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DVM, Sylhet Agricultural University, Bangladesh; not shah Jalal University of Science & Technology, BANGLADESH (Email: ferdaus.dps@sau.ac.bd)

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Animal Science, Nutrition

Animal Science, Nutrition

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PhD candidate, Northwest A&F University, Yangling, 712100, CHINA (Email: muhammad.saeed@nwsuaf.edu.cn)

Nutrition - Ruminants

Naser Maheri Sis

PhD, Assistant Prof., Dept. Anim. Sci., I.A.U.-Shabestar, IRAN ([Website](#); Emails: maherisis@iaushab.ac.ir;

nama1349@gmail.com)

Nutrition - Ruminants, Nutritive Value, Utilization of Feeds

Nilüfer SABUNCUOĞLU ÇOBAN

PhD, Professor, Department of Animal Science and Production, Faculty of Veterinary Medicine, Atatürk University, TURKEY

([Website](#); Email: ncoban@atauni.edu.tr)

Animal Hygiene, Physiology, Animal Welfare

Ömer ÇOBAN

PhD, Professor, Department of Animal Science and Production, Atatürk University, TURKEY ([Website](#);

ocoban@atauni.edu.tr)

Nutrition - Ruminants

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PhD, Associate Professor, Veterinary Pharmacology and Toxicology, University of Bologna, ITALY (Email: paola.roncada@unibo.it)

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Raga Mohamed Elzaki Ali

PhD, Assistant Prof., Department of Rural Economics and Development, University of Gezira, SUDAN (Email: ragaelzaki@yahoo.co.uk)

Animal-feed interactions, Nutritive value

Animal-feed interactions, Nutritive value

Saeid Chekani Azar

PhD, Dept. Anim. Sci., Facult. Vet. Med., Atatürk University, TURKEY (Emails: saeid.azar@atauni.edu.tr;

schekani@gmail.com)

Physiology, Product Quality, Human Health and Well-Being,

Shahin Eghbal-Saeid

PhD, Associate Prof., Dep. Anim. Sci., I.A.U., Khorasgan (Isfahan), IRAN (Email: shahin.eghbal@khuif.ac.ir)

Animal Genetics and Breeding

Shahin Hassanpour

Dept. Physiology, Facult. Vet. Med., I.A.U., Shabestar, IRAN (Email: shahin.hassanpour@yahoo.com)

Physiology and Functional Biology of Systems

Shigdaf Mekuriaw

Andassa Livestock research center, ETHIOPIA (Email: shigdafmekuriaw@yahoo.com)

Animal production and Nutrition

Tarlan Farahvash

PhD Student, Dep. Anim. Sci., I.A.U., Khorasgan (Isfahan); Tarbiat Modares University, Tehran, IRAN
Animal Genetic and Breeding

Terry Ansah

PhD student, University for Development Studies-Ghana and Harper Adams University College, UK (Email: ansahterry@yahoo.com)

Nutrition - Ruminants

Tohid Vahdatpour

PhD, Assistant Prof., Department of Physiology, I.A.U.-Shabestar, IRAN ([Website](#); [Scopus](#); [Google Scholar](#); Emails: vahdatpour@iaushab.ac.ir; tvahdatpour@gmail.com)

Physiology and Functional Biology of Systems

Ümit Acar

Research Asistant and PhD, Department of Aquaculture, Faculty of Fisheries, Muğla Sıtkı Koçman University, TURKEY (Email: umitacar@mu.edu.tr)

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Vassilis Papatsiros

PhD, Department of Porcine Medicine, University of Thessaly, Trikalon str 224, GR 43100, GREECE (Email: vpapatsiros@yahoo.com)

Dietary input, Animal and Feed interactions

Wafaa Abd El-Ghany Abd El-Ghany

PhD, Associate Prof., Poultry and Rabbit Diseases Department, Cairo University, Giza, EGYPT (Email: wafaa.ghany@yahoo.com)

Poultry and Rabbit Diseases

Wesley Lyeverton Correia Ribeiro

MSc, DVM, College of Veterinary, Medicine, State University of Ceará, Av. Paranjana, 1700, Fortaleza, BRAZIL (Email: wesleylyeverton@yahoo.com.br)

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Yadollah Bahrami

PhD, Young Researchers Club and Elites, Khorasgan Branch, Islamic Azad University, Khorasgan, IRAN (Email: bahrami97@gmail.com)

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Yavuz Gurbuz

Prof. Dr., University of Kahramanmaras Sutcu Imam, Department of Animal Nutrition, Campus of Avsar, Kahramanmaras, TURKEY (Email: yavuzgurbuz33@gmail.com)

Animal Nutrition, Feed additive, Feed Technology and Evaluation

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PhD, Department of Plant Biology, Atatürk University, Erzurum, TURKEY (Email: zohreh.yousefi12@ogr.atauni.edu.tr)

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Volume 8 (5); September 25, 2018 [Booklet]**Research Paper****Productive responses of grazing cows to feed supplementation in the Coastal Savannah zone of Ghana.**

Obese F, Adjorlolo LK and Dwumah K.

Online J. Anim. Feed Res., 8(4): 105-111, 2018; pii: S222877011800014-8**Abstract**

Daily weight gains, body condition score (BCS), milk yield, concentrations of blood metabolites and resumption of ovarian activity were evaluated in 10 Sanga and 10 Friesian-Sanga cows grazing on natural pasture and supplemented with 2.5 kg of concentrate a day for 10 weeks during the dry season. Average daily gain in weight was similar in the Sanga (293 g) and Friesian-Sanga crossbred (288 g). Body condition score was also similar in the two breeds, but Friesian-Sanga cows had higher milk yield than Sanga cows (2.23 vs 1.65 L/day; $P < 0.001$). There were no significant differences in the concentrations of all the plasma metabolites determined apart from albumin and cholesterol concentrations. Albumin concentration was significantly higher in the Friesian-Sanga crossbred cows than Sanga cows (31.0 vs 29.3 g/L; $P < 0.05$), but total cholesterol was significantly higher in the Sanga than the Friesian-Sanga crossbreds (2.33 vs 2.01 mmol/L; $P < 0.01$). The two breeds had similar interval from calving to resumption of ovarian activity and proportion of non-cycling cows. The results from this study indicate the beneficial effects of feed supplementation on milk yield in Friesian-Sanga cows. Further studies need to be carried out to determine the effects of feed supplementation on milk composition and concentrations of metabolic hormones such as insulin, insulin-like growth factor-1 and leptin that mediate the effects of nutrition on ruminant reproduction.

Keywords: Blood metabolite, Coastal Savannah, Dry Season, Nutrition, Ovarian Activity[\[Full text-PDF\]](#)**Research Paper****Composition of colostrum and milk of West African Dwarf (WAD) does fed cassava peel based- diets supplemented with African yambean (*Sphenostylis stenocarpa*) concentrate in the humid zone of Nigeria.**

Anya MI and Ozung PO.

Online J. Anim. Feed Res., 8(5): 112-119, 2018; pii: S222877011800015-8**Abstract**

Twelve pregnant West African Dwarf (WAD) does were used to determine the effect of cassava peel meal based diets supplemented with African yambean Meal (AYBM) concentrate on colostrum and milk yield composition. Four concentrate diets were formulated with AYBM at 0, 10, 20 and 30% levels designated as T1, T2, T3 and T4, respectively. The does were randomly assigned into four groups of three per treatment and assigned to the respective diets in a Completely Randomized Design (CRD) experiment. Lactation length for each doe was based on 135 days. Results showed that daily colostrum yield, total solids (TS), crude protein (CP), ash and energy compositions differed significantly ($P < 0.05$) between the treatment groups. The mean colostrum yield per day (15.68 g), TS (18.52 %) and CP (5.41 %) were highest in diet T3 (20% AYBM) than treatment T2 (10% AYBM). Diet T4 (30% AYBM) promoted the highest percent values for Butterfat (BF), solids-non-fat (SNF), ash and energy with values of 3.52%, 14.54%, 0.91% and 4.30MJ/Kg, respectively. Milk yield, TS, BF, CP, SNF, ash and energy composition differed significantly ($P < 0.05$) between treatment groups. Milk yield (3.26 kg), SNF (9.96%) and energy constituent (3.91MJ/kg) values were highest for does fed 10% AYBM diet, while Diet T4 (30% AYBM) promoted the highest TS, BF, CP and ash and the values were 15.03, 5.49, 4.89 and 1.04%, respectively. The study concludes that cassava peel meal based – diets supplemented with African yambean concentrate supported optimum colostrum and milk composition without deleterious effects. The study therefore recommends that 20% inclusion level of African yambean concentrate in cassava peel meal based- diets is ideal for effective colostrum yield and milk composition in West African Dwarf does.

Keywords: African Yambean, Colostrum, Milk Yield, Milk Composition, WAD Does[\[Full text-PDF\]](#)

Research Paper

Hide and skin quality factors and marketing systems in Gondar town, Ethiopia

Ayalew H, Tamiru E and Nega Y.

Online J. Anim. Feed Res., 8(5): 120-125, 2018; pii: S222877011800016-8



Abstract

The study was conducted with the objective of assessing the type of defect affecting quality and marketing systems of hide and skin in Gondar town. Four kebeles were selected purposively based on intensity of fattening, degree of slaughtering frequency and number of hide and skin collection and traditional processing center and 15 household from each kebeles (a total of 60) were interviewed. Data were collected by developing semi structured questionnaires and direct observation. Collected data was organized, summarized and analyzed using SPSS version 20. The result indicated that all (100%) of examined hide and skin had one or more types of pre and post slaughter defects. The major types of pre slaughter defects responsible for the decline in quality of hide and skin were by ecto parasite (28.3), yolk mark (15 %), bloat surgery (31.7%), horn rake (6.7%), rope mark (8.3), branding (5%) and the rest 5% were thorn scratches. The finding also revealed that post slaughter defects were flaying cut (51.7%), poor pattern (13.3), putrefaction (16.7%) and improper fleshing (18.3%). Most of the respondents (95%) absence of defect, freshness and size were used as criteria for assessing the quality of hide and skin. It was observed that marketing was accomplished at four levels: producer, middlemen, collection center and tannery. Majority (75%) of respondents reported to sold hide and skin to the formal market for their cash income. Producers were price takers and buyers had power on price determination. It can be concluded that the both pre and post-slaughter defects have the potential to reduce quality of hide and skin. So, training should be given for livestock holders and market actors since most of the defect can be avoided through careful management of the animal, hide and skin.

Keywords: Defects, Gondar, Hide and Skin, Marketing, Quality factors

[Full text-[PDF](#)]

Research Paper

The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats.

Eburu PO and Anya MI.

Online J. Anim. Feed Res., 8(5): 126-135, 2018; pii: S222877011800017-8



Abstract

A study was conducted at the Teaching and Research farm of the University of Calabar, Cross River State, Nigeria, on the effect of essential oil (EO) combination on bio-hydrogenation (BH) of *n*-3 polyunsaturated fatty acids (PUFAs) and fermentation activities of rumen microbes. Four West African Dwarf goats (mean weight 40.0 ±2.5kg) were offered grass (*Panicum maximum*) and water *ad libitum* and supplemented with additional 400 g/goat/day of goat pellets for a period of 14 days. A basal feedstock comprising of 70:30 grass hay and concentrate was formulated. Each serum bottles contained 20 ml inoculum, 80 ml buffer, 1 g of feed substrate and supplemented with 300 mg/l of EO or their combination and incubated at 39°C for 48 h, and samples were collected to analyse ammonia N, total volatile fatty acids (TVFA) and concentration of PUFAs using GenStat 16th edition. There were four treatments and eight replicates as follows: Control, anise oil, lavender oil and blend (150 mg:150 mg) of anise and lavender (A+LO). Relative to the control, anise oil was the only treatment that reduced ($P<0.001$) ammonia N concentration in culture by a magnitude of 66% at 24 h. The concentration of TVFA (mM) was reduced by anise oil, whilst all other treatments maintained this level relative to the control (68.3, 60.4, 67.7 and 66.1 mM, for the control, anise oil, lavender oil and A+LO, respectively). The concentrations of *n*-3 PUFAs and C18:2 *n*-6 were maintained at higher levels ($P<0.001$) with addition of all EO, but were highest ($P<0.001$) in cultures where anise oil was added. The concentrations of 18:2 *cis*-9 *trans* 11 conjugated linoleic acid (CLA) and C18:1 *trans* 11 were significantly maintained ($P<0.001$) at higher levels by anise and A+LO. At both time points, the concentration of C18:0 was lowest in the control and highest in cultures supplemented with EOs. This study indicates that combining anise and lavender oil (at 1:1 ratio) substantially reduced bio-hydrogenation but without a considerable suppression of ruminal VFA concentration. Hence, this study recommends that, if this effect is confirmed *in vivo*, using anise and lavender oil (at levels 1:1) could reduce ruminal bio-hydrogenation without affecting ruminal volatile fatty acid concentration.

Keywords: Essential oils, Polyunsaturated fatty acids, Rumen fermentation, Volatile fatty acids

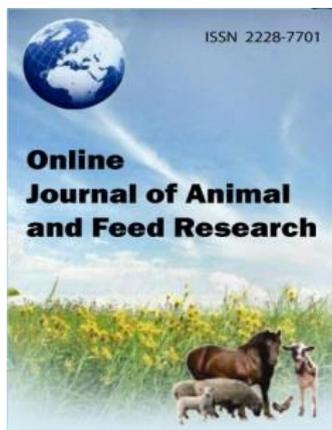
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PRODUCTIVE RESPONSES OF GRAZING COWS TO FEED SUPPLEMENTATION IN THE COASTAL SAVANNAH ZONE OF GHANA

Frederick Yeboah OBESE^{1✉}, Leonard Kofi ADJORLOLO² and Kwame DWUMAH^{1,3}

¹Department of Animal Science, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon, Ghana

²Livestock and Poultry Research Centre, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon, Ghana

³CSIR-Animal Research Institute, P.O. BOX AH 20 Achimota, Ghana

✉ Supporting Information

ABSTRACT: Daily weight gains, body condition score (BCS), milk yield, concentrations of blood metabolites and resumption of ovarian activity were evaluated in 10 Sanga and 10 Friesian-Sanga cows grazing on natural pasture and supplemented with 2.5 kg of concentrate a day for 10 weeks during the dry season. Average daily gain in weight was similar in the Sanga (293 g) and Friesian-Sanga crossbred (288 g). Body condition score was also similar in the two breeds, but Friesian-Sanga cows had higher milk yield than Sanga cows (2.23 vs 1.65 L/day; $P < 0.001$). There were no significant differences in the concentrations of all the plasma metabolites determined apart from albumin and cholesterol concentrations. Albumin concentration was significantly higher in the Friesian-Sanga crossbred cows than Sanga cows (31.0 vs 29.3 g/L; $P < 0.05$), but total cholesterol was significantly higher in the Sanga than the Friesian-Sanga crossbreds (2.33 vs 2.01 mmol/L; $P < 0.01$). The two breeds had similar interval from calving to resumption of ovarian activity and proportion of non-cycling cows. The results from this study indicate the beneficial effects of feed supplementation on milk yield in Friesian-Sanga cows. Further studies need to be carried out to determine the effects of feed supplementation on milk composition and concentrations of metabolic hormones such as insulin, insulin-like growth factor-1 and leptin that mediate the effects of nutrition on ruminant reproduction.

Keywords: Blood Metabolite, Coastal Savannah, Dry Season, Nutrition, Ovarian Activity

INTRODUCTION

Livestock farming holds enormous potential for improving food security and alleviating poverty. Considerable improvement in feeding and management strategies will be needed to increase productivity to meet increasing demand for livestock products in developing countries due largely to increasing population and income levels and rapid urbanization (Delgado, 2005; Thornton, 2010). Meeting the nutritional needs of cattle for productive purposes has been a major challenge especially in the dry season for farmers grazing cattle extensively on natural pasture in the coastal savannah zone in Ghana. The dry season is usually characterized by scarcity of good quality feed resulting in poor growth rate, low milk yield, delayed resumption of ovulation and extended calving intervals in animals consequently marginalizing profits (Obese et al., 2010). The energy content of forages does not generally meet the productive needs of cows. However, supplementing the diet of grazing cattle with concentrate has been reported to improve their productivity (Pulido et al., 2009; Filho et al., 2014; Idris et al., 2014).

The Sanga and Friesian-Sanga cattle are utilized mostly for meat and milk production in the extensive cattle production system depending greatly on natural pasture in the coastal savannah zone in Ghana. Some baseline studies conducted to assess the growth and reproductive status of these two breeds indicate less than optimum performance due to nutritional deficiencies especially in the dry season (Sottie et al., 2009; Obese et al., 2013, 2015). There is however inadequate information on the productive response of these two breeds to feed supplementation in the extensive cattle production systems in Ghana. Such information is vital in the development of strategic management practices to improve productivity.

This study therefore determined the daily weight gain, body condition score (BCS), milk yield, concentration of blood metabolites and resumption of ovarian activity in Sanga, and Friesian-Sanga cows grazing natural pasture and supplemented with concentrate diet during early lactation in the coastal savannah zone in Ghana.

ORIGINAL ARTICLE
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MATERIALS AND METHODS

Location of Study

The study was conducted at the Council for Scientific and Industrial Research - Animal Research Institute's (CSIR-ARI) Katamanso Station located in the coastal savannah zone of Ghana on latitude 05° 44' N and longitude 00° 08' W (<https://en.climatedata.org/location/777295/>). The vegetation is grassland with sparsely distributed shrubs. The area has a bimodal rainfall pattern with the major wet season occurring from April to July and a minor season from September to November. The remaining months constitute the dry period. Annual rainfall and temperatures range between 600-1000 mm and 21°C to 33°C, respectively and relative humidity ranges from 69 to 94% (Obese et al., 2015). The study received approval from the In-house Committee for Research of the CSIR-ARI.

Management of Animals

Twenty multiparous cows comprising 10 Sanga and 10 Friesian-Sanga crossbred cows which calved between January and May in the year 2017 (mostly in the dry season) were used in the study. They were supplemented with a concentrate having the composition shown in Table 1. The chemical composition of the supplement provided, and the natural pasture grazed by the cows are indicated in Table 2. Each cow received 2.5 kg per day of concentrate before grazing on natural pasture comprising mainly *Panicum insularis*, *Sporobolus pyramidalis*, *Griffonia simplicifolia*, *Stylosanthes hamata* and *Stylosanthes guineensis* for a period of 10 weeks. Water was provided twice daily and cows were milked once daily in the morning. Milk was collected from two quarters of the udder, and the other two quarters were reserved for calves (partial milking). Mating was natural with service bulls running freely with females all year round. Calves were weaned at six months of age. Cows and their calves were treated against ecto- and endoparasites once a month. They were also treated against diseases as the need arose. Weighing of cows and BCS determination (using a 9-point score of 1= very thin to 9= obese) was once a week as indicated in an earlier study (Obese et al., 2015).

Table 1 - Composition of supplement (concentrate)

Ingredient	Composition (%)
Maize	40.0
Wheat Bran	42.0
Soya bean Meal	10.0
Dicalcium Phosphate	2.0
Oyster Shell meal	5.0
Salt	0.5
Premix*	0.5
TOTAL	100

* The premix provided the following per kg of concentrate: Vit.A =30,000 IU, Vit.D = 35,000 IU, Vit.K = 33.75 mg, Vit.B=210 mg, Se = 0.375 mg, Mn =150 mg, Iodate = 5 mg, Zn = 125 mg, Cu =15 mg, Choline Chloride = 300 mg and Antioxidant 62.5 mg.

Table 2 - Chemical composition of forage (basal diet) and concentrate diet (supplement) fed to Sanga and Friesian – Sanga cows on dry matter basis

Constituent	Forage (%)	Supplement (%)
Dry matter	90.2	88.0
Crude protein	5.40	16.0
Neutral detergent fibre (NDF)	77.5	49.7
Acid detergent fibre (ADF)	49.0	13.8
Hemicellulose	28.5	35.9
Cellulose	31.9	3.97
Lignin	11.0	5.00
Silica	3.71	3.71
Digestible Energy (MJ/kg)	11.2	13.9

NDF = Neutral detergent fibre; ADF = Acid detergent fibre; MJ = Megajoules

Blood Sampling and Metabolite analyses

Blood samples were collected from cows after calving once every week for 10 weeks after morning milking at 08.30 h by jugular venipuncture into 7-mL vacutainer tubes and the concentrations of glucose, total protein, albumin, triglyceride, cholesterol, non-esterified fatty acid (NEFA), beta- hydroxybutyrate (BHB) and urea determined in the plasma at weeks 1, 3, 5, 7 and 9 using the VITROS® 5,1 FS Chemistry System auto-analyzer (Ortho-Clinical Diagnostics, U.S.A). Globulin concentration was computed as the difference between total protein and albumin concentrations. The concentration of NEFA in the plasma was determined by enzymatic calorimetric techniques using an assay kit (Diasys Diagnostic Systems, Germany). Plasma BHB concentration was measured using a BHB assay kit (Randox Laboratories, UK).

The resumption of postpartum ovarian activity was determined by measuring the progesterone concentrations in plasma samples from cows from week 1 to week 10 postpartum using a commercial ELISA Kit (DiaMetra Immunodiagnostic Systems, Boldon-UK) according to the manufacturer's instructions. The progesterone ELISA assay had a sensitivity of 0.05 ng/mL. Cows were classified as having resumed ovarian activity when plasma progesterone concentration of ≥ 1 ng/mL was recorded in two consecutive samples (Tamadon et al., 2011). Cows were classified as non-cycling if progesterone concentration remained below 1 ng/mL throughout the study period.

Statistical Analyses

The average daily weight gain (ADG), BCS, milk yield, and plasma concentration of blood metabolites (total protein, albumin, globulin cholesterol, triglycerides, NEFA and BHB) in the Sanga and Friesian-Sanga cows were determined using the GenStat Release 12th Edition software (GenStat, 2009). The chi-square test was used to determine the association between resumption of ovarian cycle and breed. Values reported are least square means and standard error of mean (SEM), unless otherwise stated.

RESULTS AND DISCUSSION

Weight gain, body condition and milk yield

Daily weight gain, BCS and partial milk yield data are presented in Table 3. Average daily weight gain in the Sanga (293 g) was not different ($P > 0.05$) from that of the Friesian-Sanga (288 g) implying the supplement fed provided enough nutrients to meet the growth needs of both breeds. This is at variance with the study of Teye et al. (2010) who observed that Friesian-Sanga crossbreds grew at a faster rate than Sanga cows when the breeds were grazed on natural pasture and offered an energy based supplement. The daily weight gains in the present study were higher than the 176 g/day and 216 g/day obtained for the Sanga and Friesian-Sanga crossbred respectively reported by Teye et al. (2010). The differences may be attributed to factors including type, amount and length of feed supplementation which can influence weight gains achieved by livestock (Detman et al., 2014; Filho et al., 2014; da Silva et al., 2017). The energy concentrate offered in the present study was based on maize and wheat bran and was fed at a rate of 2.5 kg/day, while Teye et al. (2010) used an energy concentrate based on brewers' spent malt, rice bran and wheat bran offered at a rate of 1 to 2 kg/day. The body condition score for the Sanga (7.33) was not significantly different from that obtained for the Friesian-Sanga (7.04) in the present study. These values indicate the cows were in good body condition suggesting adequate provision of nutrients from the concentrate for maintenance and productive purposes. Partial milk yield increased from week 1 to week 10 postpartum in the Friesian-Sanga crossbred, but remained stable during this period in the Sanga breed (Figure 1). The milk yield was significantly higher in Friesian -Sanga crossbred than the Sanga cows (2.23 versus 1.65 L/day; $P < 0.001$) suggesting higher partitioning of more energy for milk production in the Friesian-Sanga crossbred cows than the Sanga.

Table 3 – Daily weight gain, body condition score and milk yield in Sanga and Friesian-Sanga cows supplemented with concentrate

Parameter	Breed		SEM	Significance
	Sanga	Friesian-Sanga		
ADG of cow (g)	293	288	8.28	NS
ADG of calf (g)	682	798	1.98	NS
BCS	7.33	7.04	0.208	NS
Milk yield (L/day)	1.65	2.23	0.081	***

*** $P < 0.001$; NS = not significant; SEM = standard error of mean

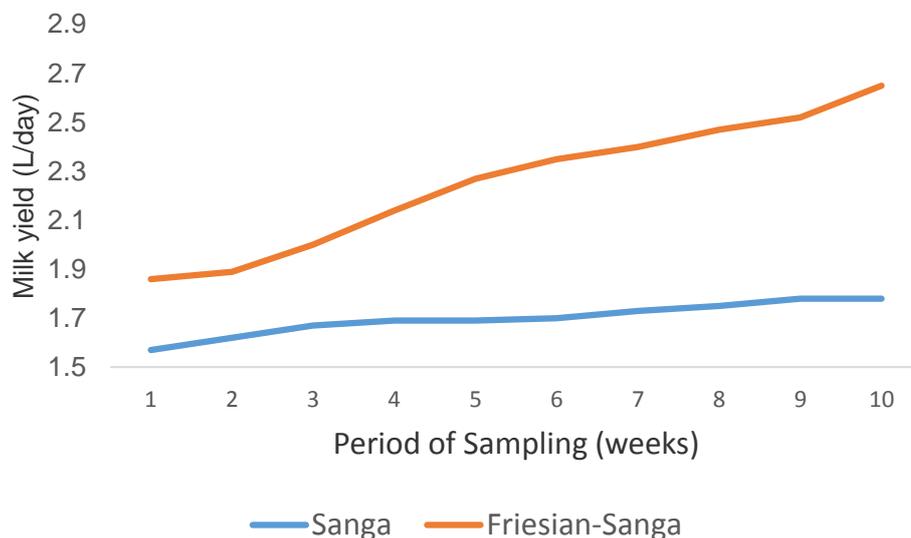


Figure 1 - Daily milk yield in Sanga and Friesian-Sanga cows during the postpartum period

Blood metabolite profiles

The concentrations of blood metabolites reflect nutritional, metabolic and health status in cattle (Ndlovu et al., 2007). The concentrations of all the plasma metabolites determined were not significantly different ($P > 0.05$) in the two breeds apart from albumin and total cholesterol concentrations (Table 4), suggesting that the supplement provided adequate nutrients for the two breeds to maintain their health and physiological status. The concentration of glucose can serve as indicator of energy balance status in cattle. The similar concentrations recorded in the Sanga (4.41 mmol/L) and Friesian - Sanga crossbred (4.16 mmol/L) cows which fell within the normal physiological range of 2.2 - 5.6 mmol/L for cattle (The Merck Veterinary Manual, 2010) implies adequate energy supply to the cows. Although total protein concentration was similar in the two breeds, the Friesian - Sanga cows had higher albumin concentration than the Sanga (31.0 vs 29.3 g/L; $P < 0.05$; Figure 2) indicating better protein status of the Friesian - Sanga crossbred cows than the Sanga. Albumin concentration in the blood is known to reflect protein status (Agenas et al., 2006). The concentrations of albumin obtained for the two breeds were within the normal range of 25 - 38 g/L reported for cows (The Merck Veterinary Manual, 2010) implying the cows were not malnourished. The similar plasma globulin concentrations in the two breeds suggest equal potential of fighting against diseases. Cholesterol levels in the plasma were significantly higher in the Sanga than the Friesian-Sanga crossbred (2.33 vs 2.01 mmol/L; $P < 0.01$; Figure 3), however these values fell within the normal physiological range of 1.6 - 5.0 mmol/L reported for cows (The Merck Veterinary Manual, 2010). Plasma concentration of urea did not differ in the Sanga and Friesian Sanga cows and the levels recorded were within the normal physiological range of 3.8 - 6.5 mmol/L reported for cattle (The Merck Veterinary Manual, 2010). The same trend was observed for triglyceride, NEFA and BHB concentrations.

Table 4 – Blood metabolite concentrations of Sanga and Friesian-Sanga cows supplemented with concentrate

Blood metabolite concentrations	Breed		SEM	Significance
	Sanga	Friesian-Sanga		
Glucose (mmol/L)	4.41	4.16	0.15	NS
Total protein (g/L)	76.5	72.9	1.08	NS
Albumin (g/L)	29.3	31.0	0.448	*
Globulin (g/L)	47.1	44.6	0.549	NS
Cholesterol (mmol/L)	2.33	2.01	0.070	**
Triglyceride (mmol/L)	0.23	2.01	0.007	NS
Urea (mmol/L)	4.92	5.23	0.148	NS
NEFA (mmol/L)	0.17	0.17	0.017	NS
BHB (mmol/L)	0.44	0.38	0.029	NS

* $P < 0.05$; ** $P < 0.01$; NS = not significant; SEM = Standard error of mean; NEFA = non-esterified fatty acid; BHB = Beta-hydroxybutyrate

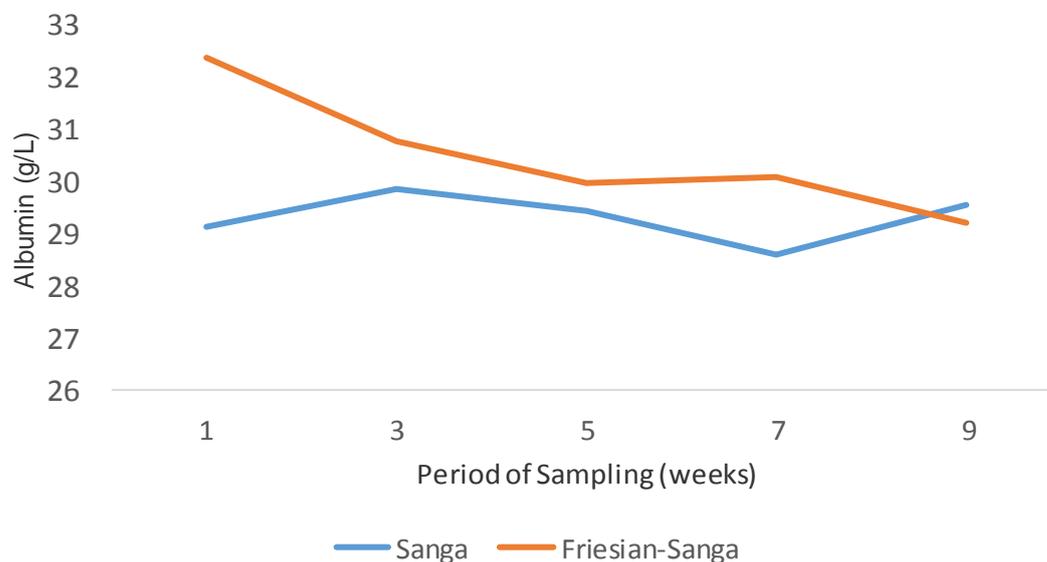


Figure 2 - Plasma Albumin concentration in Sanga and Friesian-Sanga cows during the postpartum period

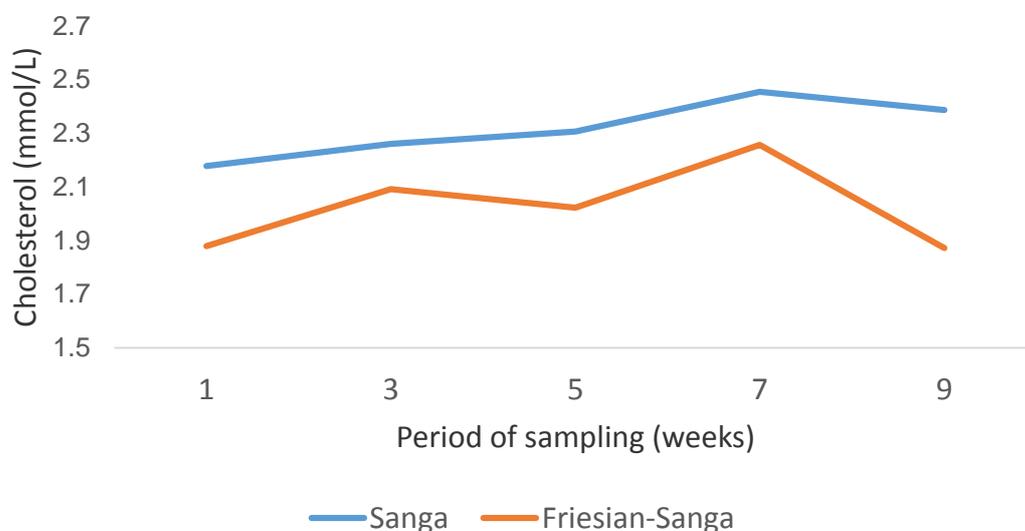


Figure 3 - Plasma Cholesterol concentration in Sanga and Friesian-Sanga cows during the postpartum period

Resumption of ovarian activity

The interval from calving to resumption of ovarian activity was not significantly ($P>0.05$) different in the Sanga (60 days) and Friesian- Sanga (63 days) cows indicating that the nutrients obtained by the two breeds of cows from the natural pasture and the supplement was adequate for resumption of ovarian activity. The 60 - day interval from calving -to -resumption of ovarian activity obtained for the Sanga in the present study was shorter than the 101.3 days reported in an earlier study for the same breed grazing extensively on natural pasture without feed supplementation in the coastal savannah zone (Obese et al., 1999). Inadequate nutrition delays the resumption of ovulation in cattle during the postpartum period by inhibiting the synthesis and secretion of luteinizing hormone, insulin-like growth factor-I and oestradiol which are necessary for ovarian follicular development and function (Diskin et al., 2003; Soca et al., 2014). The beneficial effects of feed supplementation in enhancing the onset of ovarian activity in Sanga cows in extensive grazing cattle production system in the coastal savannah zone has been reported (Okantah et al., 1998, 1999). For example, Okantah et al. (1999) supplemented Sanga cows grazing on natural pasture with 1.5 kg of wheat bran during early lactation in the coastal savannah zone and observed that the percentage of Sanga cows resuming ovarian activity after calving was higher than non-supplemented cows (46.4 vs 22%). Also in that study the interval from calving to the resumption of cyclic ovarian activity was shorter in supplemented than non-supplemented Sanga cows (64.6 vs 82.5%).

CONCLUSION

Friesian-Sanga crossbred cows produced more milk than Sanga cows when grazed on natural pasture and supplemented with energy concentrate. The similar levels of most of the blood metabolites measured and their concentrations falling within the normal physiological values for cattle indicate absence of deleterious effects of the supplement provided on the health and physiology of the cows.

The similar intervals from calving to resumption of ovarian activity indicate adequate provision of nutrients from the pasture and feed supplement for resumption of ovarian function. Further studies are required on the effects of feed supplementation on milk composition and concentrations of metabolic hormones that mediate the effects of nutrition on reproduction.

DECLARATIONS

Corresponding Author

Frederick Yeboah OBESE, *Department of Animal Science, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon, Ghana, E-mail: fobese@ug.edu.gh*

Authors' contribution

FYO designed the trial, supervised the data collection and laboratory work and contributed to the interpretation of the results and drafting of manuscript. LKA participated in the design of the study, interpretation of data and drafting of manuscript. KD contributed to data collection, laboratory and statistical analysis.

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Competing Interest

The authors declare that they have no competing interests

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COMPOSITION OF COLOSTRUM AND MILK OF WEST AFRICAN DWARF (WAD) DOES FED CASSAVA PEEL BASED-DIETS SUPPLEMENTED WITH AFRICAN YAMBEAN (*Sphenostylis stenocarpa*) CONCENTRATE IN THE HUMID ZONE OF NIGERIA

Magnus Izah ANYA and Pascal Ogar OZUNG 

Department of Animal Science, University of Calabar, Calabar, Nigeria

 Supporting Information

ABSTRACT: Twelve pregnant West African Dwarf (WAD) does were used to determine the effect of cassava peel meal based diets supplemented with African yambean Meal (AYBM) concentrate on colostrum and milk yield composition. Four concentrate diets were formulated with AYBM at 0, 10, 20 and 30% levels designated as T₁, T₂, T₃ and T₄, respectively. The does were randomly assigned into four groups of three per treatment and assigned to the respective diets in a Completely Randomized Design (CRD) experiment. Lactation length for each doe was based on 135 days. Results showed that daily colostrum yield, total solids (TS), crude protein (CP), ash and energy compositions differed significantly (P<0.05) between the treatment groups. The mean colostrum yield per day (15.68 g), TS (18.52 %) and CP (5.41 %) were highest in diet T₃ (20% AYBM) than treatment T₂ (10% AYBM). Diet T₄ (30% AYBM) promoted the highest percent values for Butterfat (BF), solids-non-fat (SNF), ash and energy with values of 3.52%, 14.54%, 0.91% and 4.30MJ/Kg, respectively. Milk yield, TS, BF, CP, SNF, ash and energy composition differed significantly (P<0.05) between treatment groups. Milk yield (3.26 kg), SNF (9.96%) and energy constituent (3.91MJ/kg) values were highest for does fed 10% AYBM diet, while Diet T₄ (30% AYBM) promoted the highest TS, BF, CP and ash and the values were 15.03, 5.49, 4.89 and 1.04%, respectively. The study concludes that cassava peel meal based – diets supplemented with African yambean concentrate supported optimum colostrum and milk composition without deleterious effects. The study therefore recommends that 20% inclusion level of African yambean concentrate in cassava peel meal based- diets is ideal for effective colostrum yield and milk composition in West African Dwarf does.

Keywords: African Yambean, Colostrum, Milk Yield, Milk Composition, WAD Does

INTRODUCTION

In Nigeria and majority of other countries in the world, milk supply is basically from bovines while that from sheep, goats and camels is negligible. The Nigerian dairy industry is highly underdeveloped, being very rural/traditional and relying heavily on the importation of dairy products worth more than US\$ 300 million per annum to meet the domestic demand of 1.45 billion litres (SAHEL, 2015).

The gap between supply and demand for dairy products is widening as a result of increase in population, urbanization, growing income, changing lifestyles and food preferences. Imports used to bridge part of the gap have also been declining as a result of devaluation of the Nigerian currency. Consequently local collection, processing and marketing of milk is becoming increasingly competitive (Any, 2012).

As at 1990, the annual collectable milk from the national herd was approximately 550,000 tonnes (Anon, 1990). In 2013 estimates show that Nigeria only produced 591, 491 metric tonnes which cannot meet the projected demand. Small ruminants (sheep and goats) also produce milk, but in Nigeria limited information is available in the milk productivity of these animals especially of the West African dwarf goat in the humid tropical rainforest zone.

In Nigeria, the population of sheep and goats was put at 22 and 34 million respectively (RIM, 1990). However, the main emphasis is on meat and skin with little emphasis on milk production. This makes it difficult to access the full potential of our indigenous small ruminants in terms of milk production. The over 440 million goats (worldwide) produce an estimated 48 million metric tonnes of milk that is predominantly consumed locally or processed into various types of milk products. However, the report of FAO (1991) showed that Asia, Africa and Europe are the leading continents in term of goat milk production.

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Goats are very important for their milk production potentials, for their size, goats have a higher relative yield than cattle or buffalo. This is partly because of the goat's relatively larger udder size and volume. The udder of the goat is a greater proportion of the total weight of the animal and contains a higher total quantity of secretory tissues than cows. This leads to a larger daily intake of feed and larger proportion of milk produced per unit of body weight (Steele, 1996). Thus, the goat is a more energetically efficient producer of meat and milk than the cow, even under conditions of good grazing (Raun, 1982; Anya, 2012). Some goats are maiden milkers and unlike cows can continue to lactate for up to two years (higher lactation persistency) which entails that they do not have to be mated each year and have a more pronounced milk ejection reflex (Jennes, 1980; Steele, 1996). In view of these facts, goats may in the long-run displace cows. Milk production data is very useful for selection purposes; it is well established that females with high milk output promote faster growth and better survivability of kids. Available information indicates that WAD goats have a low milk production potential. However, studies by some workers (Nuru, 1985; Okonkwo, 2001; Ahamefule, 2005 and Ukpabi, 2007) indicated that WAD does may produce some considerable amounts of milk especially if well nourished.

In other climes, goat colostrum is increasingly becoming important not only for goats but as a health product with a competitive demand for humans. New born mammals acquire immunity to certain infections via colostrum. Immune globulins appear in blood within 3 hours after colostrum is fed. Normally all proteins are digested, but in colostrum the inhibitor allows the immune globulins to reach the intestines without destruction. In humans, colostrum is known to burn excess fat, builds up lean nuclear growth and promotes efficient cellular function, tissue repair and skin rejuvenation (Tropical Traditions, TT; 2010). Thus, goat colostrum is now being harvested widely and sold to health product companies manufacturing several colostrum based products. This is so because there are over 90 known health enhancing components including dopamine, serotonin and lactoferrin in goat colostrum (TMN, 2009).

This study therefore evaluated the composition of colostrum and milk of West African Dwarf (WAD) Does fed cassava peel based diets supplemented with varying concentrate levels of African yambean in the high rainforest (Humid) zone of Cross River State – Nigeria.

MATERIALS AND METHODS

Location of study

The study was carried out in the Sheep and Goat Unit of the Teaching and Research Farm, Faculty of Agriculture, University of Calabar, Calabar. Calabar is located on latitude 4^o57'4"N and longitude 8^o19'4"E of the equator. Annual temperature and rainfall ranges from 25 – 30°C and from 1260 to 1280mm, respectively. The relative humidity is between 70 and 90% and Calabar is 98 metres above sea level (NMA, 2018).

Processing of cassava peel and African yambean seed meal

Cassava peels of TMS 30555 variety were collected fresh from the Department of Crop Science commercial "Garri" processing unit of the University of Calabar, Calabar. The peels were from 10-12 months old plants. The peels were properly sun dried for a period of 6 - 7 days during which they were regular turnings to give even drying to a moisture content of 10%. The peels could sometimes have tuber linings as a result of the method of removing the peels. The sun-dried cassava peels were then milled and used in the study as dried cassava peel meal (CPM). African yambean (*Sphenostylis stenocarpa*) seeds (Nsukka brown variety) were purchased from local famers in Obudu and Obanliku Local Government Areas in the Northern parts of Cross River State. The undecorticated brown seeds were boiled for 30 minutes. Water was made to boil at 100°C in a large (mammoth) cooking pot before the seeds were poured in. The seeds were allowed to boil for 30 minutes. Water was decanted using local baskets and the seeds sun-dried on aluminum roofing sheets for 3 days before being milled and used as yambean seed meal (AYBM) to formulate the experimental diets.

Experimental diets, its proximate analysis and test ingredients

Four experimental diets designated as T₁, T₂, T₃ and T₄ were formulated as presented in Table 1. Diet T₁ was the control and contained no yambean seed meal (YBSM). Diet T₂, T₃, and T₄, contained 10, 20, and 30% of AYBM, respectively. The diets were allotted randomly to the four animal groups. Each animal within a group was offered 1kg of an assigned concentrate diet daily for 56 days. The concentrate diets were fed at 0800 hour daily. Clean drinking water was provided *ad-libitum* for each animal within the period. Each animal was provided with a salt lick block (TANLICK), a product of SKM Pharma (P) Limited JF-10, City Point, Infantry Road Bangalore – 560001 India. The salt lick had the following composition: Na, 35.96%; Zn, 0.25%; Fe, 0.30%; Mn, 0.20%; I, 0.003%; Co, 0.002%; Cu, 0.10% and Mg, 0.05%. All the experimental diets including CPM and AYBM were analyzed for proximate composition using AOAC (2000) methods.

Table 1 - Gross composition of experimental diets

Ingredients	Diets (%)			
	T ₁	T ₂	T ₃	T ₄
Cassava peel	46.00	46.00	46.00	46.00
African yambean seed meal	0.00	10.00	20.00	30.00
Wheat offal	33.00	23.00	13.00	3.00
Palm kernel cake	18.00	18.00	18.00	18.00
Bone meal	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

Animal management

Twelve West African Dwarf (WAD) Does with average age of 18 months were selected from the goat herd of the Teaching and Research Farm of the University of Calabar, Calabar and used in this study. The average weight was 19.6 ± 1.12 Kg (18.5-22.7 Kg). The does which were in their second parity were randomly divided into 4 groups of 3 animals each. Each group was assigned to one of the experimental diets (Table 1) in a Completely Randomized Design (CRD) experiment. Each animal was housed separately in cement floored pens measuring 1.85 x 1.70 m (3.15m²). Dry hay material was used as bedding. During the first 4 months of pregnancy, the Does were zero-grazed with forage consisting mainly *Pennisetum purpureum*, *Pueraria phaseoloides* and *Centrosema pubesens*. Daily dry matter provision for each animal was based on 3% body weight. In the last trimester of pregnancy, each in-doe received 0.5kg of a concentrate diet in the morning (08:00hr) and 1.0 Kg *Pennisetum purpureum* in the afternoon (1400hr). This nutritional regime continued through parturition and into the 10th week of lactation for each Doe. Prior to parturition, routine spraying and deworming programmes were carried out including vaccination against PPR a viral disease of small ruminants endemic in the locality. Concentrate diets were placed in wooden troughs while water was provided *ad libitum* in plastic containers. The study was carried out in accordance with the code of ethics for animal experiments as stated in http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.

Kid management

For each pregnant doe, each feeder pen also doubled as a maternity pen. At birth, each kid had umbilical cord cleansed with disinfectant and cut at a distance of about 2cm away from the naval flap and a tincture of iodine was added to aid healing and prevent entry of pathogens. Kid weights were recorded immediately after parturition using a 5 kg capacity sensitive top loader "Salter" scale. Colostrum was collected, measured and fed back to the kids using feeding bottles for first 4 days. Thereafter, new born kids were left to suckle their dams freely for the next 6 days. The date of kidding, parity and liter sizes of does were recorded. The sex of kid born to a doe was also recorded and from this, the litter composition of the dam per kidding was determined.

Milk measurements

Does were hand-milked daily (morning). However, the total amount of milk yielded per day was recorded as the morning daily yield of the Doe. The daily milk yield was then estimated for each doe on the assumption that actual daily production of Does can be met if the animals were milked twice a day. Thereafter, based on the concept of fixed yield responses to changing milk frequency (Erdman and Verner, 1995), the constant 0.6596 was used as a weighting factor on the morning milk yield. Each day's milk yield (S) was estimated as: $S = M + 0.6596M$; where, M is the morning milk yield (once-a-day milking).

Prior to each day's milking including the first 4 day period for colostrum sampling (Anya, 2012), kids were separated from their dams at 1800hr on the evening preceding the day of milking. Within this period of separation, kids were fed milk with the aid of feeding bottles. During colostrum and normal milking, the two halves of the udder of lactating does were hand milked daily from 700 to 0800hr. The quantity of milk harvested from a doe was measured using a graduated glass cylinder (500ml capacity) and weighed back to the nearest gram on a sensitive laboratory scale. Dams were allowed to nurse their kids in the morning after milking and in the afternoon before separation at 1800hr daily.

Milk sampling

Colostrum sampling was initiated on the first day of kidding for each lactating doe and terminated on the fourth day post-partum. Samples for daily colostrum yield for each Doe were analysed daily for lactose content before being bulked (10 ml each) and analysed for total solids (TS), butterfat (BF), crude protein (CP), solids-not-fat (SNF), ash and energy. The bulked colostrum samples were stored in a refrigerator at - 5°C until required for analysis.

Lactation length for each Doe was based on 135 days. Milk sampling was initiated on the 10th day for each lactating doe and terminated on the 79th day post-partum. Samples from daily milk yield for each doe were analyzed daily for lactose content before being bulked and analyzed weekly for ST, BF, CP, SNF, ash and energy. The bulked samples were after stored in a refrigerator (-5°C) until required for analysis. The weekly milk production was a summation of each 7-day milk yield per doe while the weekly determinations (analysis) represented each doe milk profile for the week. The weekly lactose contents of milk of a doe was determined as average of daily lactose determinations.

Analytical procedure

The colostrum and milk samples were analyzed for lactose, TS, BF, CP (N x 6.38), SNF, ash and gross energy. TS were determined by drying about 5 g of milk sample to a constant weight at 105 °C for 24 hours. Lactose content was determined from fresh samples by the Marrier and Boulet (1959) procedure. BF was obtained by the Roesse-Gottlieb method (AOAC, 1980). Milk protein (N x 6.38) was determined by the semi-micro distillation method using Kjeldahl and Markham's apparatus. Ash content was obtained by drying and ashing a weighed milk sample (10 ml) to a constant weight at 550 °C for 48 hours. SNF was determined as the difference between TS and butterfat. Milk energy Y (M/kg) was computed using the multiple regression equation. $Y = 0.386F + 0.205 \text{ SNF} - 0.236$ (MAFF, 1975); where F and SNF represent percentages of fat and solids-not-fat, respectively.

Statistical analysis

The data on colostrum and milk yield and composition were analysed using the analysis of variance (ANOVA) procedures for a Completely Randomized Design (Morris, 1999). Significant means were separated using methods as outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Proximate composition of experimental diets and test ingredients (CPM and AYBM)

The result of the proximate composition of experimental diets, cassava peel meal and African yambean meal is presented in Table 2.

Colostrum yield and composition

The average daily colostrum yield and composition of Does fed concentrate diets containing varying levels of African yambean seed meal are summarized in Table 3. Colostrum yield (g) differed significantly ($P < 0.05$) among treatment groups. The colostrum yield of animals fed the control diet (T_1) was the lowest (14.28 g) followed by diet T_2 (14.43 g), diet T_3 (15.68g) and diet T_4 (14.46 g), respectively. Goats fed diet with 20 % AYBM produced the highest mean daily colostrum yield of 15.68 g which differed ($P < 0.05$) from the yield of other treatment groups. In this study, diet T_3 promoted the highest colostrum yield over the 4 day period while the control diet was the least. This showed that AYBM based-diets promoted higher colostrum yields compared to the control. Furthermore, among the AYBM based diets, it is possible that more ruminal acetate was produced in does fed diet T_3 hence the high colostrum yield recorded for this treatment group in comparison with the yield of goats fed either diets T_2 or T_4 .

Percent TS was higher ($P < 0.05$) in the colostrum of does fed AYBM diets than the control group. Does on diet T_3 had the highest percent TS (18.52) that was significantly different ($P < 0.05$) from those on control diet (17.37). However, TS was similar ($P > 0.05$) for does on diets T_3 and T_4 , while TS for does on diet T_4 was not different from those on diet T_2 . The percent TS obtained for colostrum in this study (18.08 ± 0.55) falls within the range of 19.2% reported by Akinsoyinu et al. (1977) for WAD goats.

BF percent of colostrum was highest and lowest in does fed 30% (3.52) and 10% (3.22) AYBM diets respectively while the values of 3.22% and 3.12% obtained for diet T_3 (20% AYBM) and the control group (T_1) respectively did not differ ($P > 0.05$) significantly. The butterfat percentage for colostrum obtained in this study was low when compared to 8.3% reported by Akinsoyinu et al. (1977) for WAD goats. The non-concurrent values reported may be due to differences in parity, perhaps litter size and the varying diets used in different studies.

The CP and SNF concentrations were significantly higher ($P < 0.05$) in the colostrum of does fed AYBM diets than in the control. While colostrum protein was highest (5.41%) for diet T_3 (20% AYBM), SNF values were highest (14.54%) in diet T_4 (30% AYBM). The CP and SNF concentrations in colostrum are generally influenced by diet quality. The relatively higher values obtained for these constituents in does fed AYBM diets tend to confirm that these diets were superior to the control. However, the values for total protein obtained in this study for colostrum agrees with the value of 5.1% reported by Akinsoyinu et al. (1977) for WAD goats.

Lactose concentration for colostrum in this study did not differ ($P>0.05$) significantly among the treatments. Lactose concentration in colostrum is however influenced by diet quality. Results obtained reveal that all the diets were of good quality but AYBM diets were superior to the control.

Colostrum ash (%) and energy (MJ/Kg) were fairly similar for all treatment groups. Values obtained were 0.78%, 3.87MJ/Kg; 0.80%, 3.98MJ/Kg; 0.81%, 3.98MJ/Kg; and 0.91%, 4.30MJ/Kg for diets T₁, T₂, T₃ and T₄ respectively. The mean value for ash $0.83 \pm 0.12\%$ agrees with the value of $0.82 \pm 0.01\%$ reported by Akinsoyinu (1974) for WAD goats, but higher than $0.55 \pm 0.14\%$ reported by Ahamefule et al. (2003). However, colostrum ash of diet T₄ was significantly ($P<0.05$) different compared to other diets. Milk energy (MJ/kg) of colostrum followed the same pattern like that of ash (%), with diet T₄ being superior ($P<0.05$) compared to other diets.

Table 2 - Proximate and energy composition of experimental diets, cassava peel meal (CPM) and African yambean seed meal (AYBM)

Composition (% DM)	Diets					
	T ₁	T ₂	T ₃	T ₄	*CPM	*AYBM
Dry matter	89.44	89.35	89.42	89.62	90.10	88.50
Crude protein	10.56	10.96	11.36	11.44	3.22	22.10
Crude fibre	12.47	11.05	10.31	10.11	14.73	5.92
Ether extract	4.50	4.61	4.80	4.94	0.91	7.53
N-free extract	51.38	53.61	54.33	54.62	65.67	47.67
Ash	10.35	9.12	8.62	8.49	5.57	5.28
Gross energy (kcal/g)	3.45	3.42	3.31	3.28	3.60	5.23

*Test ingredients

Table 3 - Effect of the experimental diets on colostrum yield and composition of WAD goats

Parameter	Diets				
	T ₁	T ₂	T ₃	T ₄	SEM
Colostrum yield (g)	57.50 ^b	57.63 ^b	62.71 ^a	58.26 ^b	1.23
Daily colostrum yield (g)	14.28 ^b	14.41 ^b	15.68 ^a	14.46 ^b	0.33
Total solids (%)	17.37 ^b	18.02 ^{ab}	18.52 ^a	18.42 ^{ab}	0.26
Butterfat (%)	3.12 ^b	3.22 ^b	3.22 ^a	3.52 ^a	0.09
Protein (N x 6.38) (%)	5.09 ^b	5.11 ^a	5.41 ^a	5.12 ^b	0.08
SNF (%)	14.16 ^b	14.51 ^{ab}	14.51 ^{ab}	14.54 ^a	0.09
Lactose (%)	2.28	2.29	2.30	2.30	0.02
Ash (%)	0.78 ^b	0.80 ^b	0.81 ^b	0.91 ^a	0.03
Energy (MJ/kg)	3.87 ^b	3.98 ^b	3.98 ^b	4.30 ^a	0.09

^{ab} Means on the same row with different superscripts differ significantly ($P<0.05$). SEM = Standard error of mean

Milk yield and composition

The average weekly milk yield and composition of does fed concentrate diets containing varying levels of African yambean are summarized in Table 4. Milk yield (kg) differed significantly ($P<0.05$) among treatment groups. The milk yield of goats fed the control diet (T₁) was the lowest (2.58kg) but similar ($P>0.05$) to the average yield (2.71kg) obtained for does fed diet T₄ (30% AYBM). The does fed diet T₃ (20% AYBM) had average weekly yield of 2.97kg which did not differ significantly ($P>0.05$) from the yield of the group fed diet T₂. Animals fed diet T₂ (10% AYBM) produced the highest mean milk yield of 3.26kg which differed significantly ($P<0.05$) from the yield of other treatment groups. In raising ruminant animals either for milk or meat production, there is a fundamental antagonism between milk synthesis and fattening. Diets that promote efficient weight gain, would most times naturally lead to poor milk synthesis and vice versa (Rai, 1980; Ahamefule, 2005). This according to Preston (1986); Mathewman (1995) and McDonald et al. (1995) is related to the metabolism pattern and production of ruminal volatile fatty acids associated with diets fed.

To buttress this point, Preston (1986) and Ahamefule (2005) reported that diets that lend themselves more easily to production of propionate than acetate in the rumen would tend to encourage weight gain than milk synthesis in ruminants. The converse is also true. This is an indication that more ruminal propionate than acetate was produced in does fed diet T₄; hence the low milk production recorded for this treatment group in comparison with the yields of goats fed either diets T₂ or T₃. It is also possible that more ruminal acetate than propionate was produced in goats fed diet T₂, hence the comparatively higher milk yield observed for the does placed on this diet. Generally, the inclusion of AYBM in the diets improved milk yield compared to the control. This observation was in line with the reports of Ahamefule (2005) and Ukpabi (2007).

Table 4 - Effect of the experimental diets on milk yield (kg) and composition of WAD goats

Parameter	Diets				SEM
	T ₁	T ₂	T ₃	T ₄	
Total milk yield (kg)	2.58 ^b	3.26 ^a	2.97 ^{ab}	2.71 ^b	0.15
Total solids (%)	14.70 ^b	14.71 ^b	15.01 ^a	15.03 ^a	0.09
Butterfat (%)	4.89 ^b	4.44 ^b	4.93 ^b	5.49 ^a	0.22
Protein (N x 6.38) (%)	4.34 ^b	4.66 ^b	4.67 ^b	4.89 ^a	0.12
SNF (%)	9.27 ^b	9.96 ^a	9.58 ^{ab}	9.95 ^a	0.16
Lactose (%)	4.29 ^a	4.23 ^b	4.32 ^a	4.30 ^a	0.01
Ash (%)	0.88 ^b	0.91 ^b	0.99 ^b	1.04 ^a	0.04
Energy (MJ/kg)	3.62 ^b	3.91 ^a	3.62 ^b	3.90 ^a	0.08

^{ab} Means on the same row with different superscripts differ significantly (P<0.05). SEM = Standard error of mean

The mean weekly milk yield (2.83±0.31kg) obtained for WAD goats in the first 10 weeks of lactation was rather low but comparable to the value of 3.33±1.07kg reported by Akinsoyinu (1974) for the same breed. The non-concurrent values may be due to differences in parity and perhaps litter size. Fifty percent of Does used in this study were in their second parity while fifty percent had single births. The Does used by Akinsoyinu (1974) had multiple births and were mostly in either their 2nd, 3rd or 4th parity. Litter size and parity have been identified as strong factors influencing milk yield and composition in lactating animals (Csapo et al., 1994; Steele, 1996; Akpa et al., 2001; Ahamefule, 2005; Williams et al., 2010).

Percent TS was higher in the milk of goats fed AYBM diets (T₂, T₃ and T₄) than in the control group. There was however no significant differences (P>0.05) observed in the % TS values of goats fed the 10% (14.71) and the control group (14.70) and between goats fed the 20% (15.01) and those on 30% (15.03) AYBM diets. Milk TS of goats fed 30% AYBM diet (15.03) was higher and differed significantly (P>0.05) from those on diet T₁ and T₂ but similar (P<0.05) to those on diet T₃. BF percent was highest and lowest in milk of goats fed 30% (5.49) and 10% (4.44) AYBM diets respectively while the values of 4.93% and 4.89 obtained for diet T₃ (20% AYBM) and the control group (T₁) did not differ (P>0.05) significantly. However, the values for fat content (%) obtained in this study compares favourably with the fat content of 4.82 – 5.79% reported by Ahamefule and Ibeawuchi (2005) for lactating WAD does fed pigeon pea-cassava peel based diets. BF and TS, like most other milk constituents, are generally influenced by the type of diet of lactating animals (Akpa et al., 2001; Williams et al., 2010) as well as yield (Ibeawuchi, 1985). Other investigations (Jenness, 1980; Ahamefule et al., 2003; 2004; Ahamefule, 2005; Ukpabi 2007) had confirmed negative correlations between yield and butterfat which agrees with the findings of this study. For instance, butterfat values was lowest in the milk of Does fed diet T₂ (4.44%) which promoted the highest weekly yield (3.26 kg). Also, the TS value of the group fed diet T₂ was among the lowest (14.71). In a contradictory swift, BF and TS values in this study were highest in the milk of does fed 30% AYBM (T₄) (5.49; 15.03% respectively) but the trend was not the same for the control group with the lowest yield. This peculiar contradictory trend observed in this study is in line with the reports of Ahamefule (2005) with pigeon pea-cassava peel based diets and Ukpabi (2007) with mucuna bean. This trend of high BF and TS in the milk yield of lactating WAD goats fed this legume could be due to the high crude protein and ether extract content of the seed meals used in the various diets fed. There is good evidence that in small ruminants about 75% of the fatty acids in milk arise from dietary fat. A dietary source of lipid can reduce considerably any imbalance caused by relative deficiencies of glycogenic energy and amino acids in the end products of rumen digestion. Thus, for many feeding systems in the tropics, the level of fat in the diet could be a primary constraint to milk production (Preston, 1986).

The CP, SNF and lactose concentrations were significantly higher (P<0.05) in milk of Does fed AYBM than the control. While milk protein was highest (4.89%) in diet T₄ (30% AYBM), SNF value was highest (9.96%) in goats fed diet T₂ (10% AYBM) while lactose was highest in goats fed diet T₃ (20% AYBM). The CP, SNF and lactose concentration in milk are generally influenced by diet quality. The relatively higher values obtained for these constituents in goats fed AYBM diets tend to buttress the fact that these diets were of superior quality to the control. The similar values (P>0.05) for CP of AYBM diets confirms earlier reports that increasing the CP of diets does not necessarily increase the protein content of milk (Sutton, 1981). Lactose content did not differ significantly (P>0.05) between diets T₁ and T₄. The values for does on diets T₃ and T₄ did not also differ significantly (P>0.05) but were higher than 4.29% recorded for diet T₁. The result of this study indicated that the inclusion of AYBM in the diets improved on the lactose content of goat milk. The high values obtained in this study may not be unconnected with the high concentrate intake, which probably promoted more production of propionic acid in the rumen fluid. This eventually resulted in more lactose output because of its glycogenic effects (Allen, 1977; Matenga et al., 2003). Similar results were reported by Ukpabi (2007) with mucuna seed meal.

Meanwhile, the mean value of $14.86\pm 0.25\%$; 4.94 ± 0.24 and $4.34\pm 0.16\%$ obtained respectively for TS, BF and lactose for WAD goats in a 10-week lactation, were lower than the respective values of 19.21 ± 0.46 ; 7.31 ± 0.21 and $6.60\pm 0.19\%$ reported by Akinsoyinu (1974) for WAD goats within the same lactation length. However, the values obtained for TS, BF and lactose in this study compare favourably with 14.92 ± 0.27 ; 5.21 ± 0.27 and $4.65\pm 0.13\%$ respectively reported by Ahamefule (2005) with pigeon pea meal based diets. Mean milk protein value ($4.64\pm 0.09\%$) obtained in this study was however superior to that reported by Akinsoyinu (1974) but comparable to $4.44\pm 0.07\%$ (Ahamefule, 2005) and slightly lower than $4.76\pm 0.12\%$ (Ukpabi, 2007). Differences in dietary planes and compositions have been reported to be responsible for variations in milk yield and composition observed even within the same breed (Ibeawuchi, 1985; Akpa et al., 2001; Ahamefule, 2005; Williams et al., 2010).

The ash content in the milk differed significantly between diets ($P<0.05$). However, the ash contents increased with increasing level of AYBM in the various diets. Values obtained were 0.88; 0.91; 0.99 and 1.04% for diets T₁, T₂, T₃, and T₄ respectively. The mean value of $0.95\pm 0.09\%$ obtained in this study was higher than $0.82\pm 0.01\%$ and $0.55\pm 0.14\%$ reported by Akinsoyinu (1974) and Ahamefule et al. (2003) respectively, but comparable to $0.93\pm 0.08\%$ reported by Ahamefule and Ibeawuchi (2005) for WAD goats, and 0.94 ± 0.05 obtained for WAD sheep (Ahamefule et al., 2003).

Milk energy (MJ/Kg) was significantly ($P<0.05$) higher in lactating does fed AYBM diets than the control group. Does fed diet T₂ (10% AYBM) recorded the highest energy concentration in milk (3.91) while the control group posted the least value (3.62). A strong positive relationship exists between BF, SNF and energy (Jennes, 1980). This implies that milk high in BF percent should be expected to be high in energy as well. This observation however did not seem to agree with the milk energy values obtained in this study. Milk samples from does fed 10% AYBM diet had the least BF content (4.44) but highest milk energy (3.91MJ/kg) because SNF was highest in the milk of diet T₂. Milk energy is a function of BF, SNF and milk volume in the milk of (Ibeawuchi, 1985; Ahamefule and Ibeawuchi, 2005) hence the result obtained for diet T₂. Though the goats fed 10% AYBM diet had the least BF content, the group produced averagely the highest volume of milk (3.26kg) and highest SNF (9.96%) among the treatment groups which may be responsible for the significantly higher milk energy obtained for animals in the group. However, the mean milk energy value (3.76 ± 0.15 MJ/kg) obtained for WAD goats in this study was higher than 1.96 ± 0.17 MJ/kg reported by Ahamefule (2005), but seem to agree with the findings of Ukpabi (2007).

CONCLUSION AND RECOMMENDATION

From the results of this study, it can be concluded that cassava peel meal based – diets supplemented with African yambean concentrate supported optimum colostrum and milk composition without deleterious effects. Therefore, it is recommended that 20% inclusion level of African yambean concentrate in cassava peel meal based-diets is ideal for effective colostrum yield and milk composition in West African Dwarf Does. Farmers are strongly advised to utilize these feedstuffs in diets meant for small ruminants in the tropical rainforest zone of Nigeria, where these ingredients are abundant.

DECLARATIONS

The work is original and has not been submitted elsewhere for publication. The authors declaration form has been signed by the authors as recommended.

Corresponding Author

E-mail: pascalozung@yahoo.com, Tel: +2348062246745

Authors' Contribution

Anya M.I. and Ozung P.O. contributed equally in the design and conceptualization of the research work. The field work and laboratory aspects of the research were carefully handled by both authors, likewise the statistical analyses and finally documentation of the research findings. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that there is no conflict of interest in this work.

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HIDE AND SKIN QUALITY FACTORS AND MARKETING SYSTEMS IN GONDAR TOWN, ETHIOPIA

Habtamu Ayalew✉, Elias Tamiru and Yostena Nega

University of Gondar, College of Veterinary Medicine and Animal Sciences, P.O.Box.196, Gondar, Ethiopia

✉Supporting Information

ABSTRACT: The study was conducted with the objective of assessing the type of defect affecting quality and marketing systems of hide and skin in Gondar town. Four kebeles were selected purposively based on intensity of fattening, degree of slaughtering frequency and number of hide and skin collection and traditional processing center and 15 household from each kebeles (a total of 60) were interviewed. Data were collected by developing semi structured questionnaires and direct observation. Collected data was organized, summarized and analyzed using SPSS version 20. The result indicated that all (100%) of examined hide and skin had one or more types of pre and post slaughter defects. The major types of pre slaughter defects responsible for the decline in quality of hide and skin were by ecto parasite (28.3), yolk mark (15 %), bloat surgery (31.7%), horn rake (6.7%), rope mark (8.3), branding (5%) and the rest 5% were thorn scratches. The finding also revealed that post slaughter defects were flaying cut (51.7%), poor pattern (13.3), putrefaction (16.7%) and improper fleshing (18.3%). Most of the respondents (95%) absence of defect, freshness and size were used as criteria for assessing the quality of hide and skin. It was observed that marketing was accomplished at four levels: producer, middlemen, collection center and tannery. Majority (75%) of respondents reported to sold hide and skin to the formal market for their cash income. Producers were price takers and buyers had power on price determination. It can be concluded that the both pre and post-slaughter defects have the potential to reduce quality of hide and skin. So, training should be given for livestock holders and market actors since most of the defect can be avoided through careful management of the animal, hide and skin.

Keywords: Defects, Gondar, Hide and skin, Marketing, Quality factors

INTRODUCTION

The agricultural sector in Ethiopia, engaging 85% of the population, contributes 52% to the gross domestic product (GDP) and 90% to the foreign exchange earnings (CSA, 2008). Ethiopia is believed to have the largest livestock population in Africa (CSA, 2017). The varied and extensive agro-ecological zones and the importance of livestock in livelihood strategies make Ethiopia home to large numbers of livestock. Indeed, Ethiopia has the largest livestock inventory in Africa, 59,486,667 cattle, 30,697,942 sheep and 30,200, 226 goats, 8,439, 220 donkeys, 409, 877 mules, 2,158, 176 horses and 59,495, 026 chickens (CSA, 2017).

In Ethiopia hides and skins contribute much to the export earnings from the livestock sector. In addition, it has a large contribution to the leather industry in the country. Livestock hide and skin contribute significant proportion of domestic leather. Girma (2003) reported that, Ethiopia has been exporting hides and skins in the past 100 years. The country has big potential to develop the sub-sector. In 2002, hides and skins represented major source of foreign exchange earnings for the country accounting for 14-16% of the total export revenue. Hides and skins are the basic raw materials for the leather industry. Currently there are about 27 tanneries in the country and have an average capacity of 4,000 pieces of hides and 30,000 pieces of skins per day (EEA, 2007/08). Based on the off-take rate of 7%, 33% and 35% for cattle, sheep and goat respectively, it is expected to produce 3.1 million hides, 7.8 million sheep skins and 8.2 million goat skins (CSA, 2004; 2007).

The quality of hide and skin is to large extent related to the amount of damage to the grain (or outside) surface. The damage may be due to skin parasite that affects the live animal. Husbandry practice on the farm or in transport of live animal (scratches, bruising or dry contamination, horn rake); it may be due to damage during slaughter or it may be caused by in appropriate handling or in adequate preservation techniques (Adugna, 2004).

In Gondar Ethiopia, most people do not preserve hide and skin before selling, this make putrefaction and this has a significant negative effect on the quality of the hides and skin. An in-depth study of the hide and skin quality management and marketing was necessary in order to gain an insight into marketing chain, the key factors involved and constraints attached to various stages of actors. Since the above factors have not been studied in the study area, there was a need to assess quality management and marketing of hide and skin to fill the existing problem in the study area.

MATERIAL AND METHODS

Description of the Study Area

The study was conducted in Gondar town, Amhara Regional State, Ethiopia. The area is located at a distance of 738 km away from Addis Ababa in North West of Amhara region. The area lies between latitude and longitude of 12°36'N 37°28'E with an elevation of 1966 meters above sea level. Gondar has a varied landscape, dominantly covered with ragged hills and plateau of land formations. The annual average temperature and rain fall were 19.7 °C and 1772 mm, respectively which could be categorized under mid highland climatic zone. The area is also classified mainly in to two seasons, the wet season, ranging from June to September and the dry season extended from October to May (CSA, 2017).

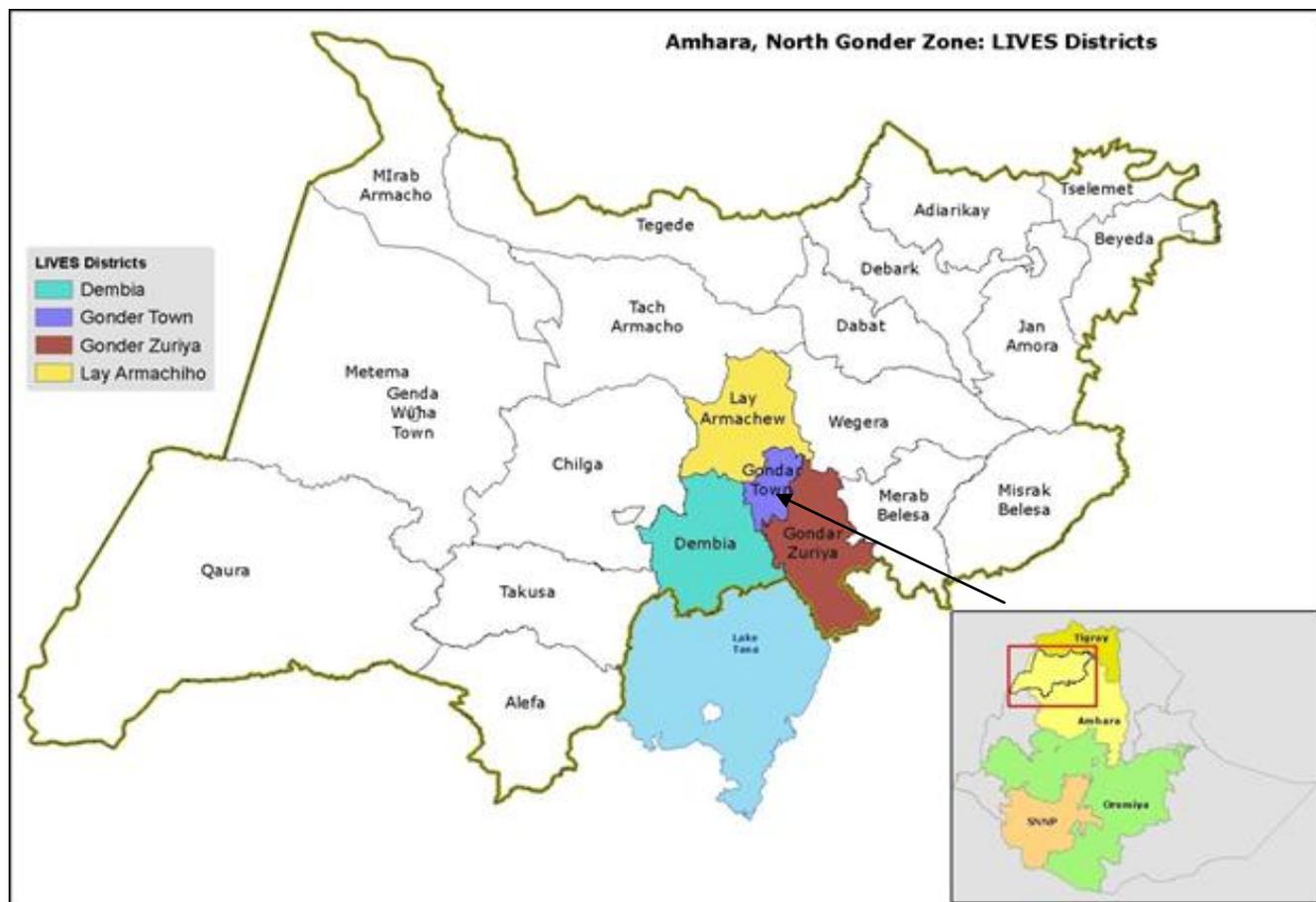


Figure 1 - Map of the study area. Source: (Kahsay, 2005)

Sampling Techniques and Methods

At the beginning of the study pilot survey were undertaken to understand and update the existing information about hide and skin management and marketing systems. During the survey, producers (farmers and butchers), middlemen, local processors and collection centers were identified. Four representative kebeles were purposively selected based on intensity of fattening, degree of slaughtering frequency and number of hide and skin collection centers. From each selected kebeles, fifty households were selected randomly for interviews. From each respondent 50 of them were producers (farmers and butchers) and rest 3 middle mens, 2 collection centers and 5 were local processors those were located in the study area.

The study was conducted by using both primary and secondary source of data collection on various aspects of hides and skins quality management practices and marketing system. The primary sources of data were collected by developing semi structured questionnaires. The questionnaire includes data on perception of major criteria for quality of hides and skins, pre and post slaughter hides and skins management practices and marketing of hide and skins.

Data Analyses

Data was done by using Microsoft excel spread sheet and analyzed using SPSS software version 20.0 and descriptive statistics was used to quantify and summarized the data.

RESULTS AND DISCUSSTIONS

Characteristics of Household

The current finding revealed that 75 % of respondents were male and 25% were female. About 58.3% respondents were found in age group 19-40, 38.3% of the respondents were between 41-65 years and 3.3% were above 65 years old. Most of respondents 50% were illiterate and the rest 33.3% completed primary school and 16.7% of respondents had joined secondary school and above. In terms of their occupation 83% were farmers or producers, 5% middlemen, 3.3% collection center and 8.3% of respondents were local processor (Table 1). In this regard, producer (farmer), middlemen, collection center and local processors were identified as major actors in the hide and skin quality management and marketing for this study.

Description		N(HH)	%(HH)
Age	Below 18	0	0
	19-40	35	58.3
	41-65	23	38.3
	Above 65	2	3.3
	Total	60	100.0
Sex	Male	45	75.0
	Female	15	25.0
	Total	60	100.0
Occupation	Farmer	50	83.3
	Middlemen	3	5.0
	Collection center	2	3.3
	Local processor	5	8.3
	Total	60	100.0
Education	Illiterate	30	50.0
	Primary school	20	33.3
	Secondary school and above	10	16.7
	Total	60	100.0

Home Base Utilizations of Hides and Skins

This study also established that some of the hides and skins remain at the production site for local use without entering the formal market (Table 2). The major raw material that was left for local use was from rejected hide and skin due to defect factors from market. Hide and skin used by respondents for domestic purposes from which majority of them (31.7%) uses hide and skin for seat cover. Whereas the rest respondents 26.7%, 23.3% and 18.3% were used for making rope, kurbet making for bed and milk container, respectively.

Judge Criteria and Quality of Hide and Skin

Based on the survey result there were different criteria used by respondents to judge the quality of hide and skin. The finding indicates that 63.3%, 30%, 6.7% of hide and skin owners were judge by size of skin and hide, absence of defect and freshness of hide and skin, respectively. As per annual hide and skin production the average quality judgment of hide and skin 61.7%, 31.6% and 6.7% of the respondents were agreed good, fair and poor, respectively.

Defects of Hide and Skin

Pre Slaughter Management Defect: Respondents raised number of problems related to livestock production and quality of hides and skins. Production Problems that constrained of respondents summarized in Table 2 indicated that 28.3% of hide and skin were affected by ecto parasite, 15% yolk mark, 31.7% bloat surgery and 6.7% horn rake, 8.3 rope mark, 5% branding and 5% affected thorn scratches. This indicates that the main problem that affects hide and skin quality before slaughtering in the study area were bloat surgery and ecto parasite. This finding agreed with Zenaw and Mekonen (2012) report that ecto parasite has the major tendency to damage skin directly.

Items	N(HH)	%(HH)
Making rope	16	26.7
Kurbet for bed	14	23.3
Seat cover	19	31.7
Milk container	11	18.3
Total	60	100.0

Table 3 - Pre Slaughter Factors affecting hide and skin quality

Factors		N(HH)	%(HH)
Defects	Ecto parasite	17	28.3
	Yolk mark	9	15.0
	Bloat surgery	19	31.7
	Horn rake	4	6.7
	Rope mark	5	8.3
	Branding	3	5
	Thorn scratches	3	5
	Total	60	100.0

Post Slaughter Defect

In the study area hide and skin of an animal can be affected by different types of defects such as flay cut (31.7), poor pattern (13.3), putrefaction (16.7) and improper fleshing (18.3). This is similar with the report of CSA (2004) which stated that poor pattern (34.79%) and knife cut (20.04%) were the major post slaughter defects of hide and skin in different parts of Ethiopia. The current finding consistent with in Ethiopian tanneries, 35% of sheep and 56% of goat skins were reported to be downgraded and rejected due to pre and post slaughter defects (Berhe, 2009; Berhanu et al., 2011).

post slaughter defects affect hide and skin quality

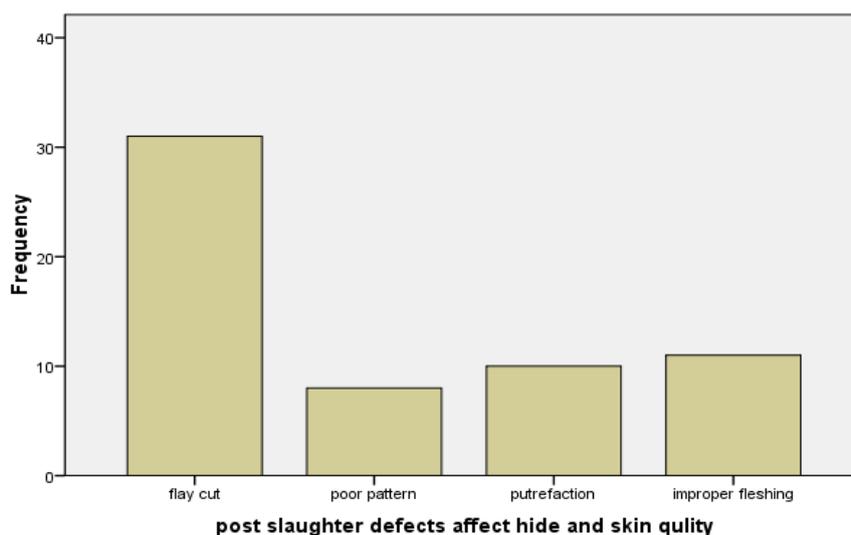


Figure 3 - Post slaughter defects affect hides and skins

Marketing systems of hide and skin

Price Determinants and price variation of Hides and Skins: Based on key informant discussion selling price of hides and skins increase as it goes from farmers to tannery (Table 3). Producer earn better price when they sold hide and skin to collection center than when they sold them to middlemen. In the study area prices of hides and skins have been reported to be determined mainly by the buyer and the seller did not determine the selling price. In fact, all producers, middlemen and collection center also respond that buyers have power on price determination. All respondents (100%) said that there was the price variation from time to time. Collector centers mentioned that it was due to the price variation of tannery and export demand of the hid and skin; market price of the hide and skin is high when the demand of the export of the product is high. And the price of hides and skins reaches high on holiday rather than other days. Because of at the time of holiday there is competition between collector of hides and skins.

Table 3 - Selling price of hide and skin to different market actors from September-April 2017

Products	Market actors and average price in birr		
	Producer to middlemen	Producer and middlemen to collection center	Collection center to tannery
Sheep skin	45	55	80
Goat skin	20	27.5	35
Cattle hide	25	30	40

Market Chain of Hide and Skin

Majority of producer sold their raw hides and skins to middlemen followed by collection centers and also some sold to local processor. On the other hand, middlemen sold their raw hides and skins to collection center and also the collection center sold their raw materials to tannery. Producer mostly sold hides and skins to middlemen (70%), local processors (20%) and collection centers (10%) that are near to collection center. All middlemen sold their raw hide and skin to collection center and the collection center sold to other collection center and Addis Ababa and Bahir-Dar tanneries.

CONCLUSION AND RECOMMENDATIONS

From the result of this study it was concluded that there were a number of pre- and post-slaughter defects that affecting the quality of the raw hide and skin. However, they have clearly ascertained that they were still causing one or more of those defects both before and after slaughtering. From pre slaughter defects the prevalent factors that can be affect hide and skin quality are skin disease and mechanical damage. Among skin disease ecto parasite was a serious problem mentioned by respondents. From mechanical damage: bloat surgery, thorn scratches, horn rake, rope mark and yolk mark were common factors of hides and skin defect in the study area. From post slaughter defect flay cut, poor pattern, improper fleshing and putrefaction defects are mentioned by respondents can reduce quality of hide and skin. The major actor of hide and skin in the study area were producers (farmer, butchers and abattoir), middle men and collection centers. Buyers played the major role in price determination during selling and buying of hide and skin. Respondents used various criteria to identify the quality of the hide and skin such as absence of defect, size, and freshness.

Based on the above conclusion the following recommendation should be undertaken to improve hide and skin quality and marketing in the study area:

- ❖ Training should be given for livestock holders and market actors since most of the defect can be avoided through careful management of the animal
- ❖ To reduce the impact of ecto parasite the appropriate and strategic control measure should be applied by animal health service.
- ❖ Bloat surgery is the dominant one from mechanical hide and skin defects in the study area. So, awareness of people for other bloat treatment and feeding strategy option should be given
- ❖ There should be forwarding adequate information for farmers to sell hide and skin to collection center in order to sell their hide and skins with better price.
- ❖ The government should create national price standards to minimize the price variation

DECLARATIONS

Corresponding Author

E-mail: habtamu.ayalew@uog.edu.et

Author's contribution

All authors contributed equally to this work from starting proposal writing up to preparation of manuscript.

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Competing Interests

The authors declare that they have no conflict of interest with respect to the research, authorship or publications of this manuscript.

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THE EFFECT OF ESSENTIAL OIL COMBINATION ON BIO-HYDROGENATION OF POLYUNSATURATED FATTY ACIDS ON WEST AFRICAN DWARF GOATS

Patrick Okara EBURU  and Magnus Izah ANYA

Department of Animal Science, University of Calabar, Calabar, CRS, Nigeria

 Supporting Information

ABSTRACT: A study was conducted at the Teaching and Research farm of the University of Calabar, Cross River State, Nigeria, on the effect of essential oil (EO) combination on bio-hydrogenation (BH) of *n*-3 polyunsaturated fatty acids (PUFAs) and fermentation activities of rumen microbes. Four West African Dwarf goats (mean weight 40.0 ± 2.5kg) were offered grass (*Panicum maximum*) and water *ad libitum* and supplemented with additional 400 g/goat/day of goat pellets for a period of 14 days. A basal feedstock comprising of 70:30 grass hay and concentrate was formulated. Each serum bottles contained 20 ml inoculum, 80 ml buffer, 1 g of feed substrate and supplemented with 300 mg/l of EO or their combination and incubated at 39°C for 48 h, and samples were collected to analyse ammonia N, total volatile fatty acids (TVFA) and concentration of PUFAs using GenStat 16th edition. There were four treatments and eight replicates as follows: Control, anise oil, lavender oil and blend (150 mg:150 mg) of anise and lavender (A+LO). Relative to the control, anise oil was the only treatment that reduced ($P < 0.001$) ammonia N concentration in culture by a magnitude of 66% at 24 h. The concentration of TVFA (mM) was reduced by anise oil, whilst all other treatments maintained this level relative to the control (68.3, 60.4, 67.7 and 66.1 mM, for the control, anise oil, lavender oil and A+LO, respectively). The concentrations of *n*-3 PUFAs and C18:2 *n*-6 were maintained at higher levels ($P < 0.001$) with addition of all EO, but were highest ($P < 0.001$) in cultures where anise oil was added. The concentrations of 18:2 *cis*-9 *trans* 11 conjugated linoleic acid (CLA) and C18:1 *trans* 11 were significantly maintained ($P < 0.001$) at higher levels by anise and A+LO. At both time points, the concentration of C18:0 was lowest in the control and highest in cultures supplemented with EOs. This study indicates that combining anise and lavender oil (at 1:1 ratio) substantially reduced bio-hydrogenation but without a considerable suppression of ruminal VFA concentration. Hence, this study recommends that, if this effect is confirmed *in vivo*, using anise and lavender oil (at levels 1:1) could reduce ruminal bio-hydrogenation without affecting ruminal volatile fatty acid concentration.

Keywords: Essential oils, Polyunsaturated fatty acids, Rumen fermentation, Volatile fatty acids

INTRODUCTION

Polyunsaturated fatty acids (PUFA) have been reported to possess the potential to reduce the risk of coronary heart diseases in humans (Department of Health, 1994; Scollan et al., 2006). The American Heart Association (AHA) recommends that most of the fat consumed during pregnancy should come from unsaturated fats because this category of fats (rich in omega-3) are required for the development as well as, sustenance of a healthy baby's brain, heart and immune systems (Salem et al., 1996). It is not new knowledge that about 60% of children's (under 3 year's) food is milk, a ruminant food product. This shows that PUFAs can significantly affect the health of both adult and young people alike. However, the concentrations of these 'all important' fatty acids are extremely low in ruminant food products such as meat, milk and their by-products. The health benefits of these PUFAs have raised the need to increase their level in ruminant food products. The fatty acid composition of ruminant food products depends on the ruminal transformation of dietary lipids (Kim et al., 2009). Conventional ruminant feeding systems do not encourage enhanced concentrations of PUFA in ruminant meat and milk (due to the problem of ruminal bio-hydrogenation of PUFAs), even though unsaturated fatty acids are in abundant supply in animal feed. Bio-hydrogenation, a process that leads to the production of saturated fatty acids (SFA) in the rumen is a microbial process (Kim et al., 2009). Hence, manipulation of rumen bio-hydrogenation of PUFA, with the aim of reducing the extent of disappearance of PUFA, through alteration of microbial activity, has received significant attention within the scientific community (Wachira et al., 2000; Scollan et al., 2001; Wang et al., 2002; Chikunya et al., 2004; Scollan et al., 2006; Gunal et al., 2013).

This dietary manipulation, if successfully achieved, would lead to the production of ruminant meat, milk and their products with a high level of PUFA, conjugated linoleic acid (CLA) and a lower content of SFA, which is of great value for consumer health.

Studies have shown that several feeding regimes have been developed over the last few decades to enhance meat and milk of ruminant origin and their products with PUFA, but have been substantially ineffective (Fievez et al., 2007; Jenkins and Bridges, 2007). A few of these studies such as the use of formaldehyde treatment of free oil and protein mixtures has been reported to be relatively efficient at increasing the flow of alpha linolenic acid (C18:3 *n*-3) to the abomasum of goats (Scott et al., 1971), as well as inhibiting the *in vitro* disappearance of C18:2 *n*-6 (Gulati et al., 1997). However, commercialization of this method has remained a challenge because of the potential criticism from regulatory authorities regarding the use of formaldehyde. Plants and plant extracts, which are natural components of animal feed, are now evaluated as potential alternatives (Zhang et al., 2009).

The possibility of using essential oils (EOs) and their individual constituent compounds (EOCs) in rumen fermentation have also been examined previously (Calsamiglia et al., 2007; Benchaar et al., 2008; Hart et al., 2008). Essential oils which are complex natural extracts from different parts of plants, are composed mainly of terpenes and phenylpropanoids (Calsamiglia et al., 2007). These compounds are responsible for the unique aroma of different plants (Szumacher-Strabel and Cieslak, 2012), and are synthesized as secondary metabolites from spices and herbal plants (Bakkali et al., 2008; Patra, 2011).

Eburu and Chikunya (2015a) reported that out of 10 EOs investigated, Anise oil (at 300 mg/L) expressed the greatest potential to maintain PUFA concentrations (229.5%) and was also having the second most inhibitory activity on VFA concentration (inducing over 86% reduction). It was also observed in that study that lavender oil had no effect on VFA concentration, but marginally maintained PUFA concentrations in vessels, relative to the control. Since the potential synergistic, antagonistic, and additive effects of EOs combination have been reported (Burt, 2004), this study intends to combine the two essential oils as they both have their individual advantage (one inhibit bio-hydrogenation without suppressing VFA concentration, whilst the other inhibited BH and VFA). Therefore, the aim of this research was to evaluate the effects of combining anise and lavender oils (1:1 ratio) on ruminal PUFA disappearance and VFA concentration on West African dwarf goats

MATERIALS AND METHODS

The study was in accordance with the code of ethics for animal experiment as stated in http://ec.europa.eu/environment/chemicals/lab_animal/legislation_en.htm.

Study area description

This study was carried out in the small ruminant units of the Teaching and Research farm of the University of Calabar, Calabar. Calabar is located on latitude 4° 57' N and longitude 8° 19' E of the equator. The average annual rainfall is between 1260 mm and 1280 mm while the average temperature is between 25° and 30°C, with a relative humidity of 70% and 90%. Calabar is located at about 98 m above sea levels (NMA, 2018).

Animal management

This study used four West African Dwarf goats (mean weight 40.0 ±2.5kg) as rumen fluid donors. The fluid donor goats were offered grass (*Panicum maximum*) and water *ad libitum* and supplemented with additional 400 g/goat/day of goat pellet, which was divided into two equal halves (200 g) and fed at 07.00 hours and 17.00 hours. The goats were housed in groups of two per pen with straw bedding. Grass (*Panicum maximum*) processed from the University of Calabar Teaching and Research farm and concentrate from a reputable dealer in Calabar, Cross River State, Nigeria, were the main feed ingredients. The goats were placed on the experimental feed for a period of 14 days before slaughter.

Feed was withdrawn from the goats at 18.00 hours on the day preceding the day of slaughter. Slaughtering was carried out at 7.30 hours in the morning. Whole rumens were collected (in tough plastic bags) and transported in insulated boxes to prevent oxygen entry to Animal Science Laboratory, University of Calabar, Calabar. The rumens were incised with a scalpel blade and rumen contents were scooped and the liquor strained through 2 layers of cheesecloth. After straining, the remaining solids were mixed with a volume of buffer (equal to the rumen liquor removed and homogenized using a kitchen blender to detach rumen microbes attached to solids (Theodorou et al., 1994). The mixture was re-strained with 2 layers of cheese cloth and the filtrate added to the rumen fluid to constitute the buffer rumen fluid mixture as the final inoculum. The mixed fluid was held in a water bath maintained at 39°C and flushed with CO₂ to expel oxygen before being dispensed into the *in vitro* incubation flasks (Theodorou et al., 1994).

Basal feedstock, treatments and *in vitro* incubation

The basal feedstuff used in the present study was a mixture of good quality grass hay (*Panicum maximum*), goat pellets, whole ground wheat grain and fish oil (SPAR SHOP, Calabar, CRS). The feed was made from the mixture of a 70:30 grass hay (*Panicum maximum*) and goat pellet concentrate, respectively. The ingredients and chemical composition of the basal feedstock used in incubation are shown in Table 1. A 70: 30 mixture of the grass hay and concentrate respectively was formulated and milled through a 1 mm screen (Glen Creston Ltd, Stammers, England). This diet was supplemented with 60% of fish oil and 40% of ground whole wheat grain. The supplementation with fish oil and ground whole wheat was to make allowance for extra sources of *n*-3 PUFAs in the diet. The composition of the concentrate used was a mixture of wheat feed (40.1%), wheat (19.6%), palm kernel extract (12%), sunflower extract (5%), molasses (3%), limestone flour (2%), salt (0.8%), mixer oil (0.5%), millspec molasses (6%), spray oil (0.5%), ammonium chloride (0.3%) and malt nuts (10%).

There were four treatments and eight replicates as follows: Control, anise oil, lavender oil and blend (150 mg: 150 mg) of anise and lavender. Incubation was done in 100 ml clear glass type bottles for 48 h. In each time point (24, 48 h), 36 (4 × 8+4) serum bottles were incubated and each bottle contained 300 mg/l of EO or their mixture, 80 ml anaerobic buffer, 1 g of feed substrate and 20 ml inoculum. Incubation bottles were sealed with rubber cork before incubation (Theodorou et al., 1994)

The effects of EOs and their combination was evaluated using the *in vitro* gas production batch culture method described by Theodorou et al. (1994). Anise and Lavender oils were purchased from SPAR SHOP, Calabar, CRS, Nigeria. The percentage composition of major constituent compounds of anise oil (*P.anisum*) are: limonene (2.3%), carryophyllene (3.8%) and 82.7% for trans-anethole (Soher et al., 2014). Similarly, the composition of lavender oil (*Lavandula angustifolia*) are: 42.74%, 23.25% and 8.03% for linalool, linalyl acetate and camphor, respectively (Danh et al., 2012).

Table 1 - The ingredients, chemical composition and fatty acid content of the basal feedstock used in incubations

Components	Composition
Feed ingredient (g/kg fresh)	
Hay	700.0
¹ Concentrate	250.0
Wheat grain	30.0
Fish oil	20.0
Chemical composition of basal feedstock (g/100g DM)	
Dry matter	93.0
Crude protein	13.1
Neutral detergent fibre	41.9
Acid detergent fibre	22.1
Ether extract	53.9
Fatty acid composition (g/100 g TFA)	
Linolenic (C18:3 <i>n</i> -3)	23.3
Linoleic (C18:2 <i>n</i> -6)	11.9
Eicosapentaenoic (C20:5 <i>n</i> -3)	4.9
Docosahexaenoic (C22:6 <i>n</i> -3)	3.9
Stearic (C18:0)	2.7
Vaccenic (C18:1)	1.8
Total fatty acids (mg/g)	59.1

¹Concentrate= Goat pellet

Sample collection and preservation

Measurement of gas pressure in the bottles during incubation was carried out from all the replicates at various time (3, 6, 9, 12, 24, 36 and 48 h) using a pressure transducer (Bailey and Mackey Ltd., Birmingham, UK) which was connected to a digital read-out voltmeter. However, only 24 and 48 h results are reported here. The pressure was read on the transducer and then the gas was released to return the head-space gas pressure to zero. The bottles were agitated by shaking before returning to the incubator. Fermentation was stopped (at 24 and 48 h) by freezing the contents of incubation bottles at -20°C for 5 minutes, serum bottles were brought to room temperature. Four replicates of each treatment (5 ml each) were sampled for ammonia and volatile fatty acids (VFA, 4 ml) determinations. The aliquots for ammonia were preserved by mixing 5 ml of 1M HCL with 5 ml of sample. Samples (4

ml) for VFAs were mixed with 1 ml of a deproteinising solution and frozen (-20°C) until required for chemical analysis, using phenol-hypochlorite method as described by Broderick and Kang (1980).

Chemical analysis

The study used the phenol-hypochlorite method (Weatherburn, 1967; Broderick and Kang, 1980), which was adopted for use on the plate reader, to determine the concentration of NH₃-N in digesta. The levels of volatile fatty acid (VFA) was analysed using the Gas chromatography (GC) method as described by Ottenstein and Bartley (1971). The concentration of free fatty acids in feed and freeze-dried samples were extracted by direct saponification method described by Enser et al. (1998).

Experimental design and statistical analysis

The study was a Completely Randomized Design (CRD) experiment, hence, data were analysed by ONE-WAY analysis of variance (ANOVA) using GenStat 16th edition. Differences between treatments were declared by Least Significance Difference (LSD) and significance was declared at P< 0.05. Data were analysed separately for each time point (24 and 48 h).

RESULTS

In vitro fermentation parameters

The effects of EOs and their combination on NH₃-N and total volatile fatty acid (TVFA) concentrations (mM) in cultures during 48 h *in vitro* incubation are shown in Table 2. After 24 h, anise oil induced about 65% reduction on the concentration of NH₃-N, relative to the control (mean values were 5.1 and 3.3 mM for the control and anise oil, respectively). At both 24 and 48 h, the average level of TVFA was lowest (P<0.001) in vessel supplemented with anise oil, whilst other treatments did not affect this concentration relative to the control.

Table 2 - Effects of EOs and EO combination on ammonia and TVFA concentrations (mM) in cultures during 48 h *in vitro* incubation

Variables	Time	Control	Anise	Lavender	A+LO	Significance	
						s.e.d	p-values
NH ₃ -N	24	5.1 ^{ac}	3.3 ^b	4.2 ^{ab}	4.2 ^{ab}	0.62	0.001
	48	7.5	7.3	7.4	8.0	0.51	NS
TVFA	24	68.3 ^a	60.4 ^b	67.7 ^a	66.1 ^a	1.17	0.001
	48	79.1 ^a	72.0 ^b	77.9 ^a	78.8 ^a	1.60	0.001

Means within row with different superscripts are significantly different (P<0.05); TVFA= total volatile fatty acids; A+LO= 1:1 mixture of anise and lavender oils; s.e.d = standard error of difference.

Effects of EOCs on fatty acid metabolism

Effects of EOs and their combination on the concentration of fatty acids (g/100 g TFA) in cultures are shown in Table 3. After 24 h, the observed concentrations of C18:0 were higher in vessels where EOs and their combination were added, with the highest content found in cultures supplemented with A+LO (mean values were: 6.9, 7.9, 9.0 and 10.1 g/100 g TFA, for the control, anise, lavender and A+LO, respectively, Table 3). However, after 48 h, the highest concentration of C18:0 was recorded in vessels supplemented with anise oil, relative to the control.

The level of C18:1 *trans* 11 found in cultures supplemented with lavender at 24 h was lower (P<0.001) than the observed concentrations in anise and A+LO, whose contents were similar to the control. After 48 h however, all EOs and their combination maintained higher (P<0.001) the concentration of C18:1 *trans* 11, relative to the control (average concentrations were: 2.1, 3.1, 3.0 and 3.0 g/100 g TFA, for the control, anise, lavender and A+LO, respectively, Table 3). Except for cultures supplemented with anise oil, inclusion of lavender and A+LO did not affect the concentration of *cis*-9 *trans* 11 CLA.

Supplementing EOs and their mixture maintained higher (P<0.001) the concentrations of all PUFAs (C18:2 *n*-6, C18:3 *n*-3, C20:5 *n*-3 and C22:6 *n*-3) at both 24 and 48 h, but potency differs, with concentrations of PUFA being maintained in the following order: Anise > A+LO > lavender > control (Table 3),

The effects of EOs and their combination on the bio-hydrogenation of selected PUFA (g/100 g) is presented in Table 4. In both the control and EOs, the bio-hydrogenation of PUFAs increased with time, being lower at 24 h than at 48 h. It is observed that, at all time (both 24 and 48 h), the inclusion of EOs and their mixture inhibited (P<0.001) the bio-hydrogenation of all PUFAs (C18:2 *n*-6, C18:3 *n*-3, C20:5 *n*-3 and C22:6 *n*-3). The potential of EOs and their combination to reduce the extent of rumen bio-hydrogenation of all identified PUFAs can be ranked in the following increasing order: Anise > A+LO > lavender > control.

Table 3 - Effects of EOs and their combination on the concentration of fatty acids (g/100 g TFA) in cultures at 24 and 48 h *in vitro* incubation

Variables	Treatments	Time	Control	Anise	Lavender	A+LO	Significance	
							s.e.d	p-values
C18:0	24	6.9 ^a	7.9 ^{ab}	9.0 ^{bc}	10.1 ^c	0.73	0.001	
	48	6.5 ^a	11.0 ^{bd}	9.2 ^c	9.5 ^{cd}	0.79	0.001	
C18:1 <i>trans</i> 11	24	1.46 ^a	1.5 ^a	1.30 ^{cd}	1.41 ^{ad}	0.06	0.001	
	48	2.1 ^a	3.1 ^b	3.0 ^b	3.0 ^b	0.30	0.001	
C18:2 c9 t11 CLA	24	0.12 ^a	0.18 ^b	0.13 ^a	0.12 ^a	0.013	0.001	
	48	0.11	0.15	0.10	0.13	0.014	NS	
C18:2 <i>n</i> -6	24	2.4 ^a	5.7 ^b	3.1 ^c	5.5 ^b	0.17	0.001	
	48	1.3 ^a	3.2 ^b	2.1 ^c	2.9 ^d	0.13	0.001	
C18:3 <i>n</i> -3	24	2.7 ^a	8.9 ^b	4.5 ^c	7.9 ^d	0.31	0.001	
	48	1.8 ^a	5.3 ^b	3.0 ^c	4.0 ^d	0.16	0.001	
C20:5 <i>n</i> -3	24	1.3 ^a	2.8 ^b	1.7 ^a	2.2 ^c	0.22	0.001	
	48	0.7 ^a	1.9 ^b	1.1 ^c	1.4 ^d	0.06	0.001	
C22:6 <i>n</i> -3	24	1.5 ^a	2.1 ^b	1.7 ^c	1.8 ^c	0.09	0.001	
	48	1.0 ^a	1.8 ^b	1.3 ^c	1.4 ^c	0.11	0.001	

Means within row with different superscripts are significantly different (P<0.05); A+LO= 1:1 mixture of anise and lavender oils.

Table 4 - Effects of EOs and their combination on the biohydrogenation (g/100 g) of selected PUFA in cultures at 24 and 48 h *in vitro* incubation

Variables	Treatments	Time	Control	Anise	Lavender	A+LO	Significance	
							s.e.d	p-values
C18:2 <i>n</i> -6	24	80.8 ^a	40.0 ^b	68.1 ^c	50.3 ^d	2.13	0.001	
	48	88.9 ^a	60.9 ^b	75.2 ^c	67.0 ^d	1.67	0.001	
C18:3 <i>n</i> -3	24	85.3 ^a	42.1 ^b	70.2 ^c	51.0 ^d	2.01	0.001	
	48	91.0 ^a	71.0 ^b	85.0 ^c	78.5 ^d	1.11	0.001	
C20:5 <i>n</i> -3	24	58.3 ^a	27.0 ^b	39.9 ^c	29.1 ^{bc}	5.70	0.001	
	48	78.3 ^a	43.0 ^b	60.9 ^c	49.7 ^d	2.31	0.001	
C22:6 <i>n</i> -3	24	31.3 ^a	16.1 ^b	22.0 ^b	17.4 ^b	4.56	0.001	
	48	58.8 ^a	30.6 ^b	55.9 ^a	41.0 ^c	4.18	0.001	

Means within row with different superscripts are significantly different (P<0.05); A+LO= 1:1 mixture of anise and lavender oils.

DISCUSSION

In vitro fermentation parameters

The EOs used were chosen based on their previous effects as reported by Eburu and Chikunya (2015a), where anise oil substantially inhibited bio-hydrogenation and significantly suppressed VFA concentration in ruminal content. In addition, lavender in that study marginally reduced the extent of bio-hydrogenation but had no effect on ruminal VFA concentration relative to the control.

In this study, anise oil which has a phenolic compound, anethole, as the most abundant compound was more effective than lavender at expression of antimicrobial action against ruminal microbes. This was evident by its ability to inhibit both bio-hydrogenation and VFA production processes. This observation confirms the assertion that phenolic compounds have greater antimicrobial strength to alter ruminal microbial composition. Modification of microbial composition results in decreased fermentation of substrates, as reduction in fermentation is due to altered microbial species composition (Van Soest, 1994).

The concentration of NH₃-N in cultures was significantly reduced by anise oil. However, inclusion of 1:1 anise and lavender oil (combination) did not affect NH₃-N concentration, suggesting a dilution/neutralization of anise by lavender oil. The chemical configuration of essential oil's compounds, proportion of individual compounds and their interactions with one another, are fundamental factors that influence the inherent activity of EOs (Dorman and Deans, 2000; Marino et al., 2001; Delaquis et al., 2002). Components of essential oils could interact synergistically, additively, or antagonistically. Synergistic effect is observed when the effect of an individual component is lesser than the combined effects of the substances (Davidson and Parish, 1989) whilst, antagonism occurs when the effect of individual substances is greater than the resultant effect of combining one or more compounds. But effect is said to

be additive when the sum of the individual effect is similar to the combined effect of compounds (Burt, 2004). Hence, the current effect of combining anise and lavender oils on $\text{NH}_3\text{-N}$ shows that the two oils are antagonistic in nature. But this has to be interpreted with caution since inclusion of only lavender did not change the levels of $\text{NH}_3\text{-N}$ relative to the control. Similar studies with blend of major compounds of essential oils show that the antibacterial potential of the whole EOs are greater than the effects of mixing their major active individual components (Gill et al., 2002; Mourey and Canillac, 2002). This suggests that the minor components of the oil are equally involved in determining the antimicrobial activity of the oil and may have a synergistic effect with the major components (Burt, 2004). Reduced concentration of $\text{NH}_3\text{-N}$ with the inclusion of anise oil could be due to inhibition of microbial enzymes responsible for amino acid deamination. Reduced concentration of or inhibition of amino acid deamination is the consequence of decreased proteolytic activity of the rumen (McInotch et al., 2003). Different groups of microbes (hyper ammonia producing bacteria, proteolytic bacteria and protozoa) are involved in ammonia production. Hyper ammonia producing bacteria (HAP) are less abundant than others but use amino acids as energy source; proteolytic bacteria are higher in abundance but possess lower rate of $\text{NH}_3\text{-N}$ producing potential (Bach et al., 2005). Therefore, the lower concentration of ammonia in cultures supplemented with anise could be due to inhibition of activities of these predominant microorganisms by anise oil (McInotch et al., 2003).

Anise oil reduced TVFAs, suggesting that the active compounds in anise oil inhibited rumen activities. Ruminants obtain majority of their energy from VFA (Bergman, 1990), hence, an inhibition of over 65% of VFA concentration would not be ideal for normal growth of animal. Inhibition of TVFA by anise oil in the current study is in tandem with some previous studies of Busquet et al. (2005), where inclusion of higher dose (3000 mg/L) of whole EO and individual components such as cinnamon oil and cinnamaldehyde (3000 mg/L) reduced TVFA; Agarwal et al. (2009), where 1.0 and 2.0 ml/L of peppermint oil depressed feed digestibility and TVFA production; Gunal et al. (2013), where different doses of citronella oil (125, 250 and 500 mg/L) reduced TVFA and that of Eburu and Chikunya (2014), where inclusion of 300 mg/L of EOCs reduced TVFA. The observation on lavender also agrees with previous studies where lavender oil (5, 50 and 500 mg/L) did not modify rumen fermentation parameters after 24 h (Castillejos et al., 2008).

Inclusion of EOs may not always elicit a consistent effect, because their expression or potency is influenced by the dose and type of EO (Busquet et al., 2006), as well as the pH of the rumen (Cardozo et al., 2005). At high doses, EOs decrease TVFA levels but concentrations may be unaffected at low doses depending on the concentration of the EOs to alter feed nutrient digestibility (Patra and Saxena, 2010).

Effect of EOs on fatty acid metabolism

Bio-hydrogenation of C18:3 *n*-3, C18:2 *n*-6 and C18:1 *n*-9 produce C18:0 as the last product. The concentration of this fatty acid (C18:0) was maintained higher in vessels where EOs and their combination were added despite high accumulation of C18:3 *n*-3 and C18:2 *n*-6 in those cultures. The high accumulation of C18:3 *n*-3 and C18:2 *n*-6 and a corresponding increased level of C18:0 is characterized by uncertainty since C18:0 is the end-product of their ruminal transformation. However, this observation agrees with the previous studies of Vasta et al. (2013), where the use of essential oils maintained higher concentrations of both PUFAs and their end-product (C18:0). A number of possibilities could be put forward to justify the high accumulation of both PUFA and their end-product (C18:0). First, although this study did not measure the concentration of oleic acid (C18:1 *n*-9) in cultures, the high level of C18:0 could be emanating from the high concentration of oleic acid in cultures with added EOs and their combination. Studies have shown that only about 30% of ruminal C18:1 *n*-9 gets transformed to ketostearic acid and hydroxystearic acid, the remaining 70% is converted to C18:0 (Jenkins et al., 2006). The second potential justification for high accumulation of C18:0 despite high levels of its precursors (C18:3 *n*-3 and C18:2 *n*-6) could be as a result of a reduced lipolytic action. It has been reported that reduced lipolytic activities may cause small amounts of dietary polyunsaturated fatty acids to reach the rumen (Buccioni et al., 2012). Finally, high accumulation of C18:3 *n*-3, C18:2 *n*-6 and C18:0 may be the result of other unidentified intermediates of bio-hydrogenation in these cultures. Accumulation of PUFAs in this study suggests that anise and lavender oils inhibit the activity of *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica*, microorganisms which are widely recognized for being responsible for the hydrolysis of the ester bond in fatty acids (Buccioni et al., 2012), hence, decreasing the processes of lipolysis and isomerization of oil. High accumulation of stearic acid in the current study would not provoke any particular concern because, reports have shown that, unlike C14:0 and C16:0 fatty acids, dietary manipulation of cholesterol through control of fatty acids should not be focused on C18:0 as though it is a SFA, it has no harmful or deleterious effects on human health (Cobb, 1992; Grundy, 1994; Pariza, 2004).

In this study, EOs and their combination maintained high concentration of vaccenic acid (*trans* 11 18:1). During the transformation of PUFA, vaccenic acid is synthesized by the reductase enzyme from the reduction of *cis*-9 *trans* 11 18:2 conjugated linoleic acid (Jenkins et al., 2008; Kim et al., 2009; Buccioni et al., 2012). This observation suggests that anise, lavender and their combination have the potential to stimulate reductase enzyme which is

responsible for the synthesis of trans-11 18:1. Secondly, it suggest that, high accumulation of C18:1 trans 11 could be that these EOs and their combination impair the activity of *Butyrivibrio proteoclasticus* (Kemp et al., 1975; Maia et al., 2007; Moon et al., 2008), the bacteria responsible for converting C18:1 trans 11 to C18:0. The implication of this observation is that, these oils could have the capacity to increase the concentration of cis-9 trans 11 18:2 conjugated linoleic acid since Vaccenic acid is the substrate for endogenous synthesis of CLA in animal tissues through the Δ -9 desaturase enzyme (Grinari et al., 2000). Studies have shown that majority of the cis-9 trans-11 CLA in cow's milk resulted from the desaturation of trans-11 18:1 (Piperova et al., 2002; Kay et al., 2004).

Anise oil increased the concentration of cis-9 trans 11 CLA, an intermediate of the bio-hydrogenation of cis-9, cis-12 18:2. This observation is consistent with previous reports (Whitney et al., 2011; Eburu and Chikunya, 2014; Eburu and Chikunya, 2015a,b). It is established that cis-9, cis-12 18:2 is the product of the transformational activities of cis-9, cis-12 18:2 by linoleic acid isomerase (LA-I) during the initial stage of bio-hydrogenation (Jenkins et al., 2008; Kim et al., 2009; Buccioni et al., 2012). The implication of this observation on cis-9, cis-12 18:2 is that the activities of lipase (LA-I) that is responsible for the synthesis of cis-9, cis-12 18:2 was stimulated by the inclusion of pure anise oil but not the mixture of the essential oils (A + L O).

The level of C18:2 n-6 in the control, anise oil, lavender oil and the combination of these oils rapidly decreased with time, being lowest at 48 h and highest at 24 h. The bio-hydrogenations of C18:3 n-3 and C18:2 n-6 were similar, however, the level of C18:3 n-3 that disappeared was slightly higher than C18:2 n-6. The bio-hydrogenation values of PUFAs in this study agrees with the bio-hydrogenation results of previous *in vivo* (Wachira et al., 2000) and *in vitro* (Beam et al., 2000) studies. The pattern of C18:3 n-3 and C18:2 n-6 bio-hydrogenation are similar, hence, the two are discussed as one.

As a direct reflection of the bio-hydrogenation of the C18 fatty acids (C18:3 n-3 and C18:2 n-6, C18-FAs) in cultures, their contents rapidly decreased with time, being higher at 24 h and lower at 48 h. The rapid bio-hydrogenation of these C18-FAs from whole ground wheat grain which was more than 80 g/100 g at 24 h agrees with the results obtained with some EOCs in the report of Eburu and Chikunya (2014). This bio-hydrogenation values obtained are also in support of other results of previous *in vivo* studies (Wachira et al., 2000; Scollan et al., 2001, Wang et al., 2002) and *in vitro* trials (Sinclair et al., 2005).

Anise oil maintained the highest concentration of C18:3 n-3 followed by AO + LO but level was consistently lowest in lavender oil treatment. As reported previously, anethole, the most active compound in anise oil possess phenolic moieties in their chemical structures (Calsamiglia et al., 2007; Bakkali et al., 2008), and have been reported previously to be among the most effective compounds to maintain the highest concentrations of C18:3 n-3 and C18:2 n-6 (Eburu and Chikunya, 2014). It is established that the antimicrobial activity and specific mode of action of an individual EO is influenced by the chemical structure of EO components (Dormans and Deans, 2000). The possibility of anise oil to exert the greatest antimicrobial characteristic by maintaining higher levels of C18:3 n-3 than lavender and their combination could be due to their phenolic properties. This could suggest that anise oil could have probably reduced isomerization of C18:3 n-3 compared to lavender and their combination. These categories of compounds are thought to exhibit a mechanism considered to generally include coagulation of cell contents, disruption of proton motive force (PMF), disturbance of cytoplasmic membrane and disruption of active transport and electron flow (Sikkema et al., 1995; Davidson, 1997).

The extent of disappearance of C20:5 n-3 and C22:6 n-3, across all treatments, in vessels increased as the time of incubation progressed, being low at 24 h and high at 48 h. These results agree with previous reports from both *in vivo* studies where the bio-hydrogenation of C20:5 n-3 and C22:6 n-3 in fish oil range from 72 to 93 g/100 g TFA (Wachira et al., 2000; Scollan et al., 2001; Chikunya et al., 2004), and *in-vitro* reports where the bio-hydrogenation of C20:5 n-3 and C22:6 n-3 was in the range of 50 g/100 g (Ashes et al., 1992; Sinclair et al., 2005). As discussed above, the bio-hydrogenation results of the fish oil fatty acids (C20:5 n-3 and C22:6 n-3) were less compared to the ruminal disappearance of C18 fatty acids (C18:3 n-3 and C18:2 n-6). Studies have shown that the inability of microbes to hydrogenate fish oil fatty acids is not due to the difference in the lipase activities but because such microbes lack the enzymes necessary to hydrogenate the long chain n-3 PUFA (Ashes et al., 1992). The potential of EOs and their combination to reduce the extent of rumen biohydrogenation of all identified PUFAs can be ranked in the following increasing order: Anise > A+LO > lavender > control.

CONCLUSION AND RECOMMENDATIONS

From this study, anise oil alone inhibited bio-hydrogenation but significantly suppressed ruminal VFA concentration, which is not beneficial to the animal. However, combining anise and lavender oil (at 1:1 ratio) substantially reduced bio-hydrogenation but without a considerable suppression of ruminal VFA concentration. Hence, this study concludes that, if this effect is confirmed *in vivo*, using anise and lavender oil (at levels 1:1) could reduce ruminal bio-hydrogenation without affecting ruminal VFA concentration which will be of utmost benefit to the animal in

terms of growth, as the level of ruminal volatile fatty acids is a direct reflection of digestibility and utilization of nutrient, and hence growth and performance. This study recommends that further *in vivo* study should be conducted to confirm these *in vitro* results.

DECLARATIONS

Corresponding Author

Email: patrickeburu@gmail.com; phone number: +234 9072199515; +234 8146977397

Authors' Contribution

All authors contributed equally.

Conflict of interests

The authors declare that they have no competing interests.

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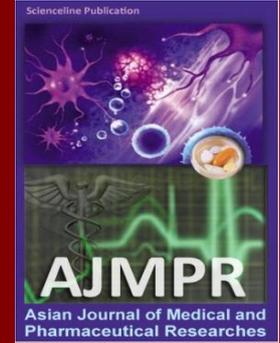
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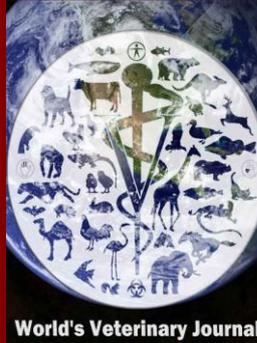
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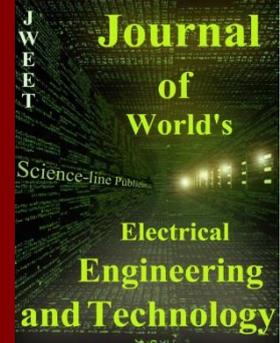
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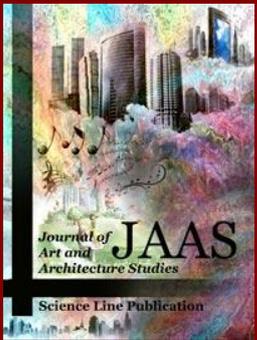
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