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Full Length Research Paper

Effect of high forage oxalate and calcium consumption on goat urine characteristics

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Goat production in Hawaii has grown steadily in the past decade and this growth necessitated a more controlled diet to sustain production. Local goat pastures on the Big Island, Hawaii, are dominated by Napier grass (*Pennisetum purpureum* Schumach.), yellow foxtail (*Seteria glauca* L.), and guava trees (*Psidium guajava* L.). These plants contain compounds such as calcium and oxalates that could have detrimental effects on growth and development of goats when consumed in large quantities. This experiment was designed to evaluate the effect of grazing Napier grass and yellow foxtail with and without added guava tree parts using two groups of 14 female goats (*Capra hircus* L.) crossbred between Boer, Spanish, and Kiko breeds by analysis of some urine characteristics such as color, turbidity, specific gravity, pH, presence of bacteria, fungi, casts, as well as concentrations of nitrite, blood, urobilinogen, protein, glucose, ketones, bilirubin, red blood cells, and white blood cells. Urinalyses were performed on four sampling periods over three months. All variables were subjected to Repeated Measures in General Linear Models and correlation analysis using SAS. Urinalysis results were similar for both the groups. Urine protein levels of the (-) guava group, however, were higher, but not statistically different than those of the (+) guava group; significant interactions existed with some of the variables with time. The increased concentrations of calcium or oxalates in guava tree bark, Napier grass, and yellow foxtail may cause calculogenic minerals to accumulate, over longer periods of grazing, causing uroliths with a calcium and (or) oxalate base in male goats. This could result in penile obstruction in male goats.

Key words: *Capra hircus*, goat, guava, Napier grass, *Pennisetum purpureum*, *Psidium guajava*, *Seteria glauca*, urinalysis, yellow foxtail.

INTRODUCTION

In Hawaii, as well as other tropic and subtropic locales, goat (*Capra hircus*) producers and farmers graze their animals in pastures inhabited by numerous guava trees (*Psidium guajava*), Napier grass (*Pennisetum purpureum*), and yellow foxtail (*Seteria glauca)*. These forages are common in yards, pastures, and low-land forests. The objective of the study was to determine the health effects on goats feeding on guava trees, Napier grass, and yellow foxtail through urinalysis. Guava tree shoots provide moderate protein and energy sources as feedstuffs than other parts of the tree. The shoot, in this case is a small protuberance on a stem or branch, enclosed in protective scales containing an undeveloped leaf. The stems or branches are low in protein content and energy values. Stems or branches have higher fiber content than the other parts of the guava tree. The bark of guava contains 12 to 30% of tannin (Burkill, 1997), resin, and calcium oxalate crystals (Nadkarni and Nadkarni, 1999). It is important to note that goats tend to

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strip the bark habitually off of guava trees in the wild and open range, often times destroying the tree.

Napier grass, also called elephant grass, on the other hand, has striking resemblance with sugarcane. A native of Zimbabwe and commonly found in tropical and subtropical countries, this grass has a strong root system with wide leaf blades of 20 to 40 mm and tubercle-based hairs (Chippendall, 1955). Napier grass thrives rainy weather; however, it can tolerate and survive times of drought because of its deep root system. The grass's root system contributes to the grass's dominance in local pastures which contain different soils and microclimates. The root system can also be beneficial for nitrogen uptake (Orodho, 2006). Napier grass is known for its high dry matter yield and its suitability for silage. As it matures, the leaf to stem ratio declines (Karanja, 1984; Kariuki, 1989) causing changes in the chemical composition and concomitant reduction in feed value (Minson, 1990). When mature, Napier grass is high in fiber, making its nutritional value poor.

Serra et al. (1996) contrasted the mineral composition of Napier grass with the required dietary concentrations for ruminants and concluded that it is likely to be deficient in most minerals (Orodho, 2006). The study found that the mineral composition of Napier grass was 3.5 g kg^{-1} DM Ca^{2+}, 2.0 g kg^{-1} DM P^{5+}, 1.7 g kg^{-1} DM Mg^{2+}, and 8.0 g kg^{-1} DM K^{+} (Serra et al., 1996). Calcium and P^{5+} concentrations of 3.2 g kg^{-1} and 1.5 g kg^{-1} DM respectively were documented by Nyambati et al. (2003). Phytochemical screening showed the presence of alkaloids, cyanogenic glycosides, flavonoids, oxalates, phytates, saponins, and tannins, in which oxalates were 1.6 g kg^{-1} (Okaraonye and Ikewuchi, 2009).

Yellow foxtail is a weed with a seedhead resembling a fox's tail that appears yellow when mature. It thrives shallow-rooted in fertile soils throughout the United States and can grow upwards one to two feet. Like giant foxtail, barnyard grass, and shepherd's purse, yellow foxtail provides relatively poor forage quality (Bosworth et al., 1985; Marten and Anderson, 1975; Temme et al., 1979). According to the Pennsylvania State University, College of Agriculture, Cooperative Extension Service's web page, yellow foxtail is nutritious. It is usually not, however, encouraged in well-managed fields because it can crowd out more desirable plants. The barbed awns can cause abscessation and infection by lodging in the mouth, nose, and eye tissues of livestock (www.weeds.cas.psu.edu, 2010). This weed is also known to have high concentrations of anti-nutritional components such as oxalates, just like Napier grass (Minson, 1990).

According to Statistics of Hawaii Agriculture (Hawaii Department of Agriculture and the United States Department of Agriculture, 2004), the goat industry in Hawaii has grown between 1992 and 2002. This trend is not only seen in Hawaii, but also other regions of the world. Currently, producers are not aware of such possible threats to their herd brought about by consumption of common forage such as Napier grass, yellow foxtail, and guava. Guava tree bark, Napier grass, and yellow foxtail contain high concentrations of calcium or oxalates. These compounds, if consumed in appreciable quantities, may cause calculogenic minerals to accumulate in the urinary systems causing uroliths with a calcium and (or) oxalate base, especially in male goats. To date, there is a dearth of information whether guava, Napier grass, or yellow foxtail consumption affects goats' health. Little to no research was found in veterinary or livestock science journals.

If excessive feeding on guava, Napier grass, and yellow foxtail affect the health of a goat, it would be critical information for the goat industry and would be vital to better educating producers and farmers in order to change their feeding and grazing programs. The study was designed to determine the effect of the consumption of oxalates and high Ca^{2+} by goats from a mixed forage diet consisting of guava, Napier grass, or yellow foxtail on goats' urinary health.

MATERIALS AND METHODS

Experimental goat subjects

The experimental goats were crossbred between Boer, Spanish, and Kiko from the University of Hawaii at Hilo Agriculture Farm. The study consisted of two separate groups of test animals, each consisting of 14 mature, female goats. The subjects were between 3 and 5 years of age, with body weights ranging between 38 to 45 kg. In this study, only female goats were used because the urinary tract diameter of a female is larger than a male's, therefore reducing the likelihood of obstructions from urinary stones. Since the formation of stones can occur in males and females, choosing all-female test groups decreased the chance of life-threatening conditions occurring.

Diet and sampling

One group of goats' diet (+ guava) consisted of pasture forage local to the University of Hawaii at Hilo Agriculture Farm in Pana'ewa, Hawaii including Napier grass, yellow foxtail, and guava tree parts: Leaves, shoots, bark, and stems or branches.

The (+) guava group's diet consisted of guava tree parts fed daily, free-choice or ad libitum for seven months. The remainder of the diet consisted of native and tropical pasturelands local to the area dominated by Napier grass and yellow foxtail. The other group (- guava) was not fed any guava tree parts and grazed on Napier grass and yellow foxtail pasture only. Urine samples were collected from each goat in each group during four collection periods. The samples were collected on April 29, 2008, May 6, 2008, May 21, 2008 and June 24, 2008. April 2008 was the fifth month of feeding. A free-catch method utilizing sterile urine plastic cups (maximum volume capacity of 120 milliliters) and a pole extension device was used for collection.

Forty-eight samples per group were collected and were processed immediately on the same day of each collection time.

Table 1. Mineral composition of guava tree parts.

	Bark sample size n = 14	Shoots sample size n = 13	Leaves sample size n = 13	Branches sample size n = 1	Stems sample size n = 2
Macro nutrients					
Calcium, %	5.9 ± 0.7	0.6 ± 0.1	1.7 ± 0.4	1.2	0.9 ± 0.0
Magnesium, %	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.1	0.1 ± 0.0
Phosphorus, %	0.0 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1	0.0 ± 0.0
Potassium, %	0.7 ± 0.3	2.1 ± 0.2	0.9 ± 0.2	0.6	0.4 ± 0.1
Sodium, ppm	148.4 ± 28.5	180.4 ± 121.9	212.0 ± 106.0	369.9	185.3 ± 73.9
Sulfur, %	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.1	0.1 ± 0.0
Micro nutrients					
Aluminum, ppm	16.8 ± 14.0	14.8 ± 4.0	37.3 ± 6.4	13.0	8.7 ± 3.0
Boron, ppm	19.1 ± 2.0	23.4 ± 1.6	37.9 ± 5.8	9.0	8.2 ± 0.6
Copper, ppm	3.2 ± 0.5	14.7 ± 1.3	20.9 ± 3.3	5.4	5.6 ± 2.3
Iron, ppm	26.7 ± 10.2	46.7 ± 6.6	50.1 ± 7.1	23.4	51.3 ± 42.1
Manganese, ppm	22.5 ± 4.0	20.6 ± 3.7	36.3 ± 10.8	12.4	15.6 ± 6.2
Zinc, ppm	23.7 ± 3.9	45.2 ± 9.6	65.4 ± 28.6	30.8	34.3 ± 18.8

Chemical analysis on urine samples was done using multi-test dipsticks (9 parameter, urine reagent strips, LW Scientific, Inc.) as described by Hillestad (2008). Urine sediment was centrifuged and examined by microscopy at 10x to 40x magnification (Swift®, Binocular Advanced Compound Microscope).

Urinalysis

Urine samples collected were subjected to chemical analysis. Multi-test reagent strips were used to determine urine characteristics such as pH, the concentration of nitrite, blood, urobilinogen, protein, glucose, ketones, and bilirubin, and specific gravity. Each characteristic was determined after the required duration (less than a minute) for reagent immersion into the samples. For example, pH was determined within 60 s. Note that urine specific gravity measurements on dipsticks are not as accurate as those measured via spectroscopy. Urobilinogen results are not considered accurate and are difficult to interpret in animals (Hillestad, 2008). About 1 mL of each urine sample was transferred to individual Pyrex® no.9800 glass test tubes using a pipette. These were labeled and centrifuged using a Unico® Power Spin™ LX at 2000 revolutions per minute (rpm) for 10 min. After centrifugation of each sample, sediments were collected using pipettes after dispensing of the supernatant.

Sediments from each sample were mounted on glass slides with cover slips for microscopy. Methylene blue stain was used to examine each sample with a compound microscope at 10x to 40x objective magnification for various crystals, and the presence of red blood cells, fungi or yeasts, casts, bacteria, and white blood cells.

Statistical analyses

Data collected from urinalyses were statistically analyzed by Repeated Measures for General Linear Model (GLM) procedures in SAS (SAS version 9.1, SAS Institute, Cary, NC, USA). Means were separated using Least Significant Difference (LSD) at P = 0.05 level of significance. Univariate or multivariate ANOVA outputs were used accordingly after the sphericity test. Correlation analysis was performed on all test variables from the urinalysis at alpha 0.05 using Proc Corr in SAS (SAS version 9.1, SAS Institute, Cary, NC, USA).

RESULTS

Preliminary mineral analysis of the guava tree was done at Louisiana State University AgCenter Plant Analysis Laboratory. Each sample sent for analysis was oven-dried and ground to 1 mm using a Wiley Mill and packed into 2 oz Nasco Whirl-Paks®. Samples were analyzed for Ca, Mg, P, K, and S, as well as the microminerals B, Cu, Fe, Mn, Mo, Na, and Zn. Tissue concentrations of P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B were determined by inductively coupled plasma emission spectroscopy (ICPES) following digestion in nitric acid and hydrogen peroxide as outlined by Jones and Case (1990). The guava tree bark had maximum Ca^{2+} in the bark of 59 g kg^{-1}. The shoot had the highest concentration of K^{+} at 21 g kg^{-1} and the branch had the highest concentration of Na^{+} at 370 mg kg^{-1}. Nutritional analysis indicated that guava tree bark contains a high concentration of Ca^{2+}; especially when comparing it to P^{5+} in a Ca^{2+}:P^{5+} (Table 1).

There was no significant difference that existed in the variables measured between (+) guava and (-) guava groups ($F \leq 2$; $P > 0.05$). There were, however, significant interactions with some of those variables with time. All of the parameters measured in urinalysis were not affected by guava in the goats' diet; such as color ($F = 0.85$; $P = 0.36$), appearance or turbidity ($F = 0.96$; $P = 0.69$),

Table 2. Urinalysis of goats as affected by diet containing guava.

	(-) Guava group n = 48 samples	(+) Guava group n = 48 samples
Color	2.92 ± 1.30	2.75± 1.12
Appearance or turbidity	1.10 ± 0.30	1.13 ± 0.33
Nitrite	1.06 ± 0.24	1.04 ± 0.20
Urobilinogen	1.00 ± 0	1.00 ± 0
Protein	1.48 ± 0.74	1.29 ± 0.65
pH	8.23 ± 0.42	8.16 ± 0.49
Blood	1.00 ± 0	1.00 ± 0
Specific gravity	1.00 ± 0	1.00 ± 0
Ketone	1.04 ± 0.20	1.04 ± 0.20
Bilirubin	1.00 ± 0	1.00 ± 0
Glucose	1.00 ± 0	1.00 ± 0
Bacteria	1.38 ± 0.49	1.42 ± 0.50
Fungi or yeast	1.00 ± 0	1.02 ± 0.14
Red Blood cells	1.08 ± 0.28	1.04 ± 0.20
White blood cells	1.06 ± 0.24	1.04 ± 0.20
Crystals	1.10 ± 0.31	1.04 ± 0.20
Casts	1.50 ± 0.51	1.50 ± 0.51

All treatment means were not different (P > 0.05). Means in the (+) guava group followed by an asterisk (*) are significantly different from the (-) guava group.

nitrite (F = 0.24; P = 0.62), urobilinogen (F = 0.21; P = 0.11), protein (F = 0.54; P = 0.46), pH (F = 0.46; P = 0.50), blood (F = 0.21; P = 0.11), specific gravity (F = 1.23; P = 0.25), ketone (F = 0.01; P = 1), bilirubin (F = 0.01; P = 1), glucose (F = 0.21; P = 0.11), bacteria (F = 0.5; P = 0.48), fungi or yeasts (F = 1; P = 0.32), red blood cells (F = 0.12; P = 0.73), white blood cells (F = 0.22; P = 0.63), crystals (F = 1.43; P = 0.23), and casts (F = 0; P = 1) (Table 2).

Both groups had an absence of nitrites and normal results for the urobilinogen test. Both groups did not show a significant presence of blood, ketones, bilirubin, and glucose. The (-) guava group demonstrated a higher protein level within the urine samples, closer to trace values interpreted as normal. The (+) guava group showed negative values for protein within the urine. The urine pH values were 8.16 to 8.23±0.4. The specific gravity values for both groups were fairly dilute and similar with the (-) guava group at a concentration of 1.007 and the (+) guava group at 1.006. Again, the results of this study showed no significant differences between the (-) guava and (+) guava groups when analyzing urine nitrites, urobilinogens, urine pH, specific gravity, and the presence of blood, ketones, bilirubin, and glucose. Protein test of the (-) guava group showed higher values compared to the (+) group, however, trace values are not considered abnormal in animals. The (+) guava group had increased levels of bacteria compared to the (-) guava group. Insignificant amounts of fungi or yeasts, red blood cells, and white blood cells were found for both the (-) and (+) guava groups. Although the results were similar for both groups, approximately half of both groups had the presence of casts which is a sign of the presence of kidney tubule debris. The (-) guava group showed an increased level of calcium oxalate crystals in the urine.

Based on critical values for Pearson Correlations Coefficients, some paired variables showed significant associations (critical value at 0.05 = 0.2). For instance, urinalysis showed a significantly positive correlation (r = 0.53; P < 0.05) between color intensity and turbidity (Figure 1). A significant positive correlation between intensity of color and presence of bacteria (r = 0.47; P < 0.05) was also found (Figure 2). Another example is that urine with the evidence of bacteria often times have a turbid appearance to it (Figure 3; r = 0.38; P < 0.05). Color also showed significantly positive relationships with ketone (r = 0.24; P < 0.05; Figure 4) and protein (r = 0.27; P < 0.05; Figure 5). A significantly positive relationship of red blood cells and protein (r = 0.24; P < 0.05; Figure 6) was noted. There also seems to be a reversed correlation between urine protein and urine pH (r = -0.007). For example, during week 2, the protein was at its highest level while the pH was at its lowest.

The opposite is also true; during week 4, while the protein registered lowest, the pH registered at its highest

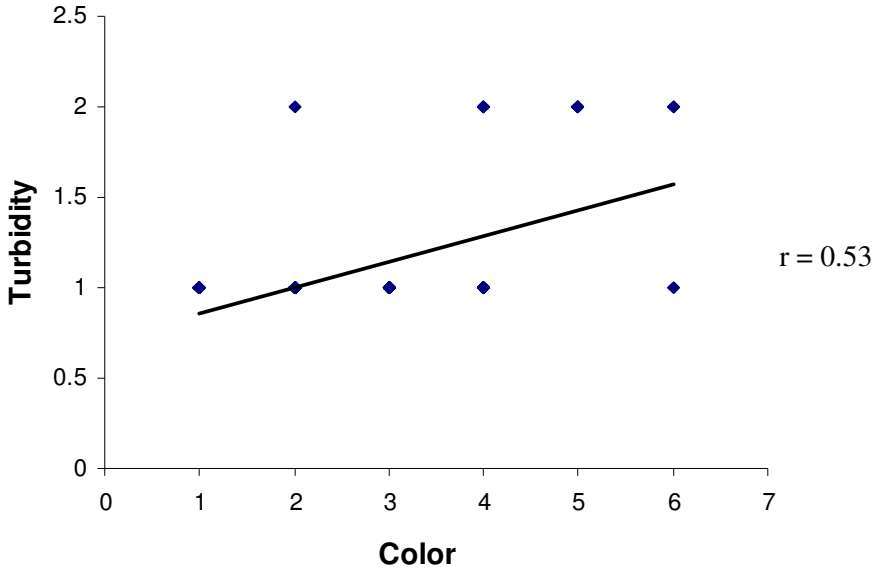

Figure 1. Relationship between color and turbidity after urinalysis.

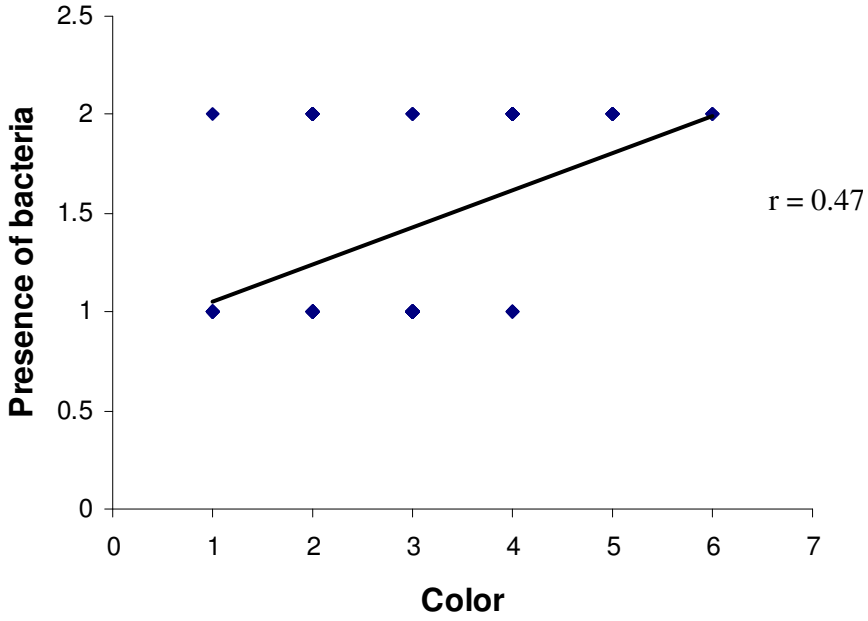

Figure 2. Relationship between presence of bacteria and color after urinalysis.

reading of the samples collected (Table 3).

DISCUSSION

Urolithiasis usually occurs in intact or castrated male goats (wethers), as well as other animals such as sheep, cattle, swine, and camelids (Van Metre and Divers, 2002). Urolithiasis is a metabolic disease and causes urinary calculi or stones which eventually results in urethral obstruction. Uroliths cause disease by prohibiting urinary outflow and cause trauma to the urinary tract (Van Metre and Divers, 2002). It is common among animals raised in management systems where the ration is composed primarily of grain or where animals graze certain types of pasture. In general, 40 to 60% of animals

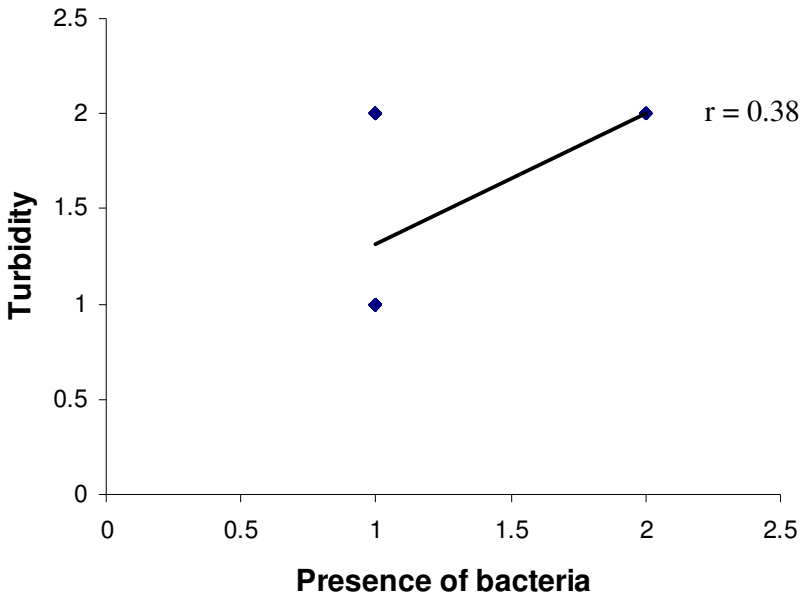

Figure 3. Relationship between turbidity and presence of bacteria.

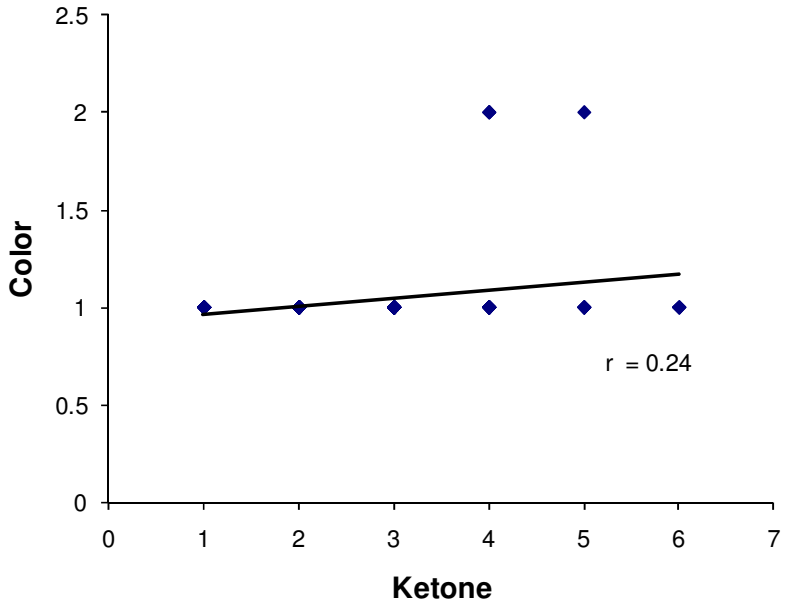

Figure 4. Relationship between color and ketone.

may form calculi or stones in their urinary tract (Radostits et al., 2000). The most common site for obstruction is the penile urethra, although obstruction can also occur within the kidney and other locations along the urinary tract. Urinary tract obstruction results in urethral perforation and rupture, bladder rupture, etc. This condition is alarming to any producer because one case of urolithiasis in a single animal suggests that all males in the population are at risk for the disease due to similar dietary and environmental factors (Van and Divers, 2002).

In most cases, goats form crystals within their urinary tract that then form stones or uroliths (Radostits et al., 2000). These stones then travel from the male goat's

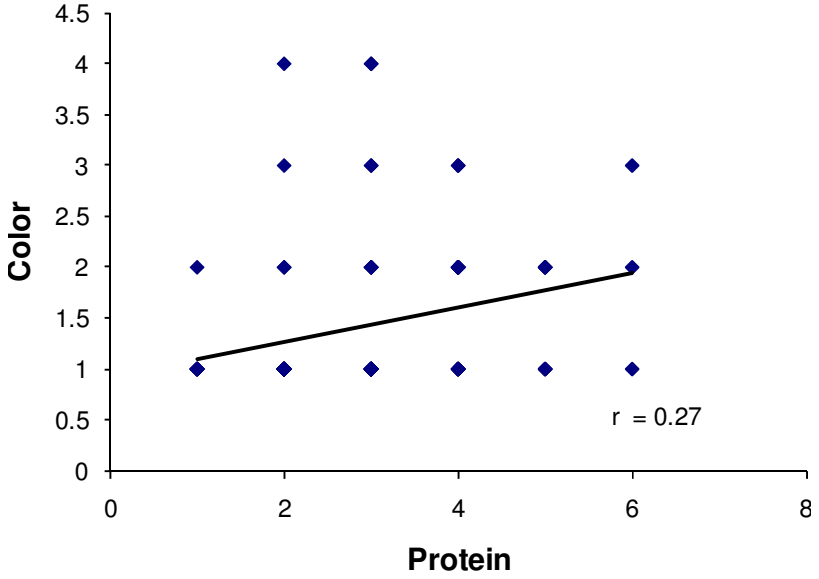

Figure 5. Relationship between color and protein.

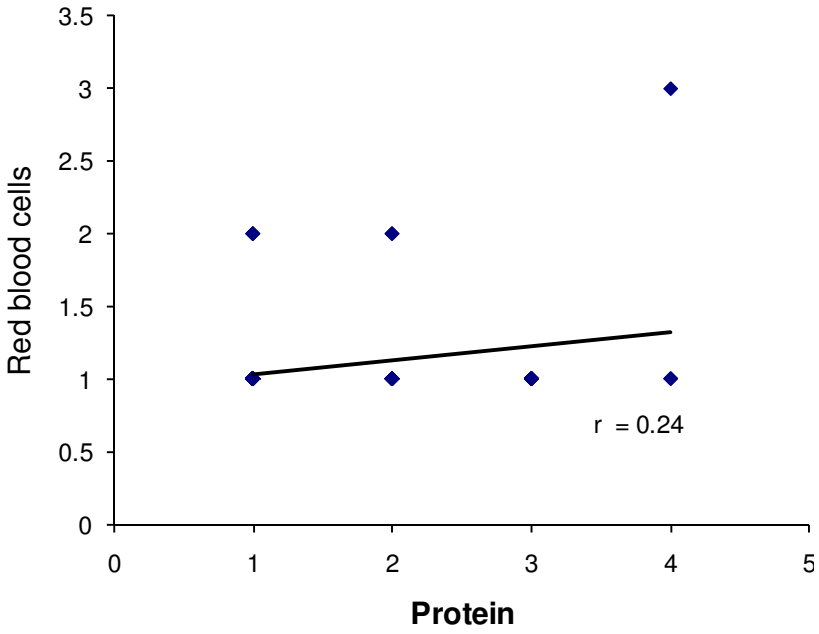

Figure 6. Relationship between red blood cells and protein.

bladder into the penis upon urination (Radostits et al., 2000). Some stones can pass, but the large ones become lodged and inhibit or block the animals, particularly males, from urinating (Radostits et al., 2000). Urine contains many self-produced toxins or waste products that are intended to be eliminated daily (Blood and Studdert, 1999). An animal that cannot urinate due to an obstruction becomes ill. If urination is not possible because of an obstruction, the animal becomes self-poisoned and enters a life-threatening state. The study had no goats with urolithiasis, or stone formation, however, only signs of crystalluria.

The reasons for the (+) guava group showing increased level of bacteria are not presently understood. Both

Table 3. Characteristics of urine samples of goats on Napier grass, yellow foxtail, and guava feedstuff.

	Collection time (Week)	Mean
Color	1	2.0c
	2	2.5bc
	3	4.2a
	4	2.7b
Turbidity	1	1.0b
	2	1.0b
	3	1.4a
	4	1.0b
Protein	1	1.2bc
	2	1.8c
	3	1.5ab
	4	1.0c
pH	1	8.3a
	2	7.9b
	3	8.2a
	4	8.3a
Specific gravity	1	1.004583c
	2	1.007708b
	3	1.011042a
	4	1.005000bc
Ketone	1	1.00000b
	2	1.00000b
	3	1.16667a
	4	1.00000b
Bacteria	1	1.08333c
	2	1.25000b
	3	2.00000a
	4	1.25000b

Means followed by same letter(s) are not statistically different at ($P < 0.05$).

groups did not show a presence of glucose within the urine which could exacerbate the numbers of bacteria and potentiate their growth. The urine collection methodology for both groups was static, therefore, the potential bacterial contaminants to be introduced to the samples of each group would be the same. Consequently, the consumption of high calcium and (or) high oxalate forage may increase bacterial numbers within goat urine due to variable urine composition, chemical metabolism, etc. The (-) guava group demonstrated an increased presence of calcium oxalate crystals within the urine probably due to a more concentrated presence of Napier grass and yellow foxtail in their diet. Both grasses are known to be high in oxalates (Minson, 1990). The differences in the variables like color, turbidity, specific gravity, ketone, and bacteria between collection times are noted to be at the highest values on week 3 of collection and the lowest on week 1 of collection (Table 3). These differences were not chronological. The highest values occurred during week 3 rather than week 4. It is not known why week 3 results were the highest versus week 4, particularly because one would expect an accumulation of or an increase in properties over time as more feed was consumed. One would generally expect with all other parameters being normal and without any disease process occurring, for

urine with a darker or more intense color to have a higher specific gravity rather than a lower one. Both support the theory that diets higher in protein may result in a lower urine pH because proteins in the diet are broken down to amino acids, thus potentiating a lower or acidic urine pH reading and acid accumulation.

It is important to note that goat metabolism and biochemistry is much more complex than stated here and can be complicated by the involvement of disease processes. Again, the protein levels found in the (-) guava group were of trace values and not considered abnormal.

Conclusion

The inclusion of guava in goats' diet that consisted of Napier grass and yellow foxtail did not demonstrate any significant effects on some urine characteristics such as color, turbidity, specific gravity, pH, presence of bacteria, fungi, casts, as well as concentrations of nitrite, blood, urobilinogen, protein, glucose, ketones, bilirubin, red blood cells, and white blood cells. Significant interactions, however, occurred relative to some variables over time. It is recommended that a follow-up study include a feeding trial utilizing known quantities of guava tree part intake of the goats in the (+) guava group in order to quantify the extent of their effects on goats' health. This same recommendation can be applied to feeding of Napier grass and yellow foxtail.

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