



## Effects of restricted feed intake on blood constituent concentrations in Dorper, Katahdin, and St. Croix sheep from different regions of the USA

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### ABSTRACT

Forty-six Dorper, 47 Katahdin, and 41 St. Croix female sheep (initial BW = 62, 62, and 51 kg, respectively, SEM = 1.43; 3.8 ± 0.18 yr) from farms in Midwest, Northwest, Southeast, and central Texas regions of the USA were used to evaluate effects of feed restriction on blood constituent levels. The amount of feed offered varied in the first 4 wk to achieve stable BW, and that in wk 5–10 was 55% of intake in wk 3–4. Blood was sampled at the end of wk 3, 4, 6, 8, and 10. There were relatively few effects and interactions involving region and no breed × time interactions. Breed affected the concentration of a small number of constituents, including urea N (14.0, 13.7, and 15.4 mg/dl; SEM = 0.31) and creatinine (0.945, 0.836, and 0.809 mg/dl for Dorper, Katahdin, and St. Croix, respectively; SEM = 0.0253). Also, the concentration of triglycerides in wk 4 and 10 was lowest for St. Croix (29.8, 29.5, and 26.7 mg/dl for Dorper, Katahdin, and St. Croix, respectively; SEM = 0.88). There was a trend for a difference ( $P = 0.051$ ) between wk 4 and 10 in the glucose concentration (51.9 and 54.2 mg/dl; SEM = 0.90), and there were differences ( $P < 0.05$ ) in lactate (23.9 and 20.3 mg/dl; SEM = 0.89), urea N (16.4 and 13.0 mg/dl; SEM = 0.25), creatinine (0.808 and 0.919 mg/dl; SEM = 0.0165), triglycerides (31.8 and 25.5 mg/dl; SEM = 0.63), and cholesterol (67.5 and 74.7 mg/dl, respectively; SEM = 1.66). In conclusion, similar responses in blood constituent levels of different hair sheep breeds to feed restriction is in accordance with comparable effects on the maintenance energy requirement.

### 1. Introduction

Breeds of ruminant livestock have developed in various ways. Some have been intensively selected for specific purposes, characteristics, environments, management practices, and/or production systems. Therefore, different breeds vary in physiological conditions that may influence how they respond to various production settings (Blackburn et al., 2011).

In part because of the relatively low value of wool in the USA, the prevalence of hair sheep has been increasing (Thomas, 1991; Wildeus, 1997). Common breeds of hair sheep in the USA are Dorper, Katahdin, and St. Croix, which vary markedly in size, conformation, body composition, resistance to internal parasitism, etc. (Bowdridge, Zajac & Notter, 2015; Burke & Miller, 2004; Thomas, 1991; Wildeus, 1997). These characteristics may influence responses to stress factors that may increase in importance due to climate change. Relatedly, environmental conditions where animals are located and have adapted to could have impacts. Therefore, the resilience to high heat load, limited availability

of drinking water, and restricted feed availability of Dorper, Katahdin, and St. Croix sheep from different regions of the USA, Midwest, Northwest, Southeast, and central Texas, has been evaluated through measures such as BW change, rectal temperature, respiration rate, and feed intake (Hussein et al., 2020; Tadesse, Puchala, Gipson & Goetsch, 2019a, b). Consideration of different origins and sources of breeds should provide a robust characterization relative to the more common approach of research with animals from one or a small number of sources. In addition to the aforementioned variables, it would also be useful to gain an understanding of how these conditions and length of exposure impact levels of blood constituents reflective of nutritional status and physiological conditions (Ndlovu et al., 2007).

Blood constituents such as urea N, total protein, albumin, creatinine, and cortisol are related to protein status, and levels of lactate, glucose, triglycerides, and cholesterol provide information regarding energy demand and supply. Blood levels of these constituents can vary with many factors, including environmental conditions, nutrient and energy requirements, etc. (Carlos et al., 2015; Marai, El-Darawany, Fadiel &

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Abdel-Hafez, 2007). Therefore, objectives of this experiment were to evaluate effects of feed restriction on concentrations of blood constituents in hair sheep differing in breed and region of the USA where they originated.

## 2. Materials and methods

### 2.1. Animals

Procedures of the study are described by Tadesse, Puchala and Goetsch (2019b), which are briefly overviewed below. Protocols for the trials were approved by the Langston University Animal Care and Use Committee. The study consisted of four animal sets and trials, conducted in the winter of 2015/2016, winter of 2016/2017, spring/summer of 2017, and summer/fall of 2017. There were 38, 34, 31, and 31 animals in trials 1, 2, 3, and 4, respectively, consisting of 46 Dorper, 47, Katahdin, and 41 St. Croix that had been obtained from 45 commercial farms in the summer of 2015. The farms were located in four regions of the USA with different climatic conditions, representing distinct 'ecotypes.' Regions were the Midwest (portions of Iowa, Minnesota, Wisconsin, and Illinois), Northwest (primarily Oregon with one farm in southern Washington and another near Seattle), Southeast (Florida and one farm in southern Georgia), and central Texas. Two farms consisted of separate flocks from the same producer, and there were two other farms from which animals of two breeds of different flocks were obtained. Most animals were ewes when procured, although a small number were ewe lambs. Age at the start of the trials averaged  $3.8 \pm 0.18$  yr, ranging from 1.2 to 11.7 yr. Initial BW was 62.9, 62.4, and 51.3 kg (SEM = 1.43) and body condition score (BCS; 1–5) was 3.77, 3.73, and 3.36 (SEM = 0.055) for Dorper, Katahdin, and St. Croix, respectively.

### 2.2. Procedures

The procedures employed were largely based on the study of Goetsch et al. (2017). Animals were housed in five  $6.1 \times 5.6$  m pens in an enclosed building that had a  $6.1 \times 1.35$  m area with a concrete floor and a  $6.1 \times 4.25$  m unpaved floor area. A group of six to eight ewes of the three breeds were maintained in each pen fitted with eight or nine Calan gate feeders (American Calan, Inc., Northwood, NH). The pens were aligned in a row adjacent to one another. Temperature and humidity monitored indicated that animals were not subjected to cold or heat stress conditions (NRC, 2007).

A 50% concentrate pelleted diet described by Tadesse et al. (2019b) was used. During a preliminary period of 2 wk for adaptation and the first few days of period 1 (i.e., Maintenance phase), 4 wk in length, feed was offered at  $44.4 \text{ g DM/kg BW}^{0.75}$ , assumed adequate to meet the average metabolizable energy (ME) requirement for maintenance. Animals were individually fed with two equal-sized meals at 08:00 and 15:00 h using Calan gate feeders and had access to a continuous supply of fresh water. Body weight was determined three times weekly on Monday, Wednesday, and Friday. There were small changes in the amount of feed offered on an individual animal basis in the Maintenance phase (i.e.,  $\leq 5\%$  of that given most recently) when BW increased or decreased in the previous few days. The intent was for BW to stabilize so that the amount of diet consumed was that required for BW maintenance. In the 6 wk of period 2 or the Restricted phase, DM offered was limited to 55% of that consumed in wk 3 and 4 on an individual animal basis.

Blood samples were collected via jugular venipuncture using 10-ml tubes at 13:00 h on the last day of wk 3, 4, 6, 8, and 10. Serum was obtained by centrifugation for 15 min at  $3000 \times g$  and frozen at  $-20^\circ\text{C}$ . Thawed serum was analyzed for glucose, lactate, urea N, creatinine, total protein, albumin, triglycerides, and cholesterol in samples of wk 4, 6, and 10 with a Vet Axcel® Chemistry Analyzer (Alfa Wassermann Diagnostic Technologies, West Caldwell, NJ, USA) according to the

manufacturer's instructions. The concentration of cortisol was determined with an ELISA kit of Enzo Life Sciences (Farmingdale, NY, USA).

### 2.3. Statistical analyses

Data were analyzed with mixed effects models of SAS (Littell, Henry & Ammerman, 1998; SAS, 2011). Different covariance structures were compared via Akaike's Information Criterion, but values were lower for Variance Components or differences were not marked. The first analysis included blood constituent concentrations in all weeks. Model fixed effects were animal set or trial, age as a covariate, breed, region, week, and all interactions other than ones involving animal set and age. Week was a repeated measure, and animal within breed and region was random. Breed and region means were separated by least significant difference when the treatment F-test was significant ( $P < 0.05$ ). For variables other than cortisol, week means were evaluated by orthogonal contrasts for the Maintenance vs. Restriction phase (mean of wk 3 and 4 vs. mean of wk 6, 8, and 10), week of the Maintenance phase (wk 3 vs. 4), and linear and quadratic effects of advancing week in the Restriction phase (wk 6, 8, and 10). Contrasts for cortisol were for phases (wk 4 vs. mean of wk 6 and 10) and week of the Restriction phase (wk 6 vs. 10). The second analysis was for concentrations in the last week of Maintenance and Restriction phases, wk 4 and 10, respectively, when BW was stable and maximal adaptation to levels of intake had been achieved.

## 3. Results

### 3.1. Concentrations in all weeks

The analysis of blood constituent concentrations in all weeks was to characterize the nature of change in some physiological conditions as animals of the different breeds and regions adapted to the levels of feed intake. There were only two constituents for which the concentration varied among breeds, urea N ( $P < 0.001$ ) and creatinine ( $P = 0.001$ ), and none were affected by region ( $P > 0.05$ ; Tables 1 and 2). Urea N was greatest among breeds for St. Croix ( $P < 0.05$ ) and creatinine was greatest for Dorper ( $P < 0.05$ ).

As generally expected, concentrations of all constituents varied among weeks ( $P < 0.001$ ), although the orthogonal contrasts revealed considerable variation in the nature of week differences. Concentrations were greater for means of weeks in the Maintenance than Restriction phase for glucose, lactate, urea N, triglycerides, and cortisol ( $P < 0.05$ ) and lower in the Maintenance phase for creatinine and cholesterol ( $P < 0.05$ ). In the Maintenance phase, values for total protein, albumin, triglycerides, and cholesterol were greater in wk 4 vs. 3 ( $P < 0.05$ ). In the Restriction phase there were linear and quadratic effects of advancing time ( $P < 0.05$ ) in concentrations of glucose, creatinine, total protein, and albumin. The level of glucose was greatest in wk 10 and similar between wk 6 and 8. The concentration of creatinine increased and then decreased from wk 6 to 8 and wk 8 to 10, respectively, ranking wk  $8 > 10 > 6$ . Concentrations of both total protein and albumin were greatest in wk 10 and similar in wk 6 and 8. There was a quadratic effect of time ( $P < 0.05$ ) for the creatinine concentration, with the value in wk 8 higher than those in wk 6 and 10.

### 3.2. Concentrations at the end of phases

As addressed before, concentrations of blood constituents in sheep of the different breeds and regions of the USA only in the final week of the phases were addressed (Tables 3 and 4) to assess potential differences after maximal physiological adaptation had taken place and little to no further change in BW was occurring. As noted for the analysis of values in all weeks, breed affected levels of urea N and creatinine ( $P = 0.003$  and  $< 0.001$ , respectively), again, with urea N greatest for St. Croix ( $P < 0.05$ ) and creatinine greatest for Dorper ( $P < 0.05$ ). There was also a breed difference in concentration of triglycerides ( $P = 0.042$ ), with the

**Table 1***P* values for effects of breed, region, and week of Maintenance and Restriction phases on blood constituent levels in hair sheep<sup>1</sup>.

Source of variation	Constituent								
	Glucose	Lactate	Urea N	Creatinine	Total protein	Albumin	Triglycerides	Cholesterol	Cortisol
Set	0.975	0.728	0.090	0.002	0.003	0.038	0.031	0.010	0.797
Age	0.543	0.425	0.970	0.605	0.046	0.864	0.459	0.941	0.988
Breed	0.434	0.361	<0.001	0.001	0.855	0.634	0.116	0.214	0.086
Region	0.405	0.600	0.176	0.348	0.499	0.660	0.890	0.359	0.599
Breed*region	0.523	0.153	0.372	0.397	0.780	0.688	0.461	0.076	0.178
Week	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Breed*week	0.773	0.544	0.463	0.187	0.199	0.559	0.102	0.390	0.118
Region*week	0.693	0.208	0.161	0.109	0.693	0.193	0.628	0.782	0.952
Breed*region*week	0.410	0.438	0.117	0.240	0.126	0.654	0.661	0.553	0.133
Week contrasts <sup>2</sup>									
Phase	0.015	<0.001	<0.001	<0.001	0.221	0.422	<0.001	<0.001	<0.001
Maintenance week	0.244	0.482	0.054	0.750	0.003	0.015	0.007	0.019	
Restriction – linear	<0.001	0.342	0.325	<0.001	<0.001	<0.001	0.764	0.228	
Restriction – quadratic	0.003	0.632	0.206	<0.001	0.027	0.045	0.848	0.003	
Restriction – week									0.687

<sup>1</sup>Maintenance (DM intake for steady BW) and Restriction (55% of Maintenance DM intake) phases of 4 and 6 wk, respectively.<sup>2</sup>Phase for variables other than cortisol = mean of wk 3 and 4 vs. mean of wk 6, 8, and 10; Phase for cortisol = wk 4 vs. mean of wk 6 and 10; Maintenance week = wk 3 vs. 4; Restriction–linear = linear change in wk 6, 8, and 10; Restriction–quadratic = quadratic change in wk 6, 8, and 10; Restriction–week for cortisol = wk 6 vs. 10.**Table 2**Effects of breed, region, and multiple weeks of Maintenance and Restriction phases on blood constituent levels in hair sheep<sup>1</sup>.

Item	Breed2				Region3					Week4					
	DOR	KAT	STC	SEM	MW	NW	SE	TX	SEM	3	4	6	8	10	SEM
Glucose (mg/dl)	49.8	49.7	51.7	1.18	49.4	51.1	49.2	52.0	1.37	50.8	51.9	47.1	48.2	54.2	0.92
Lactate (mg/dl)	23.2	22.8	20.5	1.36	23.0	23.2	20.3	22.2	1.57	24.8	23.9	21.5	20.4	20.3	1.12
Urea N (mg/dl)	14.0 <sup>a</sup>	13.7 <sup>a</sup>	15.4 <sup>b</sup>	0.31	14.1	13.9	14.8	14.7	0.36	15.8	16.4	13.3	13.4	13.0	0.26
Creatinine (mg/dl)	0.945 <sup>b</sup>	0.836 <sup>a</sup>	0.809 <sup>a</sup>	0.0253	0.830	0.861	0.861	0.902	0.0293	0.804	0.808	0.835	0.952	0.919	0.0169
Total protein (g/l)	6.83	6.72	6.82	0.154	6.58	6.93	6.79	6.86	0.178	6.68	7.01	6.43	6.90	6.94	0.112
Albumin (g/l)	2.60	2.51	2.52	0.072	2.48	2.59	2.50	2.60	0.083	2.51	2.60	2.43	2.58	2.60	0.047
Triglycerides (mg/dl)	28.4	28.5	26.2	0.84	27.8	27.8	27.1	28.2	0.47	29.6	31.9	25.8	25.8	25.5	0.72
Cholesterol (mg/dl)	76.0	71.9	69.6	2.56	70.8	69.2	74.0	76.1	2.97	63.5	67.5	76.7	80.2	74.7	2.97
Cortisol (mg/ml)	8.11	10.38	8.98	0.756	9.66	9.54	9.29	8.15	0.875		10.55	8.58		8.31	0.542

<sup>1</sup>Maintenance (DM intake for steady BW) and Restriction (55% of Maintenance DM intake) phases of 4 and 6 wk, respectively.<sup>2</sup>DOR = Dorper; KAT = Katahdin; STC = St. Croix.<sup>3</sup>MW = Midwest; NW = Northwest; SE = Southeast; TX = central Texas.<sup>4</sup>Wk 3 and 4 were the last of the Maintenance phase and the Restriction phase was in wk 5–10. <sup>5a,b</sup>Means within grouping without a common superscript letter differ (*P* < 0.05).**Table 3***P* values for effects of breed, region, and Maintenance and Restriction phases on blood constituent levels in hair sheep<sup>1</sup>.

Source of variation	Constituent								
	Glucose	Lactate	Urea N	Creatinine	Total protein	Albumin	Triglycerides	Cholesterol	Cortisol
Set	0.453	0.439	0.634	0.006	0.003	0.018	0.001	0.024	0.987
Age	0.944	0.093	0.245	0.380	0.167	0.788	0.466	0.722	0.865
Breed	0.607	0.314	0.003	<0.001	0.996	0.709	0.042	0.054	0.188
Region	0.519	0.518	0.014	0.101	0.404	0.676	0.674	0.159	0.494
Breed*region	0.698	0.176	0.483	0.370	0.985	0.867	0.545	0.140	0.142
Phase	0.051	0.001	<0.001	<0.001	0.610	0.887	<0.001	<0.001	0.001
Breed*phase	0.504	0.911	0.340	0.570	0.155	0.369	0.098	0.101	0.105
Region*phase	0.868	0.041	0.222	0.342	0.648	0.316	0.278	0.612	0.895
Breed*region*phase	0.707	0.144	0.066	0.263	0.550	0.532	0.083	0.179	0.131

<sup>1</sup>Maintenance (DM intake for steady BW) and Restriction (55% of Maintenance DM intake) phases of 4 and 6 wk, and means were of samples collected in the final week of the phases (wk 4 and 10, respectively).

lowest level for St. Croix (*P* < 0.05). A numerical effect of breed (*P* = 0.054) occurred in the concentration of cholesterol, with values ranking Dorper > Katahdin > St. Croix. Region affected the concentration of urea N (*P* = 0.014), with values for the Southeast and Texas being greater than those for the Midwest and Northwest (*P* < 0.05). Also, there was a region × phase interaction in the level of lactate (*P* = 0.041), which resulted from values greater in the Maintenance vs. Restriction phase for the Midwest and Southeast (*P* < 0.05) but similar means for the Northwest and Texas.

Most differences between phases were similar to those based on

values of all weeks, although there were some with slight deviations. For example, there was a tendency for a higher glucose concentration in the Restriction than Maintenance phase (*P* = 0.051), in contrast to the opposite difference based on means of all weeks within phases. The main effect mean for lactate concentration was greater in the Maintenance than Restriction phase (*P* = 0.001), although as noted before this difference was the result of ones for animals from two of the four regions. As noted for all weeks, the level of urea N was greater and that for creatinine was lower in the Maintenance vs. Restriction phase (*P* < 0.001). There were no phase effects on concentrations of total protein or

**Table 4**  
Effects of breed, region, and Maintenance and Restriction phases on blood constituent levels in hair sheep<sup>1</sup>.

Item	Phase	Breed <sup>2</sup>				Region <sup>3</sup>				Phase			
		DOR	KAT	STC	SEM	MW	NW	SE	TX	SEM	Maintenance	Restriction	SEM
Glucose (mg/dl)		52.5	52.6	54.1	1.21	51.7	53.3	52.8	54.5	1.39	51.9	54.2	0.90
Lactate (mg/dl)		21.7	23.6	20.9	1.28						23.9 <sup>b</sup>	20.3 <sup>a</sup>	0.89
	Maintenance					25.7 <sup>b</sup>	21.2 <sup>ab</sup>	24.4 <sup>b</sup>	24.0 <sup>b</sup>	1.78			
	Restriction					20.2 <sup>ab</sup>	21.0 <sup>ab</sup>	17.0 <sup>a</sup>	22.8 <sup>b</sup>				
Urea N (mg/dl)		14.3 <sup>a</sup>	14.1 <sup>a</sup>	15.7 <sup>b</sup>	0.34	14.0 <sup>a</sup>	14.1 <sup>a</sup>	15.4 <sup>b</sup>	15.2 <sup>b</sup>	0.39	16.4 <sup>b</sup>	13.0 <sup>a</sup>	0.25
Creatinine (mg/dl)		0.947 <sup>b</sup>	0.849 <sup>a</sup>	0.795 <sup>a</sup>	0.0258	0.807	0.861	0.884	0.902	0.0298	0.808 <sup>a</sup>	0.919 <sup>b</sup>	0.0165
Total protein (g/l)		6.97	6.97	6.99	0.139	6.76	7.09	7.07	6.98	0.161	7.01	6.94	0.101
Albumin (g/l)		2.65	2.59	2.57	0.067	2.53	2.65	2.60	2.62	0.078	2.60	2.60	0.043
Triglycerides (mg/dl)		29.8 <sup>b</sup>	29.5 <sup>b</sup>	26.7 <sup>a</sup>	0.88	28.9	29.6	27.9	28.2	1.02	31.8 <sup>b</sup>	25.5 <sup>a</sup>	0.63
Cholesterol (mg/dl)		75.6	71.0	66.7	2.52	68.4	67.1	74.3	74.6	2.92	67.5 <sup>a</sup>	74.7 <sup>b</sup>	1.66
Cortisol (ng/ml)		8.68	10.6	9.07	0.811	10.1	9.77	9.66	8.24	0.94	10.6 <sup>b</sup>	8.35 <sup>a</sup>	0.56

<sup>1</sup>Maintenance (DM intake for steady BW) and Restriction (55% of Maintenance DM intake) phases of 4 and 6 wk, and means were the final week of the phases (wk 4 and 10, respectively).

<sup>2</sup>DOR = Dorper; KAT = Katahdin; STC = St. Croix.

<sup>3</sup>MW = Midwest; NW = Northwest; SE = Southeast; TX = central Texas. <sup>a,b</sup>Means within grouping without a common superscript letter differ ( $P < 0.05$ ).

albumin ( $P < 0.005$ ), as was the case for the all-week analysis. The degree to which the concentration of triglycerides was greater in the Maintenance than Restriction phase ( $P < 0.001$ ) was similar to that based on levels in all weeks. Conversely, the magnitude of difference in cholesterol concentration between Maintenance and Restriction phases (greater for Maintenance,  $P < 0.001$ ) was less than based on values of all weeks. The difference between phases in cortisol concentration ( $P = 0.001$ ) was the same as noted earlier since wk-8 and -10 means were similar.

## 4. Discussion

### 4.1. Breed and region

One of the factors that may have contributed to the higher concentration of urea N for St. Croix than for Dorper and Katahdin sheep is the relatively low BCS for St. Croix (Tadesse et al., 2019b), implying greater mobilization of proteinaceous tissue. Although, the magnitude of difference was not substantial (1–5; initial values of 3.77, 3.73, and 3.36 and trial averages of 3.66, 3.62, and 3.30 for Dorper, Katahdin, and St. Croix, respectively). Also, the ME requirement for maintenance without and with restricted feed intake was slightly greater for St. Croix (Tadesse et al., 2019b), also inferring greater tissue mobilization. The lack of interaction between breed and week in urea N concentration, a similar breed difference for both the analysis involving all weeks and only the last week of each phase, and no differences between or among weeks within phases suggest that physiological processes affected by level of feed intake changed relatively quickly in animals of each breed and region. However, there were differences among regions in urea N concentration for the analysis of values only in wk 4 and 10. Values for the two regions with generally warmer climates (i.e., Southeast and Texas) were greater than those for the other regions (i.e., Midwest and Northwest).

The factor most likely responsible for the higher concentration of creatinine for Dorper than for Katahdin and St. Croix sheep is an assumed greater muscle mass for Dorper (Burke & Apple, 2007; Rumosa Gwaze, Chimonyo & Dzama, 2010; Kashani, Rosner & Ostermann, 2020). The similar magnitude of difference for the analysis of concentrations in all weeks and the last week of the two phases is supported by comparable change among breeds in the ME requirement for maintenance when feed intake was restricted (Tadesse et al., 2019b).

The lower triglyceride concentration for St. Croix vs. Dorper and Katahdin sheep for the analysis of wk 4 and 10, when physiological conditions should have been most stable, is in accordance with the BCS difference and generally greater body fat levels for Dorper and Katahdin (Burke & Apple, 2007). However, the numerical breed difference for the analysis of the triglyceride level in all weeks was of slightly lesser

magnitude. Also similar to the significant breed difference in triglyceride concentration for the analyses of wk-4 and -10 values but not all weeks is the tendency for breed differences in the level of cholesterol for the former analysis. Reasons why levels of both triglyceride and cholesterol with this analysis were lowest or tended to be lowest for St. Croix in contrast to phase differences, with a higher triglyceride concentration and lower level of cholesterol in the Maintenance than Restriction phase, are unclear.

The lack of breed differences in glucose, lactate, total protein, and albumin concentrations and relatively small magnitudes of difference when significant in levels of constituents are in accordance with fairly similar ME requirements for maintenance without and with feed restriction (Tadesse et al., 2019b). Moreover, the findings probably reflect the considerable capacity of each hair sheep breed to minimize energy use with feed restriction. No breed differences or interactions between breed and week or phase in the level of cortisol also imply that any stress elicited by feed restriction did not vary among breeds. Lastly, few significant differences and interactions involving region agree with findings of Tadesse et al. (2019b) in that geographic origin was not of major importance regardless of breed. However, Tadesse et al. (2019b) indicated that the period of time between when the animals arrived at the research site and conduct of the trials could have resulted in some acclimation and, thus, lessened potential for occurrence of significant region effects.

### 4.2. Week of measurement and phase

Changes in concentrations of blood metabolites with feed restriction occur to maintain homeostasis (Felix, Radunz & Loerch, 2011). The differences in concentrations of total protein, albumin, triglycerides, and cholesterol and the tendency for a difference in urea N concentration between wk 3 and 4 of the Maintenance phase indicate that even though BW was generally static in this period of time, some physiological conditions were still changing. But, magnitudes of difference were generally much less than for some constituents between these weeks and ones of the Restriction phase as well as among weeks of the Restriction phase. As noted by Tadesse et al. (2019b), the procedures of this trial were based on the study of Goetsch et al. (2017). However, that experiment did not include measures of blood constituent concentrations.

The lower concentration of glucose in the Maintenance than Restriction phase for the analysis of all weeks agrees with some but not all findings of Keogh et al. (2015). In that study, Holstein-Friesian bulls averaging 479 days of age were subjected to restricted intake of a 70% concentrate diet over a 125-day period for ADG of 0.6 kg or had ad libitum consumption resulting in ADG of 1.9 kg. The concentration of glucose was less for the restricted than ad libitum intake treatment throughout the study. However, it is notable that for some other



constituents differences were not consistent throughout the restriction phase. In this regard, in the current experiment for the analysis of constituent levels in the last week of the two phases, the glucose concentration tended to be greater for the Restriction than Maintenance phase as a result of the linear and quadratic changes as time advanced in the Restriction phase, converse to findings of Keogh et al. (2015). Certainly the difference in animal age and growth potential contributed to the disparate findings of these studies.

Although glucose and lactate metabolism are highly interrelated (Abbas et al., 2020; Huntington, 1999), in contrast to glucose the magnitude to which the concentration of lactate in the Maintenance phase was greater than when feed was restricted was similar between the two methods of analysis, with no change as week of the Restriction phase advanced. Likewise, the concentration of urea N was lower during the Restriction vs. Maintenance phase, with similar values among wk 6, 8, and 10. This is in accordance with findings of Keogh et al. (2015) on day 55, 93, and 125, although the concentration was greater for restricted vs. ad libitum intake on day 1 and 25. Higher urea N levels without than with feed restriction can be attributed to greater ruminal ammonia levels and absorption without feed restriction (Caldeira, Belo, Santos, Vazques & Portugal, 2007).

The higher concentration of creatinine during the Restriction than Maintenance phase agrees with findings of Keogh et al. (2015), although differences in the current study would not involve muscle deposition. Rather, some protein catabolism would be anticipated early in the Restriction phase, which presumably would slow and then cease at some point, perhaps in the latter week(s) of the phase as is inherent in the study design. The linear and quadratic effects of week within the Restriction phase imply little proteinaceous tissue mobilization early, followed by a rapid increase and then a decline to eventually become zero. Relatedly, with means separation by least significant difference, the concentration of creatinine was similar among wk 3, 4, and 6. Though greater muscle mass for Dorper than for Katahdin and St. Croix was postulated to be responsible for the breed difference in creatinine concentration, evidently this did not impact the magnitude or pattern of change with time during the Restriction phase.

Results for concentrations of total protein and albumin were considerably different than noted by Keogh et al. (2015), with higher levels of both variables in restricted bulls early in the period. Later, the level of albumin was similar between treatments but the concentration of total protein was less with restricted intake. In the present experiment, there were no differences between phases with either analysis. There were linear and quadratic effects of advancing week of the Restriction phase, with a lower mean in wk 6 vs. 8 and 10. Thus, it would appear that physiological conditions governing blood protein levels stabilized relatively quickly regardless of breed.

The lower concentration of triglycerides in the Restriction than Maintenance phase for both analyses agrees with findings of Keogh et al. (2015) in the early restriction period but not with similar values later for bulls on the two treatments. The lack of change in the triglyceride concentration as week of the Restriction phase of the current experiment advanced indicates that associated physiological conditions stabilized quickly, again regardless of differences among breeds such as in body composition (Burke & Apple, 2007; Burke, Apple, Roberts, Boger & Kegley, 2003). The higher concentration of cholesterol during the Restriction than Maintenance phase presumably relates to its involvement in mobilization of adipose tissue as an energy source (Carbone, McClung & Pasiakos, 2012; Ruegg et al., 1992). The quadratic effect of week of the Restriction phase on cholesterol concentration probably reflects change in the metabolic rate, with significant need for lipid mobilization early but then decreasing to eventually become negligible. Again, it would appear that these changes were similar among breeds.

The lower concentration of cortisol in the Restriction than Maintenance phase suggests that feed restriction did not create any or marked stress for sheep of any breed or region. However, blood cortisol levels are highly variable, impacted by factors including feeding time,

handling procedures, etc. (Lérias et al., 2015). It should be noted again that animals in the present study, though in groups, were fed individually. That is, it is possible that with group feeding, restriction could increase aggressive behaviors with elevated levels of stress, as more dominant animals presumably would strive to consume more feed than intended restricted amounts.

## 5. Summary and conclusions

Level of feed intake, at the requirement for maintenance and 55% of that amount, affected blood concentrations of most constituents assessed. There were some differences among breeds of hair sheep, Dorper, Katahdin, and St. Croix, such as in levels of urea N, creatinine, and triglycerides. However, region of the USA from which the hair sheep originated had few effects on blood constituent concentrations at different times without and with feed restriction. There were no interactions between breed and phase or week of the study. Thus, in accordance with a similar magnitude of change in the maintenance energy requirement previously determined, feed restriction had similar effects on blood constituent concentrations in different hair sheep breeds of Dorper, Katahdin, and St. Croix. Future research should address aspects such as change in energy utilization during a period of realimentation after feed restriction.

## Ethical statement

The protocols for the experiments were approved by the Langston University Animal Care Committee.

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