspective for the U.S. sheep industry, Table 1 shows average numbers of progeny-tested sires born in each of the five years ending in 2009 and average accuracies of weaning weight EBVs for those sires for the four breeds with the highest rates of participation in NSIP. Among breeds, this number ranges from 22 to 41. Given the small numbers of additional sires tested in other NSIP breeds (Hampshire, Dorset, Columbia, Rambouillet, Dorper, and a few others) and the increasing trend in numbers of progeny-tested Katahdin sires over this period, NSIP is thus currently producing EBVs for 130 to 140 new progeny-tested rams per year. In terms of research institutions, the two main USDA research stations with sheep (the U.S. Sheep Experiment Station and the U.S. Meat Animal Research Station) have a combined ewe inventory of approximately 5,000 ewes and would be expected to generate something in the range of 50 new progeny-tested rams per year, currently spread across 8 to 10 breeds.

Development of GBVs for quantitative traits in U.S. breeds would thus require a major coordinated effort by industry, federal research institutions, and universities. It is not clear, however, that such an effort would be cost-effective for the industry. Van der Werf et al. (2011) concluded that genomic selection for quantitative traits could be cost-effective in the Australian sheep industry, but only with a nucleus breeding structure in which intensive genetic selection (even at relatively high costs) in a few elite flocks is efficiently transmitted through multiplier flocks to serve a commercial industry of one million breeding ewes. Expansion of the nucleus-breeding segment, with more seedstock flocks serving smaller numbers of commercial ewes per nucleus-breeding ram or a smaller commercial-ewe base (both typical of the U.S. industry) reduced anticipated benefits and required, lower break-even costs for genomic testing.

Emphasis regarding benefits of GBVs shifted dramatically in the four years between the 8th and 9th World Congresses on Genetics Applied to Livestock Production in 2006 and 2010, respectively. Speculation that GBVs could replace data-based predictors within a few years was replaced by realization that genomic information would likely supplement, rather than replace, data-based approaches for BV prediction. Interest in genomic prediction increasingly focused on its capacity to provide early-life predictors of genetic merit for traits that cannot be evaluated directly until later in life, with the most notable example being the comparative evaluation of dairy bulls prior to progeny testing. A second key area is the use of GBVs for difficult-to-measure characteristics that are not amenable to improvement under normal production conditions, including traits, such as meat tenderness and resistance to such infectious diseases as foot rot and OPP.

In the beef industry, molecular BV for individual traits are provided mainly by private companies and have been shown to be useful, but imperfect, predictors of genetic merit. These GBVs are currently incorporated into national cattle evaluations as correlated phenotypes, generally with genetic correlations of 0.4 to 0.7 with the actual trait of interest (Kachman, 2008; Spangler and Van Eenennaam, 2010.)

Genomic information also can be used to determine genetic relationship among individuals evaluated in national genetic evaluation (NGE) programs. These relationships are essential for proper weighting of performance records of relatives in derivation of EBVs. Under current methodology, relationships are based on pedigree information. Information from SNP arrays, however, allows direct assessment of animal relationships. Under current NGE procedures, pedigree information is used to quantify relationships as the predicted proportions of alleles shared by pairs of individuals. With genomic information, these proportions can be measured directly as the proportion of the (e.g.) 50,000 SNP variants shared by two individuals, providing a measure of relationship that does not require pedigree information and can therefore be determined between animals in the same flock, in different flocks, or even in different breeds.

Correspondence between SNP- and pedigree-based measurements has yet to be fully validated by comparing genomic relationships in individuals with known, but differing, pedigree relationships. Legarra et al. (2009) showed that pedigree and genomic information may be combined for use in NGE and developed procedures for optimally combining the two sources of information. Hayes et al. (2011) likewise demonstrated use of genomic relationships within and among Australian sheep breeds.

**Table 1. Numbers of sires tested and average accuracies for weaning weight EBVs for progeny-tested sires of the four NSIP breeds with the largest numbers of participating flocks. Only sires with at least five progeny with weaning weights were included in the tabulation.**

| Breed | Average annual number of new progeny-tested sires[a] | Accuracy of weaning weight EBVs[b] | |
|---|---|---|---|
| | | Average | Range |
| Targhee | 23 | 0.73 | 0.34 to 0.87 |
| Suffolk | 29 | 0.68 | 0.53 to 0.84 |
| Polypay | 22 | 0.67 | 0.56 to 0.87 |
| Katahdin | 41 | 0.68 | 0.39 to 0.91 |

[a] Average for the 5 years ending in 2009.
[b] Accuracy is defined as the expected correlation between estimated and actual breeding values.

## The Way Forward

Consideration of potential benefits of genomic testing must recognize that there are a number of different situations in which DNA-based diagnostic testing may provide useful information for U.S. sheep producers. An immediate benefit of genomic testing and expanding knowledge of the sheep genome is the opportunity to rapidly identify and develop DNA-based diagnostic tests for simply inherited genetic defects, such as the Spider syndrome in Suffolks. Opportunities to manage such defects are now available for all livestock species.

Continued study of the sheep genome using breeds, families, or individuals with extreme performance characteristics will likely result in detection of other potentially useful, simply inherited mutations. A number of major genes affecting quantitative traits have been identified in sheep and are already in use around the world in various specialized-breeding programs. For example, the FecB (or Booroola) gene and the sex-linked FecX (or Invermay) gene result in an increase in litter size of approximately 0.5 lambs in heterozygotes and up to 1.0 lambs in homozygotes (Davis, 2005). Simply inherited genes controlling resistance to scrapie are central to eradication programs in Europe and the Americas, and a DNA-based, diagnostic test for genetic resistance to OPP has been developed (Heaton et al., 2012). A number of major genes influencing muscularity involve mutations in the myostatin gene or in a region of chromosome 18 that included the callipyge, Carwell, and Texel muscling genes (Cockett et al., 2005). Each of these genes is capable of increasing loin weights by 5 percent to 12 percent in heterozygotes.

In terms of gene function, most simply-inherited genes with large effects on quantitative traits result in loss of regulatory control over some aspect of development, such as ovulation rate or muscle development. These mutations were almost always deleterious in wild ancestors of domestic sheep but may be advantageous under domestication and intensive management and are generally most useful in specific production systems and carefully controlled-breeding programs.

For much of the sheep industry, however, genetic improvement requires steady, modest improvements in several quantitative traits, hopefully in association with a properly configured, multi-trait breeding objective (Borg et al., 2007). Genes of large effect may be useful in terminal-sire breeds or intensive-production systems. However, under the extensive conditions that prevail in much of the sheep industry and in the maternal and dual-purpose breeds that dominate the national ewe flock, sustained improvement in several quantitative traits is the most appropriate strategy. Many of these traits (body size, ovulation rate, muscling, milk production) have intermediate optima that are only occasionally consistent with use of genes of large effect. Existing EBV systems, which focus on use of performance records to assess genetic merit in individual breeds, flocks, and production systems, are an appropriate model for sustained, genetic improvement. The issue then becomes how molecular information can enhance the effectiveness of existing EBV systems.

Development of GBVs for quantitative traits requires a major joint effort by industry, academic, and federal research laboratories. An example of such an effort can be seen in the Australian Sheep CRC *(http://www.sheepcrc.org.au)* but seems unlikely in the United States.

An appropriate direction for the U.S. sheep industry appears to be to establish of a baseline capacity for genomic characterization of breeding animals in U.S. flocks; establish and maintain the international connections and collaborations necessary to take advantage of advances in genomic information; develop structures for testing newly developed and released genomic tools in U.S. sheep populations; continue emphasis on detection of QTLs of large effects, especially for disease resistance and meat yield and quality; study functional genomic relationships between DNA and animal performance; and expand and strengthen traditional, genetic-evaluation systems to build capacity for establishing appropriate reference populations for training. More specifically:

- DNA samples should be collected from all progeny-tested NSIP sires to provide an archive of genomic information on these animals, and genomic characterization of these individuals should be carried out using the currently available 50K chip. This action would provide access to SNP information on a nucleus of influential, progeny-tested sires and link that information to the animals' NSIP EBVs. Benefits to this approach include:
  - Use of empirical, SNP-based relationships among tested animals to quantify and improve genetic linkages among flocks. The incorporation of genomic relationships in LAMBPLAN is anticipated in the near future and will also allow use of genomic relationships in NSIP.
  - Capacity to use genomic information and EBVs to rapidly validate new genomic tools. This capacity is provided for the U.S. beef industry by the National Beef Cattle Evaluation Corsortium (*http://www.nbcec.org*) and is essential to allow objective assessment of prospective genomic tools. For example, existing EBVs for resistance to gastrointestinal parasites in Katahdin sires could be used to assess the association between OPP resistance and parasite resistance.
  - Inceased capacity for international collaboration. Without baseline capacity to relate genomic information to objective measures of animal performance (e.g., NSIP EBVs), the U.S. sheep industry risks being shut out of collaborative international efforts to use genomic information to improve quantitative traits.
  - Possibility to take advantage of future opportunities. The field of genomics will likely continue to develop rapidly, with anticipated access to higher-density SNP chips (750K or larger) and individual-animal-genome sequences. Markers for major genes influencing various performance traits will likewise periodically emerge. Access to DNA and EBVs from a diverse cross-section of U.S. sires will prepare the industry to assess and take advantage of these opportunities.
- At least one additional SY in quantitative genomics of sheep is needed to implement the activities described above. The position could be located at an ARS station or a university, but should have the mandate and capacity to interact with the sheep industry via NSIP, federal laboratories and universities involved in sheep research, and potential international collaborators. Collaboration with scientists working in other aspects of genomics and within other species also is essential. Given the relatively small economic impact of the sheep industry, an appropriate strategy would be to locate an individual with strong training and interest in quantitative-genomics theory in an existing program involving one or more other species, with the expectation that the individual would both address needs and opportunities unique to the U.S. sheep industry and also make broader contributions to the overall science of quantitative genomics.

## Conclusions

Genomic information can enhance responses to selection for quantitative traits, but only if seamlessly integrated into existing, genetic-evaluation programs. The actions described in this review would establish a baseline capacity in genomics of quantitative traits for the U.S. sheep industry. The proposed commitment is quite modest. A greater investment to actively develop genomic tools for the industry would be desirable and could be added to the above proposal, but should not be allowed to defer establishment of the proposed-baseline capacity.

## Literature Cited


Baylis, M. and W. Goldman. 2004. The genetics of scrapie in sheep and goats. Current Molec. Med. 4:385-396.

Borg, R. C., D. R. Notter, L. A. Kuehn, and R. W. Kott. 2007. Breeding objectives for Targhee sheep. J. Anim. Sci. 85:2815-2829.

Cockett, N. E., T.L. Shay, J.E. Beever, D. Nielsen, J. Albretsen, M. Georges, K. Peterson, A. Stephens, W. Vernon, O. Timofeevskaia, S. South, J. Mork, A. Maciulis, T.D. Bunch. 1999. Localization of the locus causing Spider Lamb Syndrome to the distal end of ovine Chromosome 6. Mammalian Genome 10:35–38.

Cockett, N. E., M. A. Smit, C. A. Bidwell, K. Segers, T. L. Hadfield, G. D. Snowder, M. Georges, and C. Charlier, 2005. The callipyge mutation and other genes that affect muscle hypertrophy in sheep. Genet. Sel. Evol. 37 (Suppl. 1):65–81.

Davis, G. H. 2005. Major genes affecting ovulation rate in sheep. Genet. Sel. Evol. 37 (Suppl. 1):11–23.

Davis, G. P., S. S. Moore, R. D. Drinkwater, W. R. Shorthose, I. D. Loxton, W. Barendse, and D. J. S. Hetzel. 2008. QTL for meat tenderness in the M. longissimus lumborum of cattle. Anim. Genet. 39:40-45.

Grisart, B., F. Farnir, L. Karim, N. Cambisano, J. J. Kim, A. Kvasz, M. Mni, P. Simon, J. M. Frère, W. Coppieters, and M. Georges. 2004. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. Proc. Nat. Acad. Sci. USA 101:2398–2403.

Hayes, B. J., P. J. Bowman, H. D. Kaetwyler, J. W. Kijas, and J. H. J. van der Werf. 2011. Accuracy of genotype imputation in sheep breeds. Anim. Genet. 43:72-80.

Hayes, B. and M. Goddard. 2010. Genome-wide association and genomic selection in animal breeding. Genome 53:876-883.

Heaton, M. P., M. L. Clawson, C. G. Chito-Mckown, K. A. Leymaster, T. P. L. Smith, G,. P. Harhay, S. N. White, L. M. Herrmann-Hoesing, M. R. Mousel, G. S. Lewis, T. S. Kalbfleisch, J. E. Keen, and W. W. Laegreid. 2012. Reduced lentivirus susceptibility in sheep with TMEM154 mutations. PLoS Genetics 8(1):e1002467.

Kachman, S. D. 2008. Incorporation of marker scores into national genetic evaluations. Proc. Beef Improvement Fed. 9th Genet. Prediction Workshop: Prediction of Genetic Merit of Animals for Selection, pp. 92-98. Available at: *http://www.beefimprovement.org/PDFs/Kansas%20City%20Missouri%202008.pdf*. Accessed 9 May 2012.

Knight, M. I., H. D. Daetwyler, B. J. Hayes, M. J. Hayden, A. J. Ball, D. W. Pethick, and M. B. McDonagh. 2012. Discovery and trait association of single nucleotide polymorphisms from gene regions of influence on meat tenderness and long-chain omega-3 fatty acid content in Australian lamb. Anim. Prod. Sci. (In press).

Legarra, A., I. Aguilar, and I. Misztal. 2009. A relationship matrix including full pedigree and genomic information. J. Dairy Sci. 92:4656-4663.

Marshall K., J. F. Maddox, S. H. Lee, Y. Zhang, L. Kahn, H.-U. Graser, C. Gondro, S. W. Walkden-Brown, and J. H. van der Werf. 2009. Genetic mapping of quantitative trait loci for resistance to Haemonchus contortus in sheep. Anim Genet. 40:262-272.

McClure, M. C., N. Morsci, R. D. Schnabel, J. W. Kim, P. Yao, M. M. Rolf, S. D. McKay, S. J. Gredd, R. H. Chapple, S. L. Northcutt, and J. F. Taylor. 2010. A genome scan for quantitative trait loci influencing carcass, postnatal growth and reproductive traits in commercial Angus. Anim. Genet. 41:597-607.

McClure, M. C., H. R. Ramey, M. M. Rolf, S. D. McKay, J. E. Decker, R. H. Chapple, J. W. Kim, T. M. Taxis, R. L. Weaber, R. D. Schnabel, and J. F. Taylor. 2012. Genome-wide association analysis for quantitative trait loci influencing Warner–Bratzler shear force in five taurine cattle breeds. Anim. Genet. 44:(In press).

Silva, M. V. B., T. S. Sonstegard, O. Hanotte, J. M. Mugambi, J. F. Garcia, S. Nagda, J. P. Gibson, F. A. Iraqi, A. E. McClintock, S. J. Kemp, P. J. Boettcher, M. Malek, C. P. Van Tassell, and R. L. Baker. 2011. Identification of quantitative trait loci affecting resistance to gastrointestinal parasites in a double backcross population of Red Maasai and Dorper sheep. Anim. Genet. 43:63–67.

Smit, M. A., N. E. Cockett, J. E. Beever, T. L. Shay, and S. L. Eng. 2002. Scrapie in sheep: a transmissible spongiform encephalopathy. Sheep Goat Res. J. 17(2):21-32.

Spangler, M. and A. Van Eenennaam. 2010. Utilizing molecular information in beef cattle selection. National Beef Cattle Evaluation Consortium Sire Selection Manual. Available at: *http://www.nbcec.org/producers/sire_selection/chapter14.pdf*. Accessed 9 May 2012.

Thallman, R. M. 2011. Results from the USMARC 2,000 Bull Project. Available at: *http://www.nbcec.org/ topics/2000BullProject.pdf*. Accessed 9 May 2012.

Van Eenennaam, A. 2009. Curly calf syndrome (arthrogryposis multiplex) test available. Available at: *http://animalscience.ucdavis.edu/animalbiotech/Outreach/Curly_Calf_test.pdf*. Accessed 9 May 2012.

van der Werf, J. H. J., B. J. Horton, and R. G. Banks. 2011. Optimizing sheep breeding programs with genomic selection. Proc. Assoc. Advancement Anim. Breed. Genet. 19:(In press).

Van Raden, P.M., Van Tassell, C.P., Wiggans, G.R., Sonstegard, T.S., Schnabel, R.D., Taylor, J.F., Schenkel, F.S. 2009. Reliability of genomic predictions for North American Holstein bulls. J. Dairy Sci. 92:16-24.

Sheep & Goat Research Journal
Volume 27, 2012; Special Research Symposium Papers

# Utilization and Potential of Estimates of Genetic Value from an Industry Perspective

*David L. Thomas*

*Department of Animal Sciences, University of Wisconsin-Madison, Madison, Wisconsin, 53706, email dlthomas@wisc.edu*

## Background

The U.S. sheep industry is far behind dairy cattle, beef cattle, swine, and poultry industries in the United States and the sheep industries of many other countries in the utilization of estimates of genetic values for the improvement of economically important traits. This situation is due to both 1) a general indifference on the part of the industry to utilize currently available genetic technology, as evidenced by the low number of flocks enrolled in the National Sheep Improvement Program (NSIP) and 2) the slow output of new technology to the industry by research institutions and commercial companies. The second point is due to a small U.S. sheep research effort and lack of significant profit potential for commercial companies from a small national flock.

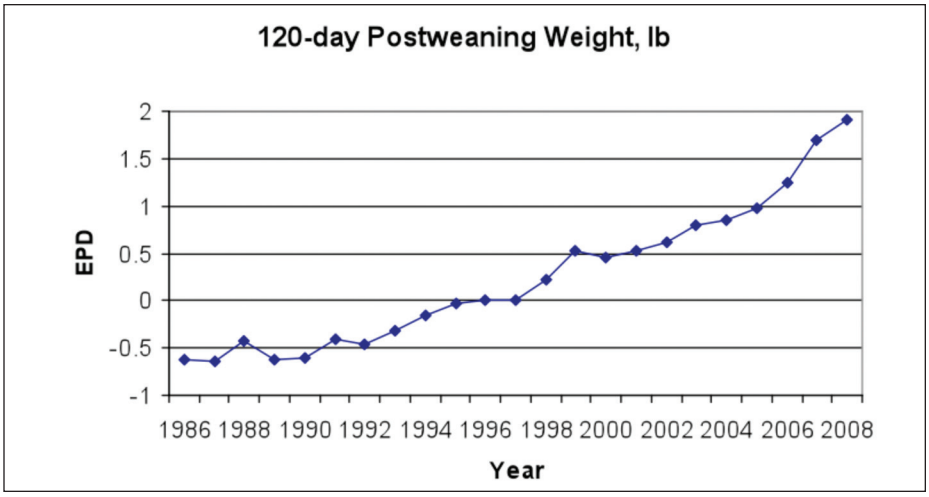
**Key Words:** Sheep industry, Genetic Selection, Economic value

## Moving Forward

There are several examples of the improvement in animal performance that can be made by the selection of replacements, especially males, on estimates of genetic value obtained from phenotypic performance records. Holstein dairy cows in the Dairy Herd Improvement program born in 2009 compared to 1990 had a 28-percent-greater milk yield (26,861 pounds vs. 20,959 pounds) (AIPL, 2011). Fifty-eight percent of this increase was due to increased genetic value of cows born in 2009 compared to cows born in 1990 – annual increases in cow breeding value resulted in a 0.8-percent-per-year increase in milk yield. Angus calves in the Angus Herd Improvement Records program born in 2010 had a yearling-weight-breeding value that was 96 pounds greater than the yearling-weight-breeding value of Angus calves born 20 years earlier in 1991 for an increase of 4.8 pounds per year or 0.5 percent per year (AHIR, 2011). There is widespread use of bulls with estimates of breeding value in commercial cattle herds, so rates of genetic change in commercial dairy and beef cattle herds would be expected to be only slightly less than the rates of change in these performance-recorded, purebred herds.

We have ample evidence that genetic change also is possible in U.S. sheep flocks if performance is recorded and selection is based on estimates of genetic value. Lambs from Suffolk flocks enrolled in the National Sheep Improvement Program born in 2008 had an average 120-day-postweaning weight estimated breeding value (EBV) that was 3.7 pounds more than the average 120-day-postweaning weight EBV of lambs born in 1996 (Figure 1), for an annual change in average, postweaning weight due to genetic improvement of approximately 0.3 percent per year (Notter, 2008). However, genetic change for economically important traits is expected to

**Figure 1. Genetic trend for 120-day weight for Suffolk sheep enrolled in the National Sheep Improvement Program (from Notter, 2008). Note: EPD = Expected Progeny difference, 2(EPD) = Estimated Breeding Value.**



be much lower for commercial flocks compared to flocks enrolled in NSIP because there is very little use of rams with estimates of genetic value in commercial flocks. This is because there is limited availability of such rams due to the small number of purebred flocks enrolled in NSIP. In 2011, among all breeds of sheep in the United States, data were submitted to NSIP on only 4,146 sheep in 70 flocks (M. Sorensen, pers. com.). One reason for the low involvement in NSIP by purebred flocks is due to the failure of the commercial sheep industry to demand, pay a premium for, and use rams with high estimates of genetic value.

Research projects and extension efforts have validated the advantages of using males with estimates of genetic value, especially in beef cattle (eg. Baker et al., 2003), but very few similar validations with sheep have been conducted in the United States. In a 1999 and 2000 study at the University of Wisconsin-Madison compared growth of lambs sired by seven NSIP Suffolk rams, average, Expected Progeny Difference (EPD) for 120-day weight = +2.6 lb., were compared to lambs sired by four non-NSIP Suffolk rams (Thomas et al., 2000). The 120-day weights of the lambs sired by the NSIP rams were 3.8 pounds greater than the 120-day weights of the lambs sired by the non-NSIP rams (Table 1). That is 380 more pounds of lamb at 120 days of age per 100 lambs or $646 additional potential income per 100 lambs from the NSIP rams if lambs are worth $1.70/lb. live weight. This is not to suggest that all rams with estimates of genetic value from NSIP-enrolled flocks are genetically superior to all rams without estimates of genetic value, but without estimates of genetic value such as EPD or EBV, there is no objective information on which to select rams in order to have a higher probability of genetic ram superiority.

In addition to the need for more validation studies and demonstrations on the advantages to be gained from using rams with desirable estimates of genetic value, there is an immediate need to make the genetic information from NSIP flocks readily available to other purebred breeders and commercial producers so they can locate flocks enrolled in NSIP and identify animals to purchase that have high estimates of genetic value for traits important in their flocks. The logical places for this information are on the NSIP web site (NSIP, 2012) and on sheep-breed-association web sites. The NSIP web site has a list of flocks enrolled in NSIP so that producers know whom to contact to purchase sheep with estimates of genetic value. In addition, NSIP recently added a listing of elite older sires and elite younger rams to their web site. While this is a very positive step, the information would be more useful to prospective ram buyers if the lists could be sorted by all traits and indexes to easily identify sires that meet minimum criteria for several traits. For the most part, U.S. sheep-breed associations have provided very little promotion of NSIP or the use of genetic evaluations in breed improvement.

The success of the Center of the Nation NSIP Sale in Spencer, Iowa is an indication that commercial producers will pay a premium for sheep with high estimates of genetic value if the sheep and their estimates of genetic value are readily available. The 6th annual sale was held on July 30, 2011 and averaged $830 on 74 head ($1017 on 48 rams and $487 on 26 ewes).

The widespread use of DNA tests for scrapie resistance at codon 171 of the prion gene (R = resistant, Q = susceptible) (Hunter, 1997) and for the skeletal deformity of Spider Syndrome (N = normal, S = Spider) (Beever et al., 2006) by the U.S. sheep industry is strong evidence that the industry will make use of new DNA tests for single genes that improve well-being, health, or performance of sheep as they become available. However, it would be desirable if DNA tests for other single genes with known effects were available from U.S. laboratories [e.g. the DNA test for the Booroola gene for increased ovulation rate (Wilson et al., 2001) is commercially available only in New Zealand at Genomnz DNA Testing Laboratory]. In addition, there is a need for U.S. research institutions to validate the effect of single genes or gene markers discovered in other countries in U.S. breeds and under U.S. production conditions to determine if DNA tests for these traits would benefit the U.S. industry. Examples are the gene markers for cold tolerance in lambs and foot-rot resistance discovered in New Zealand with DNA tests available through Lincoln University (2012).

We now have the ability to test individual sheep to determine which nucleotides are present in their DNA at several thousand locations throughout the genome. Quantitative traits, such as average daily gain, feed efficiency, longevity, milk production, and loin eye area are very likely due to the combined action of a few hundred genes, most with a small effect. Specific nucleotides at specific locations in the genome may be related to greater or poorer performance for these traits. Once this is known, sheep can be tested and then selected for the "set" of good nucleotides in order to improve the trait. This is called "Genomic Selection" and is discussed in greater detail by Cockett (2012) in a companion paper.

Genomic selection is currently being used extensively in dairy cattle to improve the rate of genetic improvement (Schefers and Weigel, 2012). It will be used in sheep in the future, but the major hurdle that we have is the lack

**Table 1. Body weights (lb.) ± SE of lambs sired by NSIP[a] and Non-NSIP UNITED STATES Suffolk rams.**

| | | Age at weighing, d | | | | |
|---|---|---|---|---|---|---|
| **Sire source** | **No.** | **Birth** | **30 d** | **60 d** | **90 d** | **120 d** |
| NSIP | 130 | 12.7 ± 0.22 | 34.6 ± 0.78 | 55.8 ± 1.26 | 79.8 ± 1.62 | 103.4 ± 1.93 |
| Non-NSIP | 115 | 12.7 ± 0.24 | 32.9 ± 0.94 | 54.0 ± 1.51 | 76.7 ± 1.94 | 99.6 ± 2.34 |

[a] NSIP = National Sheep Improvement Program
From: Thomas et al., 2000.

of large numbers of performance-recorded sheep with estimates of genetic value that can serve as a training set to determine the relationship between a sheep's nucleotide profile and its genetic value for a particular, quantitative trait. These relationships are being determined in the countries of Australia and New Zealand, where there are larger numbers of sheep with estimates of genetic value and greater research budgets for sheep than in the United States (e.g. van der Werf, 2011), but it is highly likely that genomic selection criteria developed in these and other countries in their domestic sheep populations will be much less accurate when applied to sheep in the United States. While the U.S. sheep industry may be able to benefit some from genomic selection criteria developed in non-U.S. sheep populations, genomic-selection criteria need to be developed from U.S. sheep populations in order to be of the greatest benefit to our industry. This requires an organized effort by research and industry to record performance, calculate estimates of genetic value, and determine genome nucleotide profiles on large numbers of sheep. This effort will need to start with flocks enrolled in NSIP.

## Literature Cited


AIPL (Animal Improvement Program Laboratory), USDA. 2011. Trend in milk BV for Holstein. Accessed *online:http://aipl.arsusda.gov/eval/summary/trend.cfm?R_Menu=HO.m#StartBody*.

AHIR (Angus Herd Improvement Records), American Angus Association. 2011. Performance/AHIR/BRS. Accessed online: *http://www.angus.org/ Performance/Default.aspx*.

Baker, J.F., M.E. Boyd, A.H. Brown, D.E. Franke, and C.E. Thompson. 2003. Evaluation of maternal performance of daughters from high and low milk EPD sires. J. Anim. Sci. 81:1406-1413.

Beever, J.E., C. Bottema, J. Albretsen, N.E. Cockett, M.A. Smit, S.N. Meyers, and T.S. Hadfield. 2006. A single-base change in the tyrosine kinase II domain of ovine FGFR3 causes hereditary chondrodysplasia in sheep. Animal Genetics 37:66-71.

Cockett, N.E. Sheep & Goat Research Journal, Vol. 27, 2012. Special Research Symposium. :pp. 2-6

Hunter, N. 1997. PrP genetics in sheep and the applications for scrapie and BSE. Trends. Microbiol. 5:331–334.

Lincoln University – Gene Marker Laboratory. 2012. (Accessed March 20, 2012 at *http://www.lincoln.ac.nz/gene-marker-lab*).

NSIP. 2012. National Sheep Improvement Program web site. (Accessed March 8, 2012 at *http://nsip.org/*).

Notter, D.R. 2008. Suffolk NSIP Notebook, August 13, 2008. Department of Animal and Poultry Sciences, Virginia Tech. No. 9 (Accessed March 6, 2012 at *http://nsip.org/?page_id=177*)

Schefers, J.M. and K.A. Weigel. 2012. Genomic selection in dairy cattle: Integration of DNA testing into breeding programs. Animal Frontiers 2(1):4-9.

Thomas, D. L., Y. M. Berger, B. C. McKusick, R. G. Gottfredson, R. Zelinsky, and G. Rosa. 2000. A comparison of market lambs sired by Suffolk rams of United Kingdom or UNITED STATES origin. Proc. 48th Annual Spooner Sheep Day, Dept. Animal Sci., Univ. of Wisconsin-Madison. pp. 10-15.

Van der Werf, J.H.J. 2011. Genomic selection in sheep. Sir Mark Oliphant Conferences: Applied Genomics for Sustainable Livestock Breeding. Melbourne, Australia. Book of Abstracts. pp. 11-12. (Accessed March 20, 2012 at *http://www.smogenomics.org/docs/abstracts/SMOgenomicsAbstracts.pdf*).

Wilson, T., X.Y. Wu, J.L. Juengel, I.K. Ross, J.M. Lumsden, E.A. Lord, K.G. Dodds, G.A. Walling, J.C. McEwan, A.R. O'Connell, K.P. McNatty, and G.W. Montgomery. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biol. Reprod. 64:1225–1235.

Sheep & Goat Research Journal
Volume 27, 2012; Special Research Symposium Papers

# Utilization from a Producer Perspective: Where We Have Been, Where We Are Now, Challenges and Opportunities for the Future

*Chase T. Hibbard*

*Sieben Live Stock Co, PO Box 835, Helena MT 59624-0835, email chase@siebenlivestock.com*

## Summary

This article is a discussion of the evolution of personal involvement in the sheep business. It is intended to illustrate the evolution from making selection decisions based solely upon look-good, feel-good evaluation, to rudimentary records, all the way to the use of EBVs and profitability indexes. The U.S. sheep industry is challenged to make better use of the genetic selection tools currently available, and the stage is set for moving to the next frontier of gene-enhanced selection.

**Key Words:** Breeding Values, Performance Testing, Selection, Targhee Sheep

## Where We Have Been

The Hibbard family has been in the sheep business since the late 1800s. A great grandfather, who emigrated from Germany and came into Montana on the Bozeman Trail in the 1860s, was a stockman. Although a cattleman at heart, he raised "sheep for the money". By the end of his life, Henry Sieben had established two sheep ranches in west central Montana. The Hibbard family owns and operates one of those ranches today. Initially it was stocked with ten to twelve bands, herded largely by men from the same village in Romania. In those days, record keeping was quite simple. From all indications the important numbers were recorded on the back of a snoose can (smokeless tobacco) or on a leather glove.

In the early 1950s, the decision was made to begin raising rams for use on the ranch and for sale. It was decided to use a new breed ready for release from the U. S. Sheep Experiment Station at Dubois, Idaho. Development had begun in the 1930s and 1940s of a synthetic breed called Targhee, named after the Targhee National Forest just north of the station. To meet the needs of western producers, Targhees were developed as an open-faced sheep with easy fleshing, good twinning potential, foot-rot resistance and easy herding behavior. By 1951, Targhees were ready for release. The Sieben-Hibbard family was one of the first in Montana to receive Targhee rams released from Dubois. Eight hundred ewes were selected from 10,000 Rambouillet-based, western-whiteface sheep that met visual Targhee breed standards, to prepare for the introduction of this new breed. They were top-crossed with the early rams from Dubois, and thus began one of the first Targhee flocks in the United States.

Thirty-six years ago, in 1976 Chase Hibbard took over the ranch, determined to continue the legacy of Targhee sheep. By that time, the ranch had converted to mostly cattle so that the number of purebred sheep was down to about 200 registered ewes. Record keeping was pretty much still on the back of a snoose can, and performance enhancement was inspired by the show ring. In order to progress, it was necessary to make a name for yourself in the ring. Lots of ribbons were won and eventually a National Championship, and it was thought that the ranch was well on the way to meeting the needs of the industry. "On-farm" performance testing had been gaining traction and all of a sudden record keeping began creeping in. As record keeping began to get more sophisticated, with more and more numbers, the snoose can system was actually deserted altogether.

Formal performance testing in the United States began in 1948 in Texas, at the A&M University Sonora Substation. By 1950, the University of Wisconsin began on-farm selection. In 1965, Ohio put the first computerized, record keeping system in place, and by 1980 there were a total of 23 on-farm testing programs and 14 central-ram tests. In Montana, on-farm testing began in the 1960s. As on-farm testing became more prevalent, the use of "ratios" emerged as a tool to select the best of whatever trait was being measured or weighed. Some of the subjectivity of selection was challenged.

This is when the ranch started to make real progress. "Ratios" were a great tool, allowing the user to rank all flock animals against the average for any particular trait. This of course was within your own flock only, with no way to compare a 110 ratio in my flock with a 110 ratio in your flock. Records from one flock to another flock could mean entirely different things. A 100 in Flock A could conceivably be better than a

110 in Flock B. A new ratio called "number of lambs born per ewe exposed" was introduced. An index was developed and another tool was available to make progress. This gave us a way to select the most prolific animals in the flock to move toward greater productivity.

About this time, a further challenge was received from Charles Parker, who was then at the U.S. Sheep Experiment Station. His message was that U.S. producers were getting trounced by our overseas competitors and by other meat- and milk-producing industries. In his view, we weren't being competitive with either costs of production or pounds of lamb raised per ewe. This really set us on our ear. Comparisons were made to New Zealand, where the average sheep raising family raised something like 2600 ewes, fed no hay, pasture lambed, and had little or no extra labor. And to add insult to injury, when the sheep-industry's productivity was compared to the progress that had been made in terms of pounds-of-product-produced per female, or the increases in pounds-of-milk per milk cow, the sheep industry remained way behind.

At the time, selection strategies were based upon visual evaluation, better known as "best guess", plus "performance records". This meant selection was based upon how the animal looked and felt, plus the animal's own, in-flock-performance records.

Enter genetic evaluation. The National Sheep Improvement Program (NSIP)1, introduced around 1986, gave us a vehicle to take us to the next level and really begin focusing on true genetic improvement. Through Best Linear Unbiased Prediction (Henderson, 1975), we now had the ability to make genetic comparisons through Expected Progeny Differences (EPDs). EPDs are calculated as one-half Estimated Breeding Values (EBVs, which are now reported in LAMBPLAN). They are a prediction of how an individual's offspring will perform or an estimate of the animal's genetic value. EBVs and EPDs are calculated using the animal's individual performance, the animal's family performance, and the animal's offspring performance. In other words, they provide a 360-degree view of an animal's potential.

The big advancement made by introduction of EPDs was the ability to make across-flock evaluations, something not previously possible. Across-flock comparison allowed the best animals for any given trait to be identified. No longer was it necessary to scratch our heads when my ram has a weaning weight (WW) ratio of 110 and my neighbor's WW ratio is 98 and it is not known whose is better. A powerful tool was now available to begin making much more rapid genetic improvement.

NSIP really caught on in the Targhee breed, and those using it found that the show ring began to take on a different meaning. The breed association attempted to accommodate both schools of thought and it began sponsoring an NSIP class in addition to the open show classes. NSIP records for the entered animals were analyzed and ranked upon their merit. The animals were not allowed to be fitted, but otherwise were shown in traditional show-ring fashion. A formula was devised to rank both the NSIP-performance-record results and the show-ring-judging placement. A judging template included:

- Rams ranked upon individual EPDs (EBVs after 2009),
- Rams visually placed in NSIP class by show judge, and
- NSIP score doubled and combined with show ranking.

It was likely for the top-NSIP ram to win overall unless he placed near the bottom of the show class. The winner then went on to show in the open class against the fitted-range sheep. Occasionally an NSIP ram would win the open class, but he would need tremendous phenotype to beat fitted-show rams. This dual system persists to this day at Targhee shows, and is a good example of the "art" of animal breeding, since it addresses both the objective and measurable animal-performance traits with the visual, structural, aesthetic and subjective traits, the ones you can see and feel.

## Where We Are Now

Most recently, NSIP has evolved into LAMBPLAN, which is processed in Australia. Traits that are important to commercial producers have been added, and the result is a huge number of EBVs. There is so much information reported that most breeders export only the most relevant numbers to a spreadsheet in order to simplify the process. In our case, we export nine genetic traits, plus spinning count, actual micron, actual and adjusted ribeye area, scrotal measurement, and genotype for scrapie resistance (codon.) The question for most of us now s — how do I make actual decisions with all this information, particularly when some of the traits are antagonistic?

How should the following traits be sorted out: weaning weight, maternal milk, milk + growth, yearling weight, ribeye, fleece weight, fiber diameter, staple length, number of lambs born, genotype? Fortunately, selection indexes had been crafted to synthesize and measure the most important criteria. Most cattle breeds have them. Now, with LAMBPLAN, several selection indexes are available. Selection indexes weigh the most important traits in a formula according to their importance toward the breeding goal selected. Typically, the goal focuses on "profit." Index values give us an estimate of economic merit for the breeding animal. In other words, profitability indexes provide an economic filter to help sort out the traits that will positively influence income from increased production, and traits that will negatively influence income from increased expenses. An example is, selecting for increased weaning weights, which seems desirable, yearling weight of mature ewes also goes up and the overall size of mature animals increases, thereby increasing the carrying costs of the flock. A good profitability index should sort these things out by applying an economic standard. The economic value would be the difference between the increased income from the trait and the increased expense associated with the trait.

For Targhees, two profitability indexes have been designed to fit two production scenarios. The Western Range Index fits a typical Montana range, marketing system. The Farm Flock Index fits a system in which extra market-lamb weight is valued through retained ownership. A Profitability Index:

- Simplifies what is too much data, reducing it to one number, focusing on profitability;
- Eliminates "analysis paralysis"; makes production decisions easier at home; and
- Makes purchasing decisions easier for buyers.

Have these tools made a difference? The short answer is "yes, they have."

There has been a great deal of improvement since the inception of NSIP and a huge amount of improvement since the application of profitability indexes. Both weaning weights and number of lambs weaned have seen significant increases when graphed, and depending on how they are weighted, other traits are improving as well. From 2009 to 2011, the time during which our Western Range Index has been in place, progress in profitability is at an ever-increasing pace.

Personal experiences of the ranch and evolution in the sheep business have been discussed as an example of how genetic improvement has evolved over time. In summary the ranch:

- Started making selection choices almost entirely on how the sheep looked and felt,
- Progressed to keeping crude records, was influenced by the show ring,
- Moved forward utilizing in-flock testing,
- Used central ram tests,
- Was inspired by Charles Parker's Holy Grail to raise more pounds of lamb per ewe exposed or be left in the dust, and finally
- Utilized genetic evaluation made possible by NSIP, EPDs/EBVs, across flock analysis, and profitability indexes.

This brings us to where we are today. Gene-enhanced selection, or genomics, is the next frontier. Are we ready to go there? I am sure a question that most will ask is "what will it do for me?" In short, with these more advanced tools, the possibilities should include the ability to:

- Select at an earlier age, even selecting replacements or rams as lambs;
- Use shorter generation intervals with more confidence in my selection process; and
- Select for hard-to-measure, impossible-to-see traits, such as:
  - livability
  - female reproduction
  - disease and parasite resistance
  - more efficient feed conversion
  - omega-3 health component
  - eating quality.

There are some impediments, which include:

- Lack of acceptance
  *The Hibbard Ranch has been using EPDs for 25 years. For the first several years at the Montana Ram Sale, we spent time explaining what the numbers meant, and buyers eyes would glass over, they would nod politely and climb in the pen to make a visual evaluation. The central ram test winners sold well, with no promise that their offspring would perform anything like their daddy. In the last 5 or 6 years, EBVs have really caught on and now, finally, the first and sometimes the only questions buyers ask is: "What is your best indexing sheep?", or "Which has the best ribeye?", or "Which will increase my number of lambs born?" It would be difficult to sell rams at the Montana Ram Sale at all anymore without EBVs, but it has taken a very, very long time to get to that point.*
- Most producers don't use the proven tools currently available. Is there support to move forward to the next level?
- Cost
  *In Australia, producers are talking about $5 a sample. This is a fraction of the current cost.*
- Turnaround time
  *Again in Australia, producers are talking about less than 10 days.*

There are great tools out there that work, but producers have to use these tools in order to benefit from them. As a purebred producer, the ranch is making better selections in the flock, and selling animals that will improve the bottom line for my customers in the commercial sheep industry. Most sales of commercial breeding rams around the country don't require or even post EBVs. Buyers have not recognized their value. A challenge is made to the industry to use these tremendous tools—commercial producers to require their ram suppliers to enroll in NSIP and purebred producers to take the leadership to use the proven tools available.

Enhanced selection through performance records is here now, it is real, and the results are tangible. Gene-enhanced selection is the next step. It has the potential to take us to the next level. Most of the industry is flying around in a DC-8. Those using EBVs are flying in a 747. A rocket ship for Mars is currently in the dock being fueled for the next frontier. Do you want to be on it?

## Literature Cited:

The National Sheep Improvement Program (NSIP) *http://www.nsip.org/*

Henderson, C.R. (1975). Best linear unbiased estimation and prediction under a selection model. Biometrics 31 (2): 423-447.

Sheep & Goat Research Journal
Volume 27, 2012; Special Research Symposium Papers

# Genetic Selection Specifically Utilized for Evaluating the Introduction of Outside Breeds and Measuring Their Potential

*John Helle*

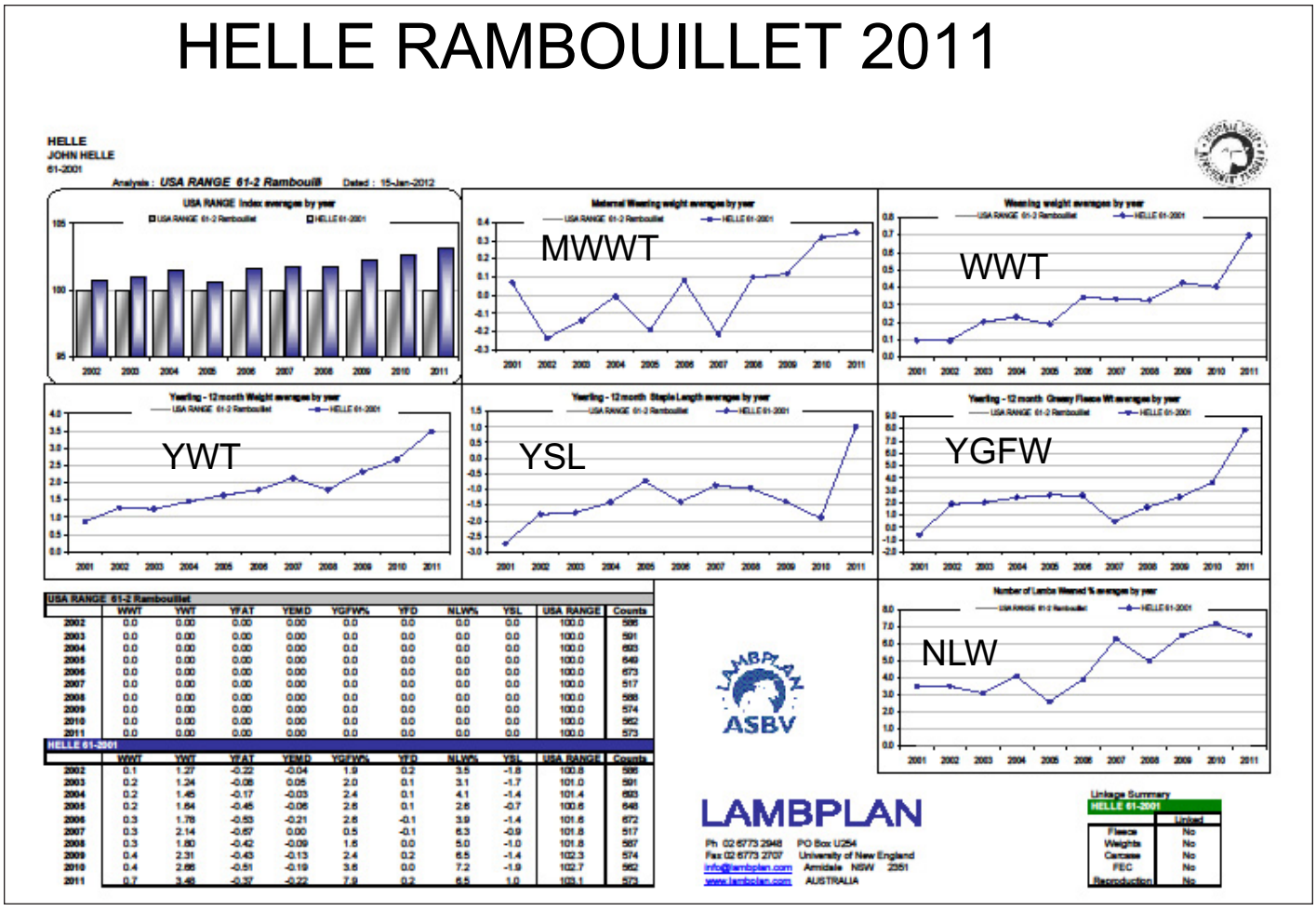
*Helle Rambouillet, 1350 Stone Creek Road, Dillon, Montana 50725-9526, email jhelle64@gmail.com*

## Background

Helle Rambouillet has utilized extensive record keeping for a number of years, placing a large emphasis on wool and maternal traits since the beginning. New traits of interest were added each year. This has been a confusing and complicated process, as data are maintained for each purebred animal; however, it has been rewarding because significant progress has been made for improved genetic selection.

**Key Words:** Genetic Selection, Traits, Breeds, Rambouillet

**Figure 1. Progress over time in USA Range Index, weight traits, staple length, greasy fleece weight and number of lambs weaned in the Helle Rambouillet Flock.**



## Forward Progress

The use of genetic records has allowed fine-tuning and careful monitoring of the genetic-selection progress. The summary report (Figure 1) from the National Sheep Improvement Program (NSIP) allows the monitoring of progress over time for some of the more important traits. In this case, the flock is a single flock in NSIP with really no genetic ties to other flocks so all analyses are within flock.

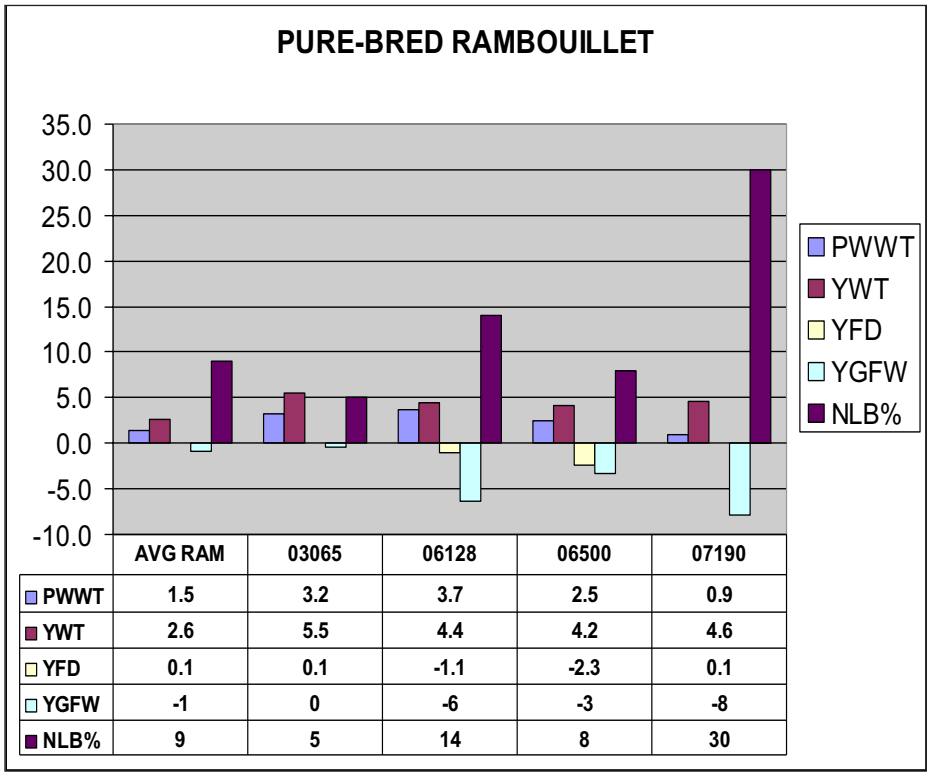
In recent years, Australian genetics was incorporated through artificial insemination. Three Merino, four SAMM (South African Mutton Merino), and two Dohne rams were selected to improve the Helle Rambouillet flock through the use of outside genetics to improve traits of interest. The use of NSIP has allowed the comparison of these rams to each other and to the pure-bred Rambouillet flock (Figures 2 and 3). It is apparent that some choices were better than others. The role of Australian genetics is still being evaluated for use in the breeding program.

The Helle Rambouillet records are now incorporated into the Australian database, and comparisons are made between rams within the Australian system, which is viewed as a huge advantage. There are some differences in adjustment factors and correlations used in the U.S. analysis versus the Australian analysis, so interpretations must be guarded, but some of the comparisons are of value.

## Conclusion

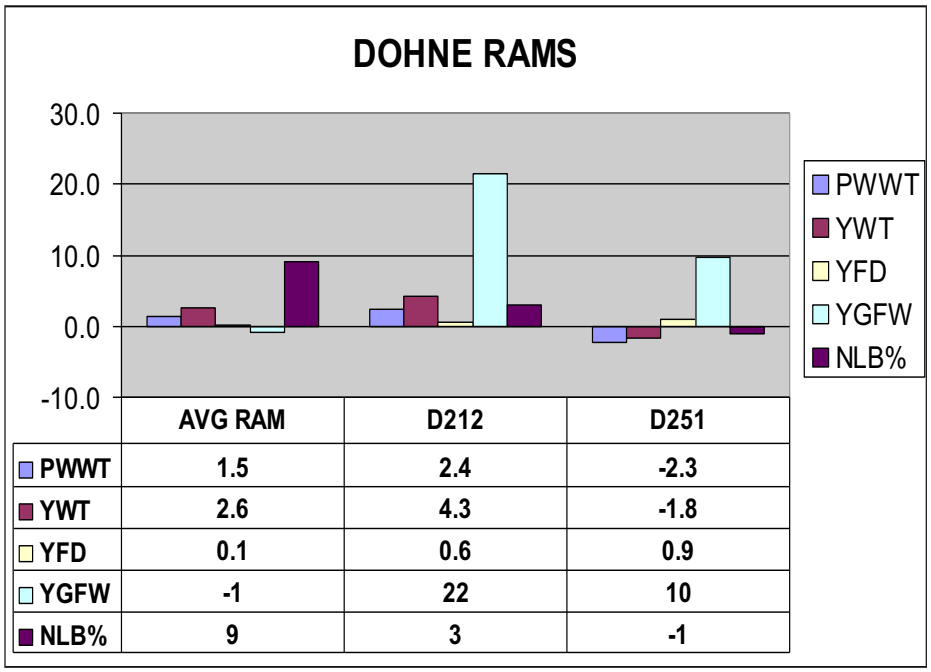
Through NSIP, Helle Rambouillet was able to more accurately interpret production data for use in a selection program. It is helping make decisions as to how Australian genetics might be effectively utilized in a selection program.

**Figure 2. Comparison of selected Rambouillet rams to the average ram in regard to post-weaning weight, yearling weight, yearling fiber diameter, greasy fleece weight and number of lambs born.**




| | AVG RAM | 03065 | 06128 | 06500 | 07190 |
|---|---|---|---|---|---|
| PWWT | 1.5 | 3.2 | 3.7 | 2.5 | 0.9 |
| YWT | 2.6 | 5.5 | 4.4 | 4.2 | 4.6 |
| YFD | 0.1 | 0.1 | -1.1 | -2.3 | 0.1 |
| YGFW | -1 | 0 | -6 | -3 | -8 |
| NLB% | 9 | 5 | 14 | 8 | 30 |

**Figure 3. Comparison of selected Dohne rams to the average ram in regard to post-weaning weight, yearling weight, yearling fiber diameter, greasy fleece weight and number of lambs born.**




| | AVG RAM | D212 | D251 |
|---|---|---|---|
| PWWT | 1.5 | 2.4 | -2.3 |
| YWT | 2.6 | 4.3 | -1.8 |
| YFD | 0.1 | 0.6 | 0.9 |
| YGFW | -1 | 22 | 10 |
| NLB% | 9 | 3 | -1 |