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Legume Addition to Grass Pastures Improved the Performance and Health Indicators of Kiko Does

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ABSTRACT

Legume addition to grass pastures is expected to improve forage quality and enhance animal performance; however, such effects on goats are not well published. We hypothesized that legume inclusion in grass pastures would improve the performance and health indicators of grazing goats. The study objective was to evaluate the impact of legume addition to grass pastures on the performance and health indicators of goats. Nineteen yearling Kiko does were divided into legume-grass (Southern peas (*Vigna unguiculata* (L.) Walp.)-browntop millet (*Urochloa ramosa* L. Nguyen) (50:50)) and sole-grass (sole browntop millet) groups and rotationally grazed in their assigned plots for 87 days. Forage samples were collected and analyzed for productivity and quality (crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF)). Does' performance data (live weight, body condition score (BCS), and FAMACHA score) were collected on Day 1, fortnightly, and at the end. Blood samples were collected and analyzed for biochemical and hematological parameters and fecal samples for nutrients (Days 1. 47, 87). Legume-grass pastures showed better quality than sole-grass pastures ($p<0.05$). Does grazing legume-grass pastures showed better performance (live wt. 5%, FAMACHA score 13%, BCS 4%) vs. sole-grass group ($p<0.05$). Legume-grass group had higher blood urea nitrogen (BUN), BUN/Creatinine ratio, and glucose on Days 47 and 87, and basophil on Day 47 vs. sole-grass group ($p<0.05$). On Day 47, fecal P was higher ($p<0.05$) in legume-grass vs. sole-grass group. Results showed that legumes enriched the pasture quality and eventually enhanced the performance and health indicators of grazing goats.

INTRODUCTION

The pasture-based goat production system is dominant in the southeast USA. More than 65 % of goat operations depend upon pastures (USDA-APHIS, 2012), which provide more than 80% of their nutrients in the United States (WSU, n.d.). However, goat pastures in most goat-producing states in the Southeast are low productive, seasonal, and poor in quality, resulting in inferior performance of goats (Karki & Karki, 2017). USDA-APHIS (2017) reported the death of 4,649 goats in 2015 due to malnourishment in most goat-producing US states. Studies have demonstrated that pasture quality and productivity can be improved by mixing two or more forage species compared to sole-grass pastures (Sturludóttir *et al.*, 2014). The inclusion of legumes in grass pastures would be an appealing alternative to improve pasture productivity and quality, and ultimately improving animals' diets as legumes consist of higher levels of crude protein and less fiber than grasses.

Putri *et al.*, (2021) stated that in high protein diet, intake and ruminal digestibility improved with increased microbial population, which in turn give rise to volatile fatty acid and microbial protein to host animals. Volatile fatty acids especially propionate is the precursor of the glucose in ruminant whereas microbial protein is an essential source of amino acids that are required for growth, health, and body maintenance in animals (Loncke *et al.*, 2020; Direkvandi *et al.*, 2020). Previous study found that cattle receiving high (CP 14.2% of dry matter) protein diet resulted in increased level of microbial protein and volatile fatty acids in the rumen than low protein diet (CP 10.2% of dry matter) (Xia *et al.*, 2018) . Consequently, the impact of microbial synthesis is reflected in the live weight gain of animals. In a study of goats, the average daily gain was 86 gram/day in high protein (76 gm/day DM) diet whereas 27 gram/day was reported in low protein diets (37 gm/day DM) (Phengvichith & Ledin, 2007). Live weight provides the nutritional status of the animals, which helps to determine the efficacy of the feeding materials. Protein metabolism in rumen not only helps with the animal growth but also has significant role in the animal health. Deficiency of dietary protein or amino acids in animal body markedly affect the immune system that makes host susceptible to the invaders and animals perform poorly (Li *et al.*, 2007). It is reported that the majority of immune components are composed of protein substrate (Coop & Holmes, 1996). For instance, cytokines and antibodies which are made up of amino acids are essential to keep the immune system functional to fight against diseases (Li *et al.*, 2007). Above findings suggested that higher protein either obtained from forages or supplemented diets plays a crucial role in the growth and proper functioning of immune system in animals. However, the beneficial impact of legume forages as supplemental protein diet in grazing sheep has not been reported well.

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LITERATURE REVIEW

A study showed bromegrass (*Bromus inermis*) and Kura clover (*Trifolium ambiguum*) mixed pastures producing greater biomass (77%) with higher crude protein (46%), resulting in improved seasonal distribution of quality forages as compared to sole bromegrass (Sleugh *et al*., 2000). Another study by Karki *et al*. (2009) reported greater biomass (40%) and N concentration (27%) for bahiagrass (*Paspalum notatum* Fluegge)-crimson clover (*Trifolium incarnatum* L.) mix vs. sole-bahiagrass stand. Enhanced productivity of legume-grass pastures is mainly contributed by the nitrogen-fixing ability of legumes (Nyfeler *et al*., 2011). Improved nitrogen content in soil eventually increases the protein content in forages, resulting in increased dry-matter intake by animals (Catanese *et al*., 2010). Bertilsson & Murphy (2003) demonstrated a higher dry-matter intake in dairy cows when they were fed the mixture of red clover (*Trifolium pratense* L.) and perennial ryegrass (*Lolium perenne* L.) (15.1 kg/day) than when fed perennial-ryegrass silage only (13.2 kg/day) in the first year of forage cutting. Unlike cattle, small ruminants are known to do well on a wide variety of forages and browse (woody species), usually not eaten by cattle (Saner, n.d.). However, when diet quality is low and/or intake is insufficient, the performance and health condition of small ruminants would be affected and warrant supplementation or pasture improvement.

Live weight, body condition score, and FAMACHA score are common parameters used to monitor the health and performance of meat goats. Negesse *et al*. (2001) reported that the body weight of growing male goat kids increased sequentially with the increased supplementation of crude protein (CP) (80, 105, 128, 155 g CP/kg dry matter) in a pen-fed trial. High-protein diet would also improve body condition scores (Mohsan *et al*., 2019). Live weight, body condition score, and FAMACHA score are common parameters used to monitor the health and performance of meat goats. FAMACHA is a chart with 5-color categories that are used to monitor the anemic condition caused by *Haemonchus contortus* in small ruminants (Karki, 2013). Bricarello *et al*. (2005) found improved FAMACHA score in lambs when supplemented with protein compared to when they were fed on a restricted diet only. A study by Zhu *et al*. (2020) discovered a reduced concentration of blood urea nitrogen (BUN) and blood glucose with a decreased amount of dietary protein (from 14.8% to 12% of dry matter) in growing goat kids. Similarly, hematocrit and albumin concentrations were increased in naturally parasite-infested lambs (under grazing and pen-fed conditions) when they were fed a high-protein (14.99% CP vs. 8.58% CP) diet (Mendes *et al*., 2018). Packed-cell volume, red blood cells, white blood cells, monocyte, and lymphocyte count of goats were improved when they were consuming legume tree (*Pterocarpus erinaceus* Poir)-grass mixed diet as compared to those on sole gamba grass (*Andropogon gayanus*) (Olafadehan, 2011). However, the impact of legume addition to grass pastures on the health and performance of goats raised solely raised on a pasture-based system is not documented well.

Besides changes in blood parameters, nutrient retention is an important aspect to explore in animals consuming high-quality diet, including pastures containing leguminous forages. Extra nutrients consumed by animals beyond their requirements or beyond their ability to use in the body would be lost in the form of urine and feces (Hristov *et al*., 2019). Such loss or retention in grazing goats may vary depending on the type and quality of forages they consume. Orellana *et al*. (2020) suggested fecal nitrogen and phosphorous as important indicators of the nutritional status of animals. A low level of fecal nitrogen indicates poor intake of dietary protein in animals (Leslie *et al*., 1989). Zhu *et al*. (2020) reported that an increased level of fecal nitrogen in pen-fed growing male goat kids was contributed by poor digestibility of crude protein, possibly resulting from the inhibitory digestive effect of pectin present in fibrous grass (Gressley & Armentano, 2005). Change in CP content in the animal diet may influence phosphorus (P) digestibility, and P concentration in the diet is correlated with fecal P concentration (Xue *et al*., 2017). Both nitrogen and phosphorus are crucial nutrients and information on their retention in grazing goats in response to the added leguminous forages is lacking. This study tested the hypothesis that legume inclusion in grass pastures would improve the health and performance of grazing goats. The objective of the study was to evaluate the impact of legume addition to grass pastures on the performance and health indicators of Kiko does.

MATERIALS AND METHODS

Study site

The study was conducted in 10 grazing plots (0.4 ha each) located at the Browse Research and Demonstration site (32°26'00.7" N 85°43'00.2" W), Tuskegee University, Tuskegee, Alabama, United States. The study site consisted of Cowarts loamy sand (89.4%; slope 5-15%) and Marvyn loamy sand (10.6 %; slope 2-5%) (USDA-NRCS, 2017). Plots were well equipped with grazing facilities for goats and sheep (shelters, water lines, watering troughs, and mineral feeders). The site was enclosed within a strong and tall perimeter fence. Study plots were fenced separately, and each plot had a 3.5-m-wide gate to move animals in and out for rotational grazing and moving planting equipment and accessories as required.

Fertilizer application and forage plantation

Soil samples were collected and tested for pH and nutrient content (N; P; Potassium, K) at the Soil, Forage, and Water testing laboratory of Auburn University, Auburn, Alabama, in the summer of 2020. The recommended amount of lime (0.5 - 3 tons/plot) based on the soil test results was applied three months before planting. Triple superphosphate (46% P) (40-60 kg/plot) and muriate of potash (60% K) (18 – 54 kg/plot) were applied in all plots at the time of seed sowing. After the application of P and K fertilizers, southern peas (*Vigna unguiculata* (L.) Walp.)-browntop millet (*Urochloa ramosa* L. Nguyen) 50:50 mix) were sown in five plots, whereas the remaining plots were sown with sole browntop millet with a no-till drill. Forage sowing began in the last week of June and was completed in the first week of July. When grass forage developed 2-4 leaves, about one month after seed sowing, ammonium nitrate (34% N) (56 - 117 kg/plot) was applied in sole-grass plots.

Forage height and biomass

The study was started once forages were established well and the average canopy height reached around 12 inches or higher. One day before bringing animals into grazing plots, before-grazing forage height was measured at 40 different spots per plot using a grazing stick. After grazing, forage heights were measured after animals were moved out of each plot. Measurement of before- and after-grazing forage heights were taken for each rotational grazing, and this information was used to find out the reduction in forage height because of animals' grazing activities. After collecting before-grazing forage height at each rotation, ten random forage samples per plot were clipped to four inches above the ground surface within 0.25 m^2 quadrats to determine the available biomass. Forage samples were brought to the Agroforestry and Grazing Ecology Laboratory of Tuskegee University and placed in the dryer set at 60°C for 72 hours. Dried samples were taken out and allowed to cool down to room temperature, then weighed to determine the dry matter. Subsequently, dry samples were ground for quality analysis.

Assessment of forage quality

For assessment of forage quality, ground samples collected at different dates from the same plot were mixed to make a composite sample. Each composite sample was replicated into eight sub-samples and analyzed for crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) using Near-Infrared Spectroscopy (NIRS). Total digestible nutrient (TDN) was derived from ADF (82.38-(.7515*ADF)) (Bath & Marble, 1989). Calibration and validation of the NIRS equation were done before developing the final NIRS model using the results obtained from both wet chemistry and NIRS for similar samples collected in 2019. A mathematical relationship was established using the spectral values obtained from NIRS and wet-chemistry results of the same set of samples. Random samples from a set of calibration samples were selected for further validation of the NIRS model. After generating a NIRS model, a rotating cup was filled with ground samples of each replicate at room temperature and allowed to pass spectra of a wavelength of 4209-6994 nm, and the resulting spectra were collected. Spectral values were exported to Microsoft Excel sheet for statistical analyses.

Research animals and groups

After forage height measurement and sample collection was completed in the designated two plots, one each from legume-grass and sole-grass pastures, the grazing study was started with 19-yearling Kiko does with an initial age of 15-16 months and 34 (± 1.4 SE) kg live weight. Does were divided into two identical groups (legume-grass and sole-grass group) based on their body weight, FAMACHA score, and body condition score (BCS). The legume-grass group of does (10) were assigned to legume-grass plots, and the sole-grass group of does (9) to sole-grass plots. Each group was rotated among their designated plots for 87 days (mid-August to early November). Animals were moved out from a plot once 50% of the vegetation was consumed. The proportion of vegetation removal was monitored visually daily, with a grazing stick and using photo plots. Does had access to clean drinking water and loose minerals designed for goats (Purina goat minerals) at all times. Animals were closely monitored every morning and evening and provided needed care throughout the study period following the protocol of the Animal Care and Use Committee of Tuskegee University.

Performance data

Live weight, BCS, and FAMACHA scores were measured on the very first day before allocating animals to the research plots, then every 14 days during the study, and on the very last day of the study. A digital weighing scale was installed at the research site's handling facility to weigh animals. Body condition score was assessed on a scale of 1 to 5 by feeling the muscles and fat along the backbone, ribs, and brisket bone. Score 1 indicated extremely lean condition and 5 represented obese condition. A FAMACHA card was used to assess the FAMACHA score (1-5) by matching the conjunctiva color on the lower eyelid on both eyes of each animal. FAMACHA scores 1 and 2 indicate non-anemic and healthy condition, score 3 represents conditional status meaning that weak animals and those with other health risks should be treated, and 4 and 5 scores indicate anemic condition caused by *Haemonchus contortus*, a blood-sucking abomasal helminth parasite. A single person monitored the BCS and FAMACHA scores throughout the study period to minimize possible inter-personal variations.

Blood sample collection and analyses

The first set of blood samples was collected right before the deployment of animals to the research plots on Day 1 of the study. The second set of samples was collected in the middle of the study (Day 47), and the final set was on the last day of the study (Day 87). Samples were collected from the jugular vein into two different vacutainers: one with anticoagulant (ethylene diamine tetra-acetic acid (EDTA)) – with a purple cap and another without it (a tube with a red cap). Samples with EDTA were gently shaken a couple of times immediately after collection to prevent coagulation. Samples without EDTA were kept undisturbed in a tube holder. Immediately after collection, blood samples were taken to the Pathobiology laboratory of Tuskegee University and processed for analysis. Samples without EDTA were centrifuged for 2-3 minutes to separate plasma, which was used to analyze the cell-free component of blood. Further, the hematological parameters of blood samples were assessed in the ProCyte Dx analyzer, and the biochemical parameters were evaluated in the Catalyst Dx analyzer (Table 1).

Table 1: List of hematological and biochemical parameters of Kiko does grazing legume-grass vs. sole-grass pastures, mid-August to early November 2020, Browse Research and Demonstration Site, Tuskegee University, Alabama, USA.

Hematological Parameters	Biochemical Parameters
Red blood cell	Glucose
Hematocrit	Creatinine
Hemoglobin	Blood urea nitrogen
Mean corpuscular volume	Blood urea nitrogen/ creatinine
Mean corpuscular hemoglobin	Albumin
Mean corpuscular hemoglobin concentration	Globulin
Reticulocyte	Albumin/globulin
White blood cell	Alanine amino transferase
Neutrophil	Alkaline phosphate
Lymphocyte	Gamma-glutamyl transferase
Monocyte	Total bilirubin
Eosinophil	Cholesterol
Basophil	Sodium
Platelets	Potassium
Mean platelet volume	Sodium/potassium
	Chlorine
	Phosphorous
	Calcium
	Serum Osmolality

Fecal sample collection and analysis

Fecal samples were collected on the same days as blood samples. Fresh fecal (12-15 grams) samples were collected from each animal and kept cold to protect it from degradation. Soon after collection, fecal samples were shipped overnight to the Texas Grazing Land Animal Nutrition Lab (GANLAB) maintaining a cold chain for the analysis of fecal nutrients: P and N concentrations. Samples were analyzed in NIRS in GANLAB using an already established NIRS equation.

Statistical analysis

All data sets were analyzed in SAS 9.4, and the significance level (alpha) for testing the hypothesis was set at 0.05. Forage dry matter data were analyzed using the Mixed Procedure with a plot as a random factor. Data were log transferred for analysis to satisfy the assumption of normal distribution and results were back transformed for presentation. The upper and lower confidence limits were derived as a measure of dispersion. The mixed procedure used to analyze forage dry matter is presented below.

$Yi = \mu + \alpha i + ei$

Where,

Yi = value of observation for ith forage type, μ = grand mean, αi = main effect of forage type, and ei = an error associated with the ith forage type, Random factor = plot.

Similarly, forage quality (CP, ADF, NDF, and TDN) data were analyzed using the following GLM Procedure with MANOVA option as these variables were correlated.

$Y(1\text{-}4)i = \mu + \alpha i + ei$

MANOVA h = Forage type, where $Y(1\text{-}4)i$ = forage quality variables, μ = grand mean, αi = main effect of the ith forage type, ei = error associated with the ith forage type.

Animal performance data were analyzed using the GLM Procedure with the following model.

$Y(1\text{-}3)i = \mu + \alpha i + ei$

MANOVA h = Animal group, repeated factor = Individual animal

Where $Y(1\text{-}3)i$ = animal performance variables, μ = grand mean, αi = main effect of the group, ei = error associated with the ith group.

Similarly, blood parameter data were analyzed using the GLM Procedure as per the model given below.

$Y(1\text{-}34)i = \mu + \alpha i + ei$

Where $Y(1\text{-}34)i$ = values of blood parameters for the ith group of animals, μ = grand mean, αi = main effect of the group, ei = error associated with the ith group.

Fecal nutrients (DOM, P, and N) were analyzed using the GLM procedure with the following model.

$Y(1\text{-}3)i = \mu + \alpha i + ei$

MANOVA h = Animal group, repeated factor = Individual animal

Where, $Y(1\text{-}3)i$ = fecal nutrient variables, μ = grand mean, αi = main effect of the ith group, ei = error associated with the ith group.

RESULTS AND DISCUSSIONS

Forage height and biomass production

Goats in this study consumed both forage types, legume-grass, and sole-grass, well from the very first day to the end of the study. Reduction in forage height because of animal grazing was significant ($p<0.05$), with 42% in legume-grass plots and 40% in sole-grass plots (Figure 1). Forage biomass production was not different between legume-grass and sole-grass pastures (Table 2).

Table 2: Forage biomass from legume-grass and sole-grass pastures, mid-August to early-November 2020, Browse Research and Demonstration site, Tuskegee University, Tuskegee, Alabama, USA.

Treatment	Forage Biomass (kg/ha)		
	LSMean	Lower CL	Upper CL
Browntop millet (*Urochloa ramosa* L. Nguyen)- southern peas (*Vigna unguiculata* (L.) Walp.) 50:50 mix	1559	1217	1996
Sole browntop millet	1411	1081	1843

Forage quality

Forages from legume-grass plots had higher CP (42%) and TDN (6%) than those from sole-grass plots ($p<0.05$) (Table 3). ADF and NDF in legume-grass pastures were lower by 18% and 16%, respectively, as compared to the sole-grass pastures ($p<0.05$).

Table 3: Quality of forages from legume-grass and sole-grass plots, mid-August to early-November 2020, Browse Research and Demonstration site, Tuskegee University, Tuskegee, Alabama, USA.

Treatment	Forage Quality (%)			
	CP	NDF	ADF	TDN
Browntop millet (*Urochloa ramosa* L. Nguyen)-southern peas (*Vigna unguiculata* (L.) Walp.) 50:50 mix	LSMean ± SE			
	17 ± 0.2^{a*}	49 ± 0.7^{b}	34 ± 0.4^{b}	57 ± 0.4^{a*}
Sole browntop millet	12 ± 0.2^{b}	57 ± 0.8^{a*}	38 ± 0.4^{a*}	54 ± 0.3^{b}

^{ab}*Values in the same column with different superscripts differ (*p<0.05). CP – crude protein, NDF – neutral detergent fiber, ADF – acid detergent fiber, TDN – total digestible nutrients.*

Animal performance

The live weight of does stocked in legume-grass pastures was 5% higher as compared to the sole-grass group ($p<0.05$) (Table 4). However, when analyzing the observation dates, there were no significant differences between the two groups on any of the live weight measurement dates. Overall, the body condition score was better in the legume-grass group (2.6 ± 0.02) than in the sole-grass group, while the interaction effect of the group and observation date was found towards the end of the study ($p<0.05$) (Table 4). Similarly, the FAMACHA score of the legume-grass group (2.1 ± 0.06) was improved than the sole-grass group does ($p<0.05$). While looking at different observation dates, FAMACHA scores between the two groups differed only on the last day of the study (Table 4).

Table 4: Performance (live weight, body condition score, and FAMACHA score) of does grazing legume-grass or sole-grass pastures, mid-August to early-November 2020, Browse Research and Demonstration site, Tuskegee University, Tuskegee, Alabama, USA.

Observations	Live Weight (kg)		BCS		FAMACHA Score	
	LGG	GG	LGG	GG	LGG	GG
1	34.3 ± 1.32	34.4 ± 1.39	2.5 ± 0.06	2.5 ± 0.06	2.5 ± 0.17	2.6 ± 0.18
2	35.3±1.32	34.3 ± 1.39	2.5 ± 0.06	2.5 ± 0.06	2.4 ± 0.17	2.4 ± 0.18
3	36.4 ± 1.32	34.2 ± 1.39	2.7 ± 0.06	2.6 ± 0.06	1.9 ± 0.17	2.3 ± 0.18
4	37.9 ± 1.32	35.9 ± 1.39	2.5 ± 0.06	2.7 ± 0.06	2.4 ± 0.17	2.4 ± 0.18
5	38.8 ±1.32	36.2 ± 1.39	2.7 ± 0.06 ^{a*}	2.4 ± 0.06 ^{b}	2.0 ± 0.17	2.4 ± 0.18
6	39.6 ± 1.32	37.0 ± 1.39	2.7 ± 0.06	2.6 ± 0.06	1.9 ± 0.17	2.2 ± 0.18
7	39.6 ± 1.32	37.8 ± 1.39	2.8 ± 0.06 ^{a*}	2.5 ± 0.06 ^{b}	1.6 ± 0.17^{b}	2.1 ± 0.18^{a*}
Overall Mean	37.4 ± 0.50^{a*}	35.7 ± 0.53^{b}	2.6 ± 0.02^{a*}	2.5 ± 0.03^{b}	2.1 ± 0.06^{b}	2.4 ± 0.07^{a*}

^{ab}*Values in the same row within each variable with different superscripts differ (*p<0.05).*
LGG: Legume-grass group, GG: Sole-grass group

Hematological parameters

Overall, hemoglobin was not different between the legume- and a sole-grass group of does; however, interaction effect between the group and observation date occurred on Day 47 of the study, when the sole-grass group had a higher concentration of hemoglobin than the legume-grass group (p<0.05) (Table 5). Overall, the basophil count was higher in the legume-grass group does than in the sole-grass group does. While looking at values obtained on different sampling dates, the legume-grass group had a higher concentration of basophil on Day 87 (p<0.05) than the sole-grass group. However, basophil concentration in both groups remained within the normal range (0-0.12 K/μL).

Table 5: Hematological parameters of Kiko does grazing legume-grass vs. sole-grass pastures, mid-August to early November 2020, Browse Research and Demonstration Site, Tuskegee University, Alabama, USA.

Parameters	Day 1		Day 47		Day 87		Normal range
	LGG	GG	LGG	GG	LGG	GG	
	LSMean ± SE						
Red blood cell (M/μL)	16 ± 0.7	17 ± 0.7	16 ± 0.7	18 ± 0.7	18 ± 0.7	18 ± 0.7	10 - 23
Hematocrit (%)	27 ± 1.43	27 ± 1.6	31 ± 1.4	32 ± 1.5	36 ± 1.5	32 ± 1.5	22 - 39
Hemoglobin (g/dL)	8.0 ± 0.20	9.0 ± 0.30	8.8 ± 0.22[b]	9.4 ± 0.24[a*]	9.6 ± 0.23	9.5 ± 0.23	8.9 - 13.8
MCV (fL)	17 ± 1.3	16 ± 1.4	20 ± 1.3	19 ± 1.4	21 ± 1.4	18 ± 1.4	14 - 22
MCH (pg)	5.0 ± 0.10	5.0 ± 0.20	5.5 ± 0.16	5.3 ± 0.17	5.4 ± 0.17	5.2 ± 0.17	5 - 7
MCHC (g/dL)	32 ± 1.2	33 ± 1.3	29 ± 1.2	30 ± 1.29	27 ± 1.3	30 ± 1.2	32 - 34
Reticulocyte (K/μL)	6.1 ± 1.25	7.6 ± 1.40	4.9 ± 1.25	4.6 ± 1.32	6.5 ± 1.32	3.9 ± 1.32	NA
WBC (K/μL)	15 ± 1.2	14 ± 1.4	16 ± 1.2	14 ± 1.3	15 ± 1.3	13 ± 1.3	6.03 - 15
Neutrophil (K/μL)	6.0 ± 0.63	7.0 ± 0.70	5.5 ± 0.63	4.6 ± 0.66	4.7 ± 0.66	4.4 ± 0.66	1.72 - 10.61
Lymphocyte (K/μL)	7 ± 0.6	5 ± 0.7	7 ± 0.6	7 ± 0.6	7 ± 0.6	7 ± 0.6	2.68 - 11.54
Monocyte (K/μL)	1 ± 0.1	1 ± 0.1	1 ± 0.1	1 ± 0.1	1 ± 0.1	1 ± 0.1	0.06 - 0.89
Eosinophil (K/μL)	0.6 ± 0.27	0.4 ± 0.30	2.1 ± 0.27	1.5 ± 0.28	1.2 ± 0.28	0.8 ± 0.28	0.03 - 1.29
Basophil (K/μL)	0.04 ± 0.020	0.03 ± 0.022	0.05 ± 0.020	0.02 ± 0.021	0.12 ± 0.021[a*]	0.03 ± 0.021[b]	0 - 0.24
Platelets (K/μL)	382 ± 30.2	378 ± 33.7	268 ± 30.2	327 ± 30.8	365 ± 31.8	389 ± 31.8	246 - 912
MCV (fL)	7.6 ± 0.19	7.2 ± 0.23	7.2 ± 0.20	7.3 ± 0.20	7.5 ± 0.20	7.2 ± 0.20	NA

*[ab]Values in the same row under the same day with different superscripts differ (*p<0.05), LGG: Legume-grass group, GG: Sole-grass group, MCV: Mean corpuscular- volume, MCH: Mean corpuscular-hemoglobin, MCHC: Mean corpuscular- hemoglobin concentration, WBC: White blood cell, MCV: Mean platelet volume, NA: Not available.*

Biochemical parameters

Overall glucose concentration was higher in the legume-grass group does than in the sole-grass group does (p<0.05). However, an interaction effect occurred between the sampling date and group, showing a higher glucose level in the legume-grass group does on Day 47 but a lower level on Day 87 than in the sole-grass group does (p<0.05). The concentration of blood urea nitrogen (BUN) and the ratio of blood urea nitrogen to creatinine were greater in the legume-grass group does on Days 47 and 87 as compared to the sole-grass group does (p<0.05) (Table 6). Overall creatinine level was not affected by legume inclusion; however, an interaction effect between the group and sampling date occurred, showing an elevated level of creatinine on day 47 in the grass- than the legume-grass group does (p<0.05). The creatinine level was reduced below the normal range in legume grass on Day 47 and in both groups on Day 87. Cholesterol level was within the normal range on Day 1 and 47; however, the level increased more than normal on Day 87 in both groups, even though there was no difference within group (Table 6).

Table 6: Biochemical parameters of does grazing legume-grass vs. sole-grass pastures, mid-August to early November 2020, Browse Research and Demonstration Site, Tuskegee University, Alabama, USA.

Parameters	**Day 1**		**Day 47**		**Day 87**		**Normal range**
	LGG	**GG**	**LGG**	**GG**	**LGG**	**GG**	
	LSMean ± SE						
Glucose (mg/dL)	35 ± 1.6	34 ± 1.8	58 ± 1.6^{a*}	38 ± 1.7^{b}	39 ± 1.7^{b}	52 ± 1.7^{a*}	54 - 93
Creatinine (mg/dL)	0.70 ± 0.030	0.70 ± 0.040	0.48 ± 0.037b	0.61 ± 0.039a*	0.57 ± 0.039	0.58 ± 0.039	0.6 - 1.4
BUN (mg/dL)	5 ± 0.9	4 ± 1.0	23 ± 1.0^{a*}	15 ± 1.0^{b}	17 ± 1.0^{a*}	5 ± 1.0^{b}	10 - 21
BUN/ Creatinine	9 ± 2.2	6 ± 2.3	48 ± 2.3^{a*}	25 ± 2.4^{b}	30 ± 2.4^{a*}	8 ± 2.4b	NA
Albumin (g/dL)	2.4 ± 0.05	2.4 ± 0.06	2.5 ± 0.05	2.6 ± 0.06	2.6 ± 0.06	2.6 ± 0.06	2.8 - 3.8
Globulin (g/dL)	4.7 ± 0.08	4.8 ± 0.09	4.1 ± 0.08 ^{b}	4.4 ± 0.09 ^{a*}	4.4 ± 0.09	4.5 ± 0.09	NA
Albumin/ Globulin	0.5 ± 0.02	0.5 ± 0.02	0.6 ± 0.02	0.6 ± 0.02	0.6 ± 0.02	0.6 ± 0.02	NA
ALT (U/L)	25 ± 2.2	27 ± 2.5	12 ± 2.2	15 ± 2.3	13 ± 2.3	20 ± 2.3	23 - 44
Alkaline phosphate (U/L)	89 ± 9.3	77 ± 10.4	159 ± 9.3	147 ± 9.3	146 ± 9.3	129 ± 9.3	NA
GGT (U/L)	61 ± 5.5	64 ± 6.1	54 ± 5.5	56 ± 5.8	56 ± 5.9	64 ± 5.8	60 - 101
Total bilirubin (mg/dL)	0.5 ± 0.03	0.5 ± 0.03	0.5 ± 0.03	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.03	0.1 - 0.3
Cholesterol (mg/dL)	80 ± 6.0	63 ± 6.7	70 ± 6.0	87 ± 6.3	133 ± 6.3	122 ± 6.3	63 - 108
Sodium (mmol/L)	151 ± 0.9	149 ± 1.0	151 ± 0.9	151 ± 0.9	149 ± 0.9	151 ± 0.9	NA
Potassium (mmol/L)	4.9 ± 0.09	4.7 ± 0.10	4.6 ± 0.09	4.8 ± 0.10	4.8 ± 0.10	4.8 ± 0.10	NA
Sodium/ Potassium	31 ± 0.6	32 ± 0.7	33 ± 0.6	31 ± 0.6	31 ± 0.6	31 ± 0.6	NA
Chlorine (mmol/L)	108 ± 0.5	109 ± 0.6	106 ± 0.5	106 ± 0.6	109 ± 0.6	110 ± 0.6	NA
Phosphorous (mg/dL)	5.8 ± 0.29	5.5 ± 0.33	5.2 ± 0.29	5.8 ± 0.31	4.3 ± 0.31	5.1 ± 0.31	NA
Calcium (mg/dL)	8.4 ± 0.11	8.3 ± 0.13	8.3 ± 0.11	8.5 ± 0.12	9.1 ± 0.12	8.8 ± 0.12	NA
Serum Osmolality (mmol/kg)	293 ± 1.7	290 ± 1.9	300 ± 1.7	297 ± 1.8	295 ± 1.8	294 ± 1.8	NA

^{ab}*Values with different superscripts within a day in the same row differ (*p<0.05), LG: Legume-grass group, GG: Sole-grass group, BUN: Blood urea nitrogen, ALT: Alanine amino transferase, GGT: Gamma-glutamyl-transferase, NA: Not available*

Fecal nutrients

Fecal P was higher (20%) in the legume-grass group on Day 47 ($p<0.0001$), whereas the grass group had 20% higher fecal P on Day 87 ($p<0.05$). There was no difference in fecal N concentration between the groups (Table 7).

Table 7: Fecal nutrients of does grazing legume-grass and sole-grass pastures on Days 1, 47, and 87 of the study, mid-August to early November 2020, Browse Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

Fecal nutrients	Day 1		Day 47		Day 87	
	LGG	GG	LGG	GG	LGG	GG
	LSMean ± SE					
Phosphorous	0.5 ± 0.03	0.4 ± 0.03	0.6 ± 0.03a****	0.5 ± 0.03b	0.5 ± 0.02b	0.6 ± 0.02 a*
Nitrogen	1.9 ± 0.09	1.8 ± 0.1	2.6 ± 0.09	2.7 ± 0.1	2.2 ± 0.07	2.2 ± 0.07

[ab]*Values with different superscripts in the same row within the same observation day differ (*p<0.05, ****p<0.0001), LGG: Legume-grass group, GG: Sole-grass group.*

Discussion

Animal performance

The hypothesis that legume inclusion in grass pastures would improve the health and performance of grazing goats has been accepted as the legume-grass group of does showed better live weight (5%), BCS (4%), and FAMACHA score (14.2%) compared to the sole-grass group. The better performance of the legume-grass group of does was as expected because of the higher quality of legume-grass pastures with better CP (42%) and TDN (5.5%) but lower NDF (16.3%) and ADF (11.7%) vs. the sole-grass pastures. The similar forage height reduction (41- 42%) because of grazing found for both groups of does indicates that the forage intake might not have been impacted by the magnitude of quality difference found in the current study. Karki & Karki (2017) found goats efficiently consuming standing forages in pastures with low fiber (ADF 194 -241 g kg−1 dry matter) and moderate to high protein content (115 -170 g kg−1 dry matter).

The improved live weight in the legume-grass group of does found in the current study is consistent with the findings of our previous study where does grazing on hairy vetch (*Vicia villosa Roth*)-Marshall ryegrass (*Lolium* L.) or hairy vetch-rye (*Secale* L.) 50:50 mixed pastures gained heavier live weight (5%, p<0.05) as compared to those grazing sole-Marshall ryegrass or rye pastures (Tiwari *et al*., 2020). Similarly, Mohsan *et al*. (2019) reported that Beetal goats raised in pens had more daily weight gain (36.5%) when supplemented with a diet containing 31.6% vs. 18% CP. In addition, greater TDN (5.5%) found in legume-grass pastures substantiated the improved live weight, BCS, and FAMACHA score of the legume-grass group of does, as TDN concentration is associated with animal performance (Ahn *et al*., 2019). The current result is consistent with that of Ghani *et al*. (2017), who found improved BCS in growing goats when fed a diet containing 16% CP vs. 11.39% CP. The better FAMACHA score of the legume-grass group of does observed in the current study is in agreement with Konwar *et al*. (2015), who found improved anemic condition of goats when CP concentration in the diet was increased from 16 to 24%. Animals receiving an adequate amount of dietary protein develop a strong immune system to fight against invaders, including blood-sucking helminth parasites (Ha *et al*., 2021), and remain healthy.

Hematological parameters

Unlike performance, no difference in hematological parameters was observed between groups, except basophil concentration on Day 87, although it was within the normal range. We also found better improvement in hemoglobin levels in the legume-grass group (20%) from the beginning to the end of the study vs. the sole-grass group (5%), even though group difference was not detected on any observation day. This result aligns with the findings of a previous study, which reported improved hemoglobin levels by 5%, 3%, and 3.2% when goats were fed concentrate feed containing, respectively, 16%, 20%, and 24% CP as compared to those raised without concentrate feed (p<0.01) (Konwar *et al*., 2015).

Biochemical parameters

Interaction effect (p<0.05) occurred in blood glucose levels with a higher level seen in the legume-grass group on Day 47 (53%) and a lower level seen on Day 87 (25%) vs. the sole-grass group. This effect could be because of the variation in the plant community characteristics in the grazing plots assigned to the groups of does. However, we did not monitor the forage quality by observation days. Further study is recommended to monitor the plant community characteristics in alignment with sampling dates of animal parameters. Higher glucose in the legume-grass group on Day 47 might be attributed to legume addition to pastures as it increases the digestibility of forages, which ultimately enhances the production of volatile fatty acids (VFA) (Dal Pizzol *et al*., 2017); VFA produced in the rumen by microbial fermentation later supports the formation of glucose in the liver (Martinez *et al*., 2022). This result agrees with the previous finding of Przemysław *et al*. (2015), who reported lambs showing higher levels of blood glucose (5%) after feeding alfalfa silage (CP - 167 g/kg of DM) vs. grass silage (CP - 155 g/kg of DM). Improved level of glucose ensures a good intake of diets and substantial energy available to meet animals' daily requirements. In the current study, glucose levels in both groups on Day 87 were below the normal range, possibly because of forage maturity. The maturation process may have resulted in reduced nutrient quality, poor digestibility, and, consequently, a decline in dietary intake (Chapman, 2013).

Higher BUN levels (p<0.05) found on both Days 47

(53%) and 87 (240%) in the legume-grass group vs. the sole-grass group were as expected. Blood urea nitrogen (BUN) is an indicator of protein metabolism and utilization in ruminants, showing a positive correlation with dietary crude protein (CP) levels (Hammond, 1998). Saro *et al*. (2020) reported that BUN level increased by 5.1% in heavy fattening Assaf lambs when fed a diet containing medium-protein (157 g/kg DM CP) as compared to a low-protein diet (134 g/kg DM CP). A similar study on Holstein bull found increased BUN levels when dietary CP was increased from 10.21 to 14.24 (% of DM) (Xia *et al*., 2018). The BUN level in the legume-grass group of does on Day 47 in the current study slightly exceeded the normal range, suggesting that they had consumed high-quality forages beyond their requirement and/or capacity to utilize. This result indicates that the proportion of leguminous forages can be reduced from the currently used ratio (50:50) while mixing with grass forages and save on the cost of production since most legume seeds are more expensive than grass seeds.

A lower level of creatinine found in the legume-grass vs. sole-grass group on Day 47 could be due to increased glomerular filtration because of higher quality forage available for the former group. A high-protein diet is reported to cause an increased glomerular filtration rate, eventually causing more creatinine removal from blood (Juraschek *et al*., 2013). Creatinine is a waste product of creatine catabolism, which is released into the blood and excreted via a glomerular filter in the kidney (Wyss & Kaddurah-Daouk, 2000). Greater BUN to creatinine ratios (BUN: creatinine) observed in the legume-grass vs. sole-grass group in the current study was the result of improved BUN and reduced creatine level in the former because of their access to higher quality forages. The higher ratio of BUN to creatinine in the legume-grass group of does in the current experiment aligns with the findings of the previous study on Jersey cattle during the mid-lactation period, where BUN/creatinine ratio was higher in legume-based pastures (BUN:creatinine -114) (*Trifolium pratense* cv. Raven, *Lotus corniculatus* cv. Bruce, *Trifolium alexandrinum* cv. Frosty and *Trifolium michelianum* cv. Fixation) compared to grass-based pasture (BUN:creatinine - 65) (*Festulolium braunii* cv. Perun, *Festuca arundinacea* cv. Rustler, *Dactylis glomerata* cv. Sundown, *Trifolium repens* cv. Domino), and forb-based pasture (BUN:creatinine - 46) (*Cichorium intybus* cv. Antler, *Plantago lanceolata* cv. Boston, and *T. repens* cv. Domino) (Ford *et al*., 2021).

Young-stage green forages contain higher levels of fatty acids and linoleic acids compared to when they mature (Whetsell & Rayburn, 2022). High levels of fatty acids and linoleic acids help maintain healthy cholesterol levels in grazing animals (Van Vliet *et al*., 2021). The high level of cholesterol found in both groups of does on Day 87 in the current study might be due to more mature forages, compared to those in earlier measurement dates, they might have consumed, resulting in increased acetate production by microbial fermentation and giving rise to an elevated level of cholesterol as acetate is the precursor of cholesterol in ruminants (Bergman, 1990). High fiber diet favors the growth of fiber-degrading bacteria such as Ruminococcus albus, thereby resulting in the production of acetate in large amounts (Wang *et al*., 2020). Cholesterol level in the blood is mostly associated with the composition and fatty acid profile of the diets. Calves showed high cholesterol (104 mg/dL vs. 76.8 mg/dL) when fed Timothy (*Phleum pratense* L.) hay (NDF 401 g/kg DM) containing high fiber concentrate vs. high starch concentrate diet (NDF 388 g/kg DM) (Jeon *et al*., 2019).

Fecal nutrients

A higher level of fecal P was found in the legume-grass group on Day 47 than in the sole-grass group, which may be due to the presence of phytate content in the legume. Legumes are richer in phytic acid than grass. This natural compound binds with P and makes P less available for intestinal absorption as a result more P might have excreted into the feces (Uribarri & Calvo, 2003). Additionally, legumes are richer in P content than grass (Wei *et al*., 2022); this could have resulted in more P in the legume-grass group in our study. Paulson *et al*. (2008) reported that leguminous forage alfalfa (Medicago sativa L.) accumulates more micro- and macro-minerals than grass species such as orchard grass (*Dactylis glomerata* L), tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire) and perennial ryegrass. A study found that P excretion increased (12, 15, and 23 g/day) with increments of P level (13, 20, and 24 g/day) in the diet of cattle (Dillard *et al*., 2015). Nevertheless, toward the end of the current study, mature forages may have affected the digestibility of forages in the gut, resulting in more phosphorus in the sole-grass group of does compared to the legume group. Dillard *et al*. (2015) found that the vegetative stages, digestibility, and production season of forage can affect the absorption of phosphorus in the gut of cattle.

Similar fecal N concentration found in both groups of does in the current study indicates better N retention in the legume-grass group vs. the sole-grass group since the former had access to higher quality forages (17% vs. 12% CP). This result contradicts the finding of Shrestha *et al*. (2023), who found 2 - 4% greater fecal N concentration in does with supplemental grazing in silvopastures containing good quality forages (14% CP) than does supplemented with hay (12% CP) and corn (9% CP). However, Morales *et al*. (2021) reported that the integration of *Leucaena leucocephala* (Lam.) and *Tithonia diversifolia* (Hemsl.) forages in *Brachiaria hybrid* cv. Mulato and *Brachiaria decumbens* (Stapf) R. Webster pastures lowered the nitrogen excretion in the feces of beef cattle as compared to those grazing sole-grass pastures. Similarly, Farghaly *et al*. (2019) reported that nitrogen retention was almost doubled in rams when fed Egyptian clover (*Trifolium alexandrinum*) (CP - 18.78%) vs. barley sprouts (*Hordeum vulgare* L.) (CP - 15.23%).

CONCLUSIONS

The research demonstrated that introducing legumes to grass pastures resulted in better live weight (5%) and both FAMACHA and body condition scores (4%) in the legume-grass group compared to a group of does grazing solely on grass (p<0.05). This outcome was attributed to higher crude protein content (42%) and lower fiber levels (ADF 11.7%, NDF 16.3%) in legume-grass pasture as compared to sole-grass pasture (p<0.05). In addition, the legume-grass group exhibited higher levels of blood urea nitrogen (BUN) (93%), BUN: creatinine ratio (122%), and blood glucose (6.8%) (p<0.05) compared to does grazing sole-grass pastures. The findings suggested that incorporating legumes into grass pastures at a 50:50 ratio enhances the dietary value and productivity of animals. Further research on higher grass: legume ratios to maintain the desired forage quality for goats at their various physiological stages would be worthwhile.

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