Milk Composition, Minerals and Fatty Acid Profile of Milk of West African Dwarf Does Goats under Different Feeding Time

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Summary

This study aims to evaluate the milk composition, minerals and fatty acid profile of milk of West African Dwarf Does Goats fed under different feeding time. Twelve lactating pregnant West African Dwarf (WAD) goat-does on their parity were randomly allocated into the morning, afternoon, and evening time of feeding in a Complete Randomized Design. This experiment lasted four weeks. The result shows that milk proximate composition of evening-fed does had the highest milk lactose (9.84%) and the highest milk fat (10.86%), while afternoon-fed does had the highest milk protein (8.26%). Afternoon-fed does had the highest milk phosphorus ($P = 0.05$) while evening-fed does had the lowest. Tridecanoic, myristic, myristoleic, stearic, ϒ - linolenic, total linolenic acid, PUFA, PUFA/SFA and atherogenicity were statistically ($P < 0.05$) influenced by the time of feeding. Change in the time of feeding can invariably determine the milk content and pattern of fatty acid synthesis.

Key words

atherogenicity index, fatty acids profile, feeding time, milk mineral, total linolenic acid

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Introduction

Goat milk and its products are important daily food source of protein, phosphate, and calcium in developing countries (Slačanac at al., 2010). But beyond their use as a source of protein, the fatty acid profile of the animal products is as important as the nutritional values due to its impact on consumers' health. Milk fat secretion and milk fatty acid constituents are of great interest regarding human nutrition. Apart from their contribution to dairy products sensorial quality and the amount of dietary energy, different lipid and fatty acids (FA) compounds (short and medium chain saturated branched, mono- and polyunsaturated, cis and trans, conjugated etc.), present in milk fat have a potentially positive or negative effect on the health of consumers (Parodi, 2004). Milk fat synthesis is well known to be responsive to nutrition and the specific trans fatty acid isomers produced in the rumen decrease milk fat synthesis in the mammary gland by inhibiting enzymes involved in lipid synthesis (Harvatine at al., 2009). Interpretation of the milk fat response to feeding is difficult because of changes in both rumen fermentation and the temporal pattern of nutrient absorption (Rottman at al., 2014). The prospects for improving the fatty acid profile of milk and meat from ruminants using different techniques such as feed additives, oil supplementation etc., represent a growing market for the global livestock sector (Or-Rashid at al., 2009). However, there is a scarce report on the impact of such feeding time on the fatty acid profile, composition and minerals of goat milk. Introducing seemingly new management practice (variation in feeding time) requires proper investigation before it is recommended to farmers. Thus, this study aims at evaluating the impact of feeding at different times of the day on milk chemical composition, minerals and fatty acid profile of milk of West African Dwarf (WAD) goat.

Materials and Methods

Experimental Site

This experiment was conducted at the Small Ruminant Unit of the Teaching and Research Farm, Federal University of Technology, Akure, Ondo State, Nigeria. The area is geographically situated between latitude 7° 15' north of the Equator and longitude 5° 15' east in Ondo State, Nigeria. It is about 370m above sea level.

Management of Goat-Does, Feeding and Experimental Design

Twelve lactating West African Dwarf (WAD) goat-does were randomly allocated into three experimental treatments, in a completely randomized design. The does were fed once daily in the morning, noon, or evening at 06:00h, 12:00 h or 18:00 h, respectively. All the does were in their first parity, the stage of milking was early lactation and the does were 36 months old. Furthermore, the does were offered the experimental diet of grass (*Panicum maximum*) and concentrate (Table 1, Table 2) at 50:50 dry matter ratio once daily. The grass and concentrate were placed in the feeding trough at the same time for the animals in each feeding period.

Table 1. Chemical composition of concentrate and grass (g 100 g^{-1})

Parameter	Concentrate	Grass
Dry matter	86.96	27.91*
Crude protein	10.70	7.81
Crude fiber	15.73	32.55
Ether extract	3.46	3.39
Ash	13.82	12.50
Acid detergent lignin	16.12	19.99
Acid detergent fiber	30.07	26.55
Neutral detergent fiber	72.03	63.94
Hemicellulose	41.96	37.39
Cellulose	13.95	6.55
Nitrogen free extract	56.30	43.75
Metabolizable energy (Kcal kg^{-1})	2677.58	2119.03
Minerals (mg kg^{-1})		
Na	91.40	41.20
K	234.00	67.00
Ca	210.50	53.60
Mg	197.00	69.00
P	6.25	5.08

Note: *- dry matter of fresh grass

Table 2. Gross composition (g 100 g^{-1}) of the concentrate

Ingredient	**Diet**
Cassava peel	40.00
Palm kernel cake	26.00
Wheat offals	14.00
Brewer dried grains	15.00
Bone meal	2.00
Urea	1.00
Premix	1.00
Salt	1.00

Determination of Milk Yield and Milk Composition

The milk yield was measured by an indirect method of Williams et al. (1979). The milk yield records were taken three times in a week (Monday, Wednesday, and Friday) and five times per day (06:05, 09:00 h, 12:05 h, 15:0 h, and 18:05 h). Prior to the day milk yield records were to be taken, the kids of all dams were withdrawn at 18:00 h the previous day irrespective of the feeding time of their dam. The kids suckled their mother five times a day at 06:05, 09:00 h, 12:05 h, 15:0 h, and 18:05 h and were withdrawn 15 min later for weighing.

The milk sample for analysis was collected once a week on Thursday from each animal thrice a day at 06:00-06:30 h, 12:00-12:30 h and 15:30-16:00 h. and mixed. The mixed samples were refrigerated at -5 °C until they were analyzed for milk constituents. The milk from the goats was analyzed for milk lactose, milk protein, milk fat, total solid and solid nonfat. The milk fat was analyzed with (AOAC, 1997) method while the solid non-fat (SNF) and total solid (TS) were calculated. Milk lactose was determined by colorimetric method (Wahba, 2013). The milk protein was determined by Lowry method (Lowry at al., 1951). The total solid was calculated by subtracting the moisture content from 100.

The milk was further analyzed for selected minerals (Na, Ca, P, K, and Mg) using the Atomic Absorption Spectrophotometer. Five minerals (Na, K, Ca, Mg and P) were analyzed using the atomic absorption spectroscopy (AAS) Buck Scientific 210 VGP. The minerals were analyzed with different wavelengths such as Na (589.0 nm), K (766.5 nm), Ca (422.7 nm), Mg (285.2 nm) and P (graphite furnace).

The Fatty Acid Methyl Esters (FAME)

Milk fat was extracted according to the Soxhlet method with hexanes ACS (AOAC, 2000). 50 mg of the extracted fat content of the sample was saponified (esterified) for five minutes at 95 °C with 3.4 mL of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCL. 3ml of the 14% boron triflouride in the methanol was added. The mixture was concentrated for five minutes at the temperature of 90 °C to achieve a complete methylation process. The content was concentrated to 1 ml for gas chromatography analysis and 1 µl of the sample was injected into the port of GC FID (Gas chromatography flame ionization detector). The fatty acid methyl esters (FAME) were extracted from the mixture with redistilled n-hexane for analysis. FAMES were then analyzed by a gas-chromatography Buck Scientific 910, equipped with a flame ionization detector (FID), column type RESTEK 10m MXT-2887.I The initial oven temperature was 50 °C and finally was adjusted to 250 °C. The carrier gas was nitrogen at a flow rate of 35 psi and hygrogen gas pressure of 15psi. Fatty acids were identified by comparison of their retention times with those of a standard FAME mixture (Supelco® 37 Component FAME Mix, Sigma Aldrich). Fatty acids were expressed as a percentage of total fatty acids identified. The sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) was reported. Atherogenicity index was calculated as the content ratio of SFA/unsaturated FA, using the following formula proposed by Ulbricht and Southgate (1991): atherogenicity index: [C12 + 4 * (C14) + C16] / (Σ sum of unsaturated FA).

Data Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) using SPSS version 23.0., using the model of: $Y_{ij} = \mu + a_i + + e_{ij}$ where: Y_{ij}= any of the response variables; μ = the overall mean; a_i= effect of the ith feeding time; e_{ij}= random error due to experimentation. The differences between treatment means were examined by Duncan multiple range test of the same package.

Results

Performance of WAD Does under Different Feeding Time

The result in Table 3 shows that time of feeding influenced ($P < 0.05$) the milk yield, feed intake and feed efficiency of does fed at different time of the day. The result showed that does fed in the evening and morning had similar milk yield which was higher ($P < 0.04$) than from the does fed in the afternoon. Record showed that does fed in the afternoon had the highest ($P < 0.03$) feed intake while those fed in the evening had the lowest. However, in term of efficiency, evening fed does had the highest ($P < 0.04$) feed efficiency while those fed in the afternoon had the least.

Milk Composition (%) and Milk Minerals (mg kg^{-1}) of WAD Does under Different Feeding Time

Milk ash in morning-fed does was higher ($P = 0.017$) than in afternoon and evening-fed does (Table 4). Milk fat concentration was the highest ($P = 0.05$) in evening-fed does while afternoon-fed does produced the least. However, time of feeding had no impact ($P > 0.05$) on total solid, solid nonfat and lactose. Feeding in the afternoon improved ($P = 0.05$) the milk phosphorus (P) more than in the milk produced by evening-fed lactating does. However, other mineral parameters of milk were not significant ($P > 0.05$) but there were numerical variations.

Table 3. Performance parameters of WAD does under different feeding time

Parameter	Morning feeding	Afternoon feeding	Evening feeding	SEM	P-Value
Milk yield (g)	415.89^{a}	342.00^{b}	427.22^{a}	13.60	0.04
Feed intake (g)	667.13^{ab}	712.81^{a}	604.92^{b}	18.48	0.03
FE milk yield	0.62^{a}	0.48^{b}	0.71^{a}	0.02	0.04

Note: Means in the same column but with different superscripts are significantly ($P < 0.05$) different according to Duncan multiple range test ($P < 0.05$); SEM - Standard Error of the Mean

Table 4. Milk composition (%), milk component (g/d) and milk minerals (mg/kg) of WAD does under different feeding time

Parameter	Morning feeding	Afternoon feeding	Evening feeding	SEM	P-Value
Proximate					
Protein	7.29	8.26	7.69	0.66	0.974
Lactose	8.61	6.61	9.84	0.57	0.131
Total Solid	21.31	22.24	23.1	0.46	0.889
Solid nonfat	13.00	14.20	13.16	0.85	0.972
Fat	8.3^{ab}	8.00^{b}	10.86^{a}	0.64	0.05
Ash	1.99^{a}	1.08^{b}	1.06^{b}	0.15	0.017
Minerals					
Sodium	18.39	19.12	19.17	0.40	0.730
Potassium	38.48	38.06	37.41	0.99	0.908
Calcium	20.98	20.98	21.57	0.34	0.756
Magnesium	21.13	20.81	21.77	0.35	0.537
Phosphorous	3.16^{ab}	3.60^{a}	2.87^{b}	0.13	0.050

Note: Means in the same column but with the same letter are not significantly different according to Duncan multiple range test ($P < 0.05$); SEM - Standard Error of the Mean

Fatty Acid Profile of Milk of Does under Different Feeding Time

The fatty acid profile of milk of does under different feeding regimes is presented in Table 5. The result showed that tridecanoic, myristic, myristoleic, stearic, ϒ - linolenic, total linolenic acid, PUFA, PUFA/SFA and atherogenicity were significantly ($P < 0.05$) influenced by the time of feeding. Tridecanoic and myristic of evening-fed lactating does were significantly higher than of the morning and afternoon-fed does. Total linolenic acid of morning and afternoon-fed does was significantly higher than of evening-fed does. Furthermore, the PUFA of the afternoon was higher than of other treatments while evening-fed does had the lowest value. In contrast, atherogenic index of evening-fed does was higher than morning and afternoon-fed does.

Discussion

Performance of WAD Does under Different Feeding Time

The increased feed intake in does fed during the day may be associated with the availability of daylight during the feeding time while the lower intake in the evening-fed does in our study may be due to lower accessibility to light in the evening-fed does, which reduced their intake. The total darkness at night means that there is increased melatonin secretion and possibility for early night sleep, which will reduce the time spent at the feeder. However, the result in our study contradicts the report of Nikkhah at al. (2006) and Nikkhah at al. (2008) where feed intake during lactation was higher in evening-fed than morning-fed dairy cows. This may be due to the illumination that was provided for 3h post-feeding in the dairy cows fed at 21:00 h in those studies.

The increase in milk yield of evening-fed does despite lower feed intake showed that does that were fed in the evening were more efficient in their feed utilization. This could mean that there was less energy expenditure in the evening when the does were fed. In addition, the increase in milk yield is related to dry matter digestibility, resulting in increased milk efficiency (Socha at al., 2007; Linn at al., 2009). This means that despite the lower feed intake and milk yield in evening-fed does, the increase in feed efficiency may be associated with improved feed digestibility. Melatonin secretion affects cellular nutrient intake, increasing peripheral nutrient availability resulting in reduced nutrient use by peripheral tissue, which is redirected to mammary tissues in favour of milk production (Nikkhah at al., 2008). Thus, increase in milk efficiency in evening-fed animals might be associated with greater insulin resistance, as well as melatonin secretion which prevents much nutrient uptake at the cellular level (like adipose tissue and muscular cells) leading to higher availability of precursor for milk synthesis in the mammary gland (Corl at al., 2006). Feeding at night reduces energy expenditure in diurnal mammals (Coomans at al., 2013; Adamovich at al., 2014; Yasumoto at al., 2016). Other factor that could also improve the milk efficiency despite reduction in peripheral nutrient uptake may be due to conservation of energy resulting in reduced energy expenditure, which negates the effect of lower feed intake. Heat stress affects milk yield and quality and production efficiency, causing metabolic shift and changes in nutrients partitioning (Sejian at al., 2018; Sammad at al., 2020). The reduction in milk yield and efficiency in afternoon-fed does may be due to heat stress which causes increased energy expenditure and partitioning of nutrient for other uses than milk production alone. The milk yield in this study agrees with the report of Nikkhah at al. (2006)

Table 5. Fatty acid profile (g 100 g^{-1}) of milk of does under different feeding time

Parameter	Morning feeding	Afternoon feeding	Evening feeding	SEM	P-Value
C4:0 Butyric	3.19	3.09	3.11	0.045	0.669
C6:0 Caproic	3.25	3.10	3.00	0.079	0.497
C8:0 Caprylic	3.20	3.074	3.19	0.039	0.389
C10:0 Capric	10.49	9.93	8.65	0.348	0.06
C12:0 Lauric	4.19	3.58	3.55	0.172	0.249
C13:0 Tridecanoic	1.39^{b}	1.83^{ab}	2.18^{a}	0.141	0.041
C14:0 Myristic	10.99^{b}	11.70^{ab}	13.71^{a}	0.501	0.041
C14:1 Myristoleic	0.91^{a}	1.04^{a}	0.26^{b}	0.141	0.022
C16:0 Palmitic	24.10	24.61	27.72	0.726	0.061
C16:1 Palmitoleic	0.72	0.65	0.06	0.135	0.059
C18:0 Stearic	11.05^{a}	10.85^{a}	8.87^{b}	0.434	0.048
C18:1 Oleic	19.39	19.33	21.65	0.694	0.341
C18:2 Linoleic	0.60	0.75	0.28	0.087	0.057
C18:3 ϒ-Linolenic	1.39^{a}	1.53^{a}	0.53^{b}	0.186	0.026
C18:3 α Linolenic	0.48	0.54	0.07	0.105	0.128
Total Linolenic	1.87^{a}	2.062^{a}	0.59^{b}	0.825	0.027
C20:1 cis-11-Eicosanoic	0.38	0.40	0.001	0.137	0.473
C20:2 cis-11-14-Eicosadienoic	0.007	0.020	0.002	0.006	0.58
C20:4 Arachidonic	4.26	4.00	3.17	0.24	0.145
C21:0 Heneicosanoic	0.001	0.00015	0.0028	0.0007	0.335
SFA	71.87	71.76	73.99	0.541	0.167
UFA	28.13	28.24	26.01	0.541	0.167
PUFA	6.73^{a}	6.83^{a}	4.05^{b}	0.542	0.025
MUFA	21.40	21.41	21.97	0.549	0.915
UFA/SFA	0.39	0.39	0.35	0.01	0.173
PUFA/SFA	0.094^{a}	0.095^{a}	0.055^{b}	0.008	0.019
Atherogenicity	2.57^{b}	2.66^{b}	3.32^{a}	0.144	0.033

Note: Means in the same column but with the same letter are not significantly different according to Duncan multiple range test ($P < 0.05$); PUFA: Polyunsaturated fatty acids; MUFA: Monounsaturated fatty acids; UFA: Unsaturated fatty acid; SFA: Saturated Fatty Acid; atherogenicity index = (C12:0 + 4 × C14:0 + C16:0)/Σ of total unsaturated fatty acids calculated according to Ulbricht and Southgate (1991) and Nikkhah at al. (2008) where dairy animals fed in the evening had higher milk yield than the morning-fed ones.

Milk Composition and Mineral of WAD Does under Different Feeding Time

Timing of feed intake can alter the circadian rhythms of peripheral tissues. Alteration in the rhythm of peripheral tissues could also affect the derivatives or metabolites controlled by the peripheral tissues such as milk yield and milk composition. An increase in milk ash in morning-fed does when compared to other treatments may be due to higher feed intake. Furthermore, milk composition varies across the day because of the rhythmicity of milk synthesis (Lubetzky at al., 2007; Cubero at al., 2007). Thus, the variation in milk minerals may be due to the rhythmicity of ash production or feed intake. Higher milk fat in milk produced by evening-fed does may be due to higher postprandial peak of peripheral blood metabolites such as beta-hydroxybutyrate resulting in increased and efficient uptake by mammary tissues. Other milk components between feeding time are similar, but the result in this study is higher than the 8-19% total solids, 7-8.3% fat, 5.1-5.3% protein, 4.9-5.19% lactose, 6.11-13.24% solid nonfat and 0.64-0.91% total ash reported by (Mba at al., 1975; Akinsoyinu at al., 1977; Tona at al., 2017). This variation may be due to feeding intake, or the method used in collecting our milk samples (Adegbeye at al. 2021). Milk is a good source of calcium and phosphorus. Industrially, their component plays an important role in the coagulation of milk during cheese production, especially phosphorus (Summer at al., 2019). The reason for the increase in P in afternoon-fed animals is not known. However, the higher milk P in afternoon and morning-fed does compared to evening may be related to the pH of the gut at the time the milk was collected. Serna and Begwitz (2020) report that gut pH affects the avalability of P in the diet. This is because alkaline pH reduces P transport while acidic ones increase it. In this study, the time that the milk samples were collected (06:00-07:00 h, 12:00-13:00 h and 15:00-16:00 h) coincides with the period the morning and afternoon-fed does consumed their diet when the gut pH would be lower due to the feed digesta in the gut, which favored P absorption. However, in the evening-fed does, at the time the milk was collected, the gut pH might have increased because digesta flow was reduced because they did not eat during the daytime when the milk was collected, hence the lower milk P.

Fatty Acid Profile of Milk of Does under Different Feeding Time

Milk fat secretion and milk fatty acid constituents are of great interest to human nutrition, due to the potential impact of the lipid profile on consumers' health.

Polyunsaturated acids are not synthesized by ruminants so their concentration in milk depends on the quantity absorbed from the intestines, hence the availability of unsaturated fatty acids to be used by the mammary gland to syntheses milk lipids (Mesquita at al., 2008). In this study the lower level of PUFA in evening-fed does milk compared to morning-fed does milk may be associated with either lower forage intake in evening-fed does, compared to morning and afternoon-fed does, because fresh forage intake increases dietary PUFA supply (D'Urso at al., 2008), or it could be due to lower level of PUFA absorption in the gut of evening-fed does due to decreasing level of intestinal nutrient absorption at dusk (Hussain and Pan, 2009), or because of slowdown in gut motility at dawn, thus giving time or room for more biohydrogenation processes which reduce the PUFA reaching the intestine. The proportion of oleic acid in milk is controlled by its plasma uptake and partly from the desaturation of stearic acid by mammary D9-desaturase (Chilliard at al., 2001). In this study, the lower level of stearic acid in milk compared to morning and evening-fed does suggests that evening feeding favors the increased activity of mammary D9-desaturase. PUFA/SFA and n-6/n-3 PUFA ratios are commonly used to measure the nutritional value and consumer health of animal fat (Pilarczyk at al., 2015). Furthermore, the PUFA/SFA ratio in the milk indicate that morning and afternoon-fed does milk contains less SFA compared to evening-fed does, which suggests that they are good for consumers' health. This also reflects on the atherogenic index of goats. The values of atherogenicity index observed for the WAD does are related, for example, to the increased concentration of the conjugated linoleic acid content, which is now described as having anti-atherogenic properties (Cieslak at al., 2010). Diets with a high atherogenicity index and n-6/n-3 ratio are considered harmful to health (Tsiplakou and Zervas, 2008; Pilarczyk at al., 2015). Nevertheless, it was observed that C14:0 in the study was higher than that reported by Kholif at al. (2017) in the range of 9.64 - 10.1 g 100 g^{-1}, the PUFA and atherogenic index in this study is higher than the 0.84 - 0.93 g 100 g^{-1}, 2.30 - 2.44 g 100 g^{-1} respectively reported by Kholif at al. (2017), and Kholif at al. (2020), while the linoleic acid in our study is higher than that reported by Kholif at al. (2020) as 0.52 - 0.53 g 100 g^{-1}.

Conclusion

In conclusion, morning-fed does have high ash content, while evening-fed does have more fat. Afternoon-fed does have higher phosphorous content of mineral. Morning and afternoon feeding time influenced the polyunsaturated fatty acid profile of milk. However, evening-fed does have influence on atherogenicity score. To produce milk with a healthy component that can aid human development regarding fatty acid profile, morning or afternoon feeding of a dairy goat is recommended.

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