Full Length Research Paper

Effect of parity on the proximate composition and fatty acid profile of milk from Nguni cattle grazing on natural pastures

Zikhona T. Rani¹, Michael Chimonyo², Arno Hugo³, Upenyu Marume¹ and Voster Muchenje^{1*}

¹Department of Livestock and Pasture Science, University of Fort Hare, P. Bag X1314, Alice, Eastern Cape, Republic of South Africa.

²University of KwaZulu Natal, Discipline of Animal and Poultry Science, School of Agricultural Sciences and Agribusiness, P. Bag X01, Scottsville, Petermaritzburg, South Africa.

³Department of Microbial Biochemical and Food Biotechnology, University of the Free State, P. O. Box 339, South Africa.

Accepted 2 May, 2011

The objective of the study is to establish the effect of parity on milk composition and fatty acid profiles of Nguni cattle milk. Forty-four Nguni cows with parities ranging from 1 to 13 were included in this study. The cows were grouped to three parity groups: parity group 1 (parity 1 to 5), parity group 2 (parity 6 to 9) and parity group 3 (parity 10 to 13). Samples of the milk were collected in a single day and the milk was analyzed using gas chromatography. Parity had no effect of proximate composition (fat, DMC and moisture %) on milk. The fatty acids (FA) present in the milk of the three parity groups were primarily palmitic (33.3 to 40.5%), oleic (16.3 to 20.3%), myristic (12.5 to 13.2%) and stearic (10.8 to 11.7%) acids, in decreasing order of proportion. Milk from parity group 3 cows contained significantly (p < 0.05) higher proportions of butyric, myristoleic, oleic, eicosenoic, conjugated linoleic acid (CLA), arachidonic and eicosopentaenoic acids when compared with the other two parity groups. Parity group 3 also had significantly (p < 0.05) higher values for total SFA, MUFA, PUFA, omega 3 fatty acids and n-6/n-3 ratio than the other two parity groups. Cows in higher parities generally have higher values for individual fatty acids when compared to those in lower parities. Parity is therefore, an important factor that must be considered when assessing milk quality in Nguni cows.

Key words: Conjugated linoleic acid, fat content, harsh environment, fatty acid profile, milk composition.

INTRODUCTION

Nguni breed is a known beef breed (Muchenje et al., 2008a; 2009a) with low milk production (Mapiye et al., 2007). In most communal production systems of South Africa, low milk production and high mortality rates in recognized dairy breeds have been observed and these

have been attributed to lack of adaptation to the harsh local environments, ticks and tick-borne diseases and low plane of nutrition prevalent in these areas (Mapiye et al., 2007). Ndlovu et al. (2009) and Muchenje et al. (2008a) however reported that the Nguni cattle are well adapted to the communal production systems of South Africa and that unlike other breeds, it has its own unique features that makes it advantageous as a beef breed compared with other breeds.

Nguni cattle are resistant to infection such as trypanosomiasis, ophtalmia, heart water and other tick-borne diseases and tick infestations (Muchenje et al., 2008b, Marufu et al., 2010). They are also tolerant to heat and light. The breed exhibit exceptionally good fertility under harsh conditions, with excellent reproductive performance

^{*}Corresponding author. E-mail: vmuchenje@ufh.ac.za. Tel: +27 40 602 2059. Fax: +27 86 628 2967.

Abbreviations: FA, Fatty acid; CLA, conjugated linoleic acid; EFC, extractable fat content; FFDM, fat free dry matter; FAME, fatty acid methyl esters; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids.

(Nqeno et al., 2009). Heifers, particularly have an early age at sexual maturity and the calving percentage is normally high (Collins-Lusweli, 2000) with a low calf mortality (Ramsay et al., 2000). Nguni cattle are less prone to dystocia, this being ascribed to their slopping rumps, small uterus and low birth weight. They are excellent foragers and will graze and browse on steep slopes and in thick bush. They fatten well on natural grazing (Muchenje et al., 2008a) as well as in the feedlot. They have long productive lives; cows calve regularly and produce 10 or more calves.

In developing countries, such as South Africa, unemployment levels are high. More people are now living under the poverty datum line (NDA, 2005) and can no longer afford basic commodities for survival. The situation is further exacerbated by inflation, which is being caused by an increase in production costs. In South Africa, for example, there has been a row over the pricing of milk to the consumers between the producers and the government. Producers argue that, the cost of producing milk from the major exotic dairy breeds is high and the issue of government controlling price has led to their enterprises not been viable. This calls for the need to come up with alternatives which will benefit the producers and the consumers living under the poverty datum line (NDA, 2005) to be able to buy cheap milk products. Adopting cattle breeds indigenous to the region can be an alternative and the Nguni cattle have been recognized as one such breed. Nguni cattle have been considered inferior to exotic breeds in terms of both beef and milk production (Hofmeyer, 1994; Bester et al., 2003). In rural communities, milk is utilized as both fresh and sour (Mapekula et al., 2009). Although, the chemical composition of milk from other breeds is known but there is paucity of information on the chemical composition of milk from Nguni cow particularly the fatty acid component. Fatty acids in animal products such as milk and meat are of paramount importance as nutrients for human health and are associated with hormone production, reproduction, and nervous tissue development (Hames et al., 1997). However, many studies have proved that diseases such as Type 2 diabetes and coronary heart disease are associated with a high intake of saturated fatty acids (Krauss et al., 1996). Mapekula et al. (2009) studied the fatty acid profiles of Nguni but did not measure them according to parity. Differences in milk composition and quantities are expected in cow in different parities. The objective of the current study is therefore, to determine how parity affects milk composition and fatty acid profiles in Nguni cows.

MATERIALS AND METHODS

Site description

The study was conducted at the University of Fort Hare farm, Eastern Cape, South Africa. The farm is situated at about 520 m above sea level and is located 32.8°S and 26.9°E. The area

receives an annual rainfall of about 480 mm and has a mean temperature of about 18.7 °C. The area has dry winters and wet summers. Acocks (1988) described the vegetation of the area as false Thornveld of the Eastern Cape. Some of the browse species found in this vegetation type include *Acacia karroo, Scutia myrtina* and *Portulaclaria afra* while the common grass species include *Themeda triandra, Panicum maximum, Digitaria erientha* and *Eragrostis* spp. The topography of the area is generally flat with a few steep slopes.

Milk sample collection

Forty four Nguni cows of different parities from parity (1 to 13) which were raised on natural pasture were used in this study. The cows were divided into three parity groups: Parity group 1(parity 1 to 5) parity group 2 (parity 6 to 9) and parity group 3 (parity 10 to 13). The cows were milked early in the morning at around 08:30 h and the milk samples were put into vacutainer containers. The first milk drawn from the udder is low in fat while the last milk (or strippings) is always quite high in fat. Therefore, to avoid any bias, milk was thoroughly mixed before taking any samples. From each cow, two samples were taken, the first was used for determining milk proximate composition. The samples were then put in a freezer, awaited transportation. The milk samples were then sent to the University of Free State in Bloemfontein for fatty acid analysis.

Determination of fatty acid composition

Total lipid from milk samples was quantitatively extracted, according to the method of Folch et al. (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001% to the chloroform/ methanol mixture (Figure 1). A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were also dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as moisture adsorbent. Total extractable fat content (EFC) was determined gravimetrically and expressed as percentage fat (w/w) per 100 g milk. The fat free dry matter (FFDM) content was determined by weighing the residue on a preweighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as percentage FFDM (w/w) per 100 g milk. The moisture content of the milk was determined by subtraction (100% - % lipid - % FFDM) and expressed as percentage moisture (w/w) per 100 g milk.

The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at -20℃ until further analyzed. Approximately 10 mg of total lipid (from Folch extraction) was transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park and Goins, 1994). Fatty acid methyl esters (FAME) were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thickness). Column temperature was 40 to 230°C (hold 2 min; 4°C/min; hold 10 min). Fatty acid methyl esters in hexane (1 μl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian star chromatography software recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19). Fatty acids were expressed as the relative percentage of each individual fatty acid to



Figure 1. Determination of fatty acids composition.

a percentage of the total of all fatty acids present in the sample. The following fatty acid combinations and ratios were calculated by using the fatty acid data: total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total omega-6 fatty acids, total omega-3 fatty acids, PUFA/SFA and omega-6/omega-3 ratio.

Statistical analysis

 $Y_{ii} = \mu + P_i + e_{ii}$

General linear model (GLM) procedure of SAS (2003) was used to analyze the data. The model used was:

Where, Y_{ij} , the response (Individual milk composition variables and individual fatty acids); μ , overall mean common to all observations; P_i , effect of parity; e_{ij} , residual error. Least square means were separated using the PDIFF procedure (SAS, 2003).

RESULTS

Parity had no effect on the proximate composition (fat, DMC and moisture %) of milk. Proximate composition of milk of cows in different parities are shown in Table 1, while the composition of individual fatty acids in milk of the Nguni cows in the three parity groups are presented in Table 2. The fatty acid (FA) present in milk of the three parity groups were primarily palmitic (33.3 to 40.5%), oleic (16.3 to 20.3%), myristic (12.5 to 13.2%) and stearic (10.8 to 11.7%) acids in decreasing order of proportion (Figure 2). Significant variations in individual fatty acid compositions were observed among the three parity groups for butyric, caproic, myristoleic, palmitic, oleic, eicosenoic, conjugated linoleic acid (CLA) and arachidonic and eicosopentaenoic acids (Table 3). Notably, parity group 3 contained significantly (p <0.05) higher proportion of butyric, myristoleic,

Percentage (%)	Parity		
	One	Тwo	Three
Fat	3.27 ± 0.42	2.50 ± 0.39	2.72 ± 0.73
DMC	7.84 ± 0.47	6.68 ± 0.44	7.80 ± 0.82
Moisture	88.1 ± 1.01	90.3 ± 0.93	88.8 ± 1.74

Table 1. Least square means $(\pm S.E.)$ depicting proximate composition of the milk for the three parity groups of Nguni cows.

DMC, Dry matter content.

Table 2. Least square means $(\pm$ S.E.) depicting the effect of parity on fatty acid composition for milk of Nguni cows.

	Parity			
Fatty acid	One	Тwo	Three	
Butyric	0.77 ± 0.55 ^a	1.22 ± 0.50 ^b	$3.08 \pm 0.94^{\circ}$	
Caproic	0.42 ± 0.03^{a}	$1.43 \pm 0.03^{\circ}$	1.33 ± 0.06 ^b	
Caprylic	1.13 ± 0.04	1.12 ± 0.03	1.11 ± 0.06	
Capric	2.81 ± 0.13	2.83 ± 0.12	2.85 ± 0.22	
Lauric	3.61 ± 0.16	3.67 ± 0.15	3.63 ± 0.27	
Tridecoic	0.09 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	
Myristic	13.2 ± 0.46	12.8 ± 0.42	12.5 ± 0.80	
Myristoleic	0.95 ± 0.10^{a}	0.85 ± 0.85^{a}	1.13 ± 0.18 ^b	
Pentadecylic	1.54 ± 0.08	1.53 ± 0.07	1.55 ± 0.13	
Palmitic	39.6 ± 1.05 ^b	$40.5 \pm 0.97^{\circ}$	33.3 ±1.81 ^ª	
Palmitoleic	2.21 ± 0.14	2.24 ± 0.13	2.27 ± 0.24	
Margaric	1.04 ± 0.04	1.04 ± 0.03	1.09 ± 0.06	
Heptadecenoic	0.11 ± 0.04	0.05 ± 0.04	0.02 ± 0.08	
Stearic acid	10.8 ± 0.64	11.6 ± 0.59	11.7 ± 1.11	
Elaidic	0.21 ± 0.02	0.17 ± 0.02	0.24 ± 0.04	
Oleic	17.2 ± 0.83^{a}	16.3 ± 0.76^{a}	20.3 ± 1.42 ^b	
Linoleic	1.01 ± 0.05	0.98 ± 0.05	1.03 ± 0.09	
Arachidic	0.37 ± 0.02	0.39 ± 0.02	0.41 ± 0.04	
Eicosenoic	0.01 ± 0.03^{a}	0.03 ± 0.02^{b}	$0.05 \pm 0.04^{\circ}$	
αLinolenic	0.60 ± 0.04	0.59 ± 0.04	0.70 ± 0.07	
CLA	0.76 ± 0.07^{a}	0.75 ± 0.06^{a}	0.92 ± 0.12^{b}	
Heneicosanoic	0.07 ± 0.01	0.05 ± 0.01	0.09 ± 0.01	
Behenic	0.19 ± 0.02	0.18 ± 0.01	0.21 ± 0.03	
Arachidonic	0.17 ± 0.02^{a}	0.16 ± 0.02^{a}	0.20 ± 0.03^{b}	
Docosadienoic	0.01 ± 0.01	0.01 ± 0.01	0.003 ± 0.01	
Lignoceric	0.09 ±0.1	0.09 ± 0.01	0.12 ± 0.02	
Eicosopentaenoic	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.05 ± 0.02^{b}	

 abc ,Values with different superscript are significant different (p < 0.05); CLA, conjugated linoleic acid.

oleic, eicosenoic, conjugated linoleic acid (CLA) arachidonic and eicosopentaenoic acids when compared to the other two parity groups.

Parity group 3 had significantly (p < 0.05) higher values for total SFA, MUFA, PUFA and omega 3 fatty acids than the other two parity groups (Table 3). No differences were, however, observed in SFA, MUFA, PUFA and omega 3 fatty acids between parity group 1 and 2. No differences in PUFA/SFA ratio were also observed among the three parity groups. Milk from parity group 3 cows, however, had significantly higher n-6/n-3 ratio followed by parity group 2 cows, with cows in parity



Figure 2. Fatty acid GC chromatogram of Nguni cattle milk.

group1 having the lowest ratio.

DISCUSSION

There are various factors that affect the amount and fatty acid composition of milk in a cow. These include

genotype (breed and selection), nutrition and seasons (Lindmark-Mànsson, 2008). Parity however, could also have a significant effect on milk fatty acid composition. In the current study, the hypothesis that animals in later parities have better milk quality than animals in early parities did not prove to be true for Nguni cows in this study. Studies have shown that, increase in body weight

Fatty acid —	Parity		
	One	Two	Three
SFA	20.7 ± 090^{a}	19.7 ± 0.83^{a}	24.1 ± 1.55 ^b
MUFA	2.52 ± 0.16^{a}	2.51 ± 0.15 ^ª	3.01 ± 0.28^{b}
PUFA	1.90 ± 0.12 ^a	1.90 ± 0.11 ^ª	2.26 ± 0.21 ^b
Omega3	0.62 ± 0.04^{a}	0.61 ± 0.04^{a}	0.75 ± 0.08^{b}
PUFA/SFA	3.10 ± 0.11	3.14 ± .010	2.99 ± 0.20
n6/n3	16.1 ± 4.73 ^a	24.9 ± 4.35^{b}	$32.1 \pm 8.16^{\circ}$

Table 3. Least square means (± S.E.) depicting the effect of parity on fatty acid categories for milk of Nguni cows.

 abc , Values with different superscript indicate differences (p < 0.05).

of animals with high number of lactations leads to a greater availability of body reserves for the synthesis of milk components and also result in an increased synthesis of milk constituents (Sevi et al., 2000). A second hypothesis could be that, there can be an improved efficiency of homeorhetic dynamics involved in the partitioning of nutrients for the processes of lactogenesis and galactopoiesis as the number of lactations increases (Hart, 1983). However, parity in cows has been found to have an effect on milk composition. Cows on third parity have significantly higher milk protein, casein and fat contents when compared to ewes in their first and second parity (Sevi et al., 2000).

Conjugated linoleic acid (CLA) is a functional food component with health benefits to human beings and milk is a major dietary source (Kelsey et al., 2003; Muchenje et al., 2009b, c). In a study carried out by Kesley et al. (2003), they reported that, parity and days in milk also had little or no relationship to the individual variation of CLA in Holstein and Brown Swiss cows. In another study carried out by Stanton et al. (1997) were parity was a variable. The study investigated the effect of diet on milk fat content of CLA; they found no consistent effect, although cows with greater than four parities did have a higher milk fat content of CLA than cows of two to four parities. The current study also found significant effects of parity on most of the fatty acids including CLA which is in line with other studies. Palmitic acid is the only fatty acid quantity that was significantly affected by the parity of the cow. Foods with high palmitic acid have been associated with high incidence and severity of fatty liver (Rukkwamsuk et al., 2000). Therefore, cows in late parities could reduce the production of palmitic acids thereby reducing the incidence of fatty liver.

The mean fat percent of the Nguni milk for this study was 2.83%. Reports of other cow breeds for fat percent are 3.9, 4.9 and 4% for Ayrshire, Jersey and Brown Swiss, respectively (Raw Milk Facts, 2010). This clearly shows that, Nguni cows have low percentage fat milk. This also concurs with the study carried out by Mapekula et al. (2009) who reported that, milk fat content in Nguni cows were lower than in crossbreds. The reason for this is that, Nguni cows are adapted to graze and digest poor quality forages (Mapekula et al., 2009). Raats et al. (2004) reported that, Nguni is adapted to low nutrient intake and it still manages to synthesize the desired fatty acids for both human consumption and calf milk.

There are no significant results in this study which show that parity has got an effect on milk composition and fatty acid profiles of milk from Nguni cows. A possible reason is that the Nguni cows are adapted to the harsh environmental conditions (Mapiye et al., 2007). They could be producing the minimum required fatty acids to meet maintenance and the extra that is secreted in milk is not different for different parities as priority might be given to maintenance needs.

In conclusion, the results of the study show that parity significantly affect fatty acid compositions. Cows in higher parities have higher values for individual fatty acids when compared to those in lower parities. Therefore, parity could also be an important factor to be considered during the assessment and improvement of milk quality in Nguni cows.

REFERENCES

- Acocks JPH (1988). Veld types of South Africa. Government Printer. Mem. Bot. Survey S. Afr. Pretoria, 57 (1988), pp. 1–46 3rd edn.
- Bester J, Matjuda LE, Rust JM, Fourie HJ (2003). The Nguni: A case study: Animal Improvement Institute, Irene, South Africa, pp. 1-36.
- Collins-Lusweli E (2000). Performance of Nguni, Afrikaner and Bonsmara cattle under drought conditions in North West province of Southern Africa. S. J. Anim. Sci. 30(1): 33-38
- Folch J, Lees M, Sloane-Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. (226): 497-509.
- Hames BD, Hooper NM, Houghton JD (1997). Instant Notes in Biochemistry. BIOS Scientific Publishers Ltd., Oxford, UK.
- Hart IC (1983). Endocrine control of nutrient partition in lactating ruminants. Proc. Nutr. Soc., 42: 181-194.
- Hofmeyer JH (1994). Finding of the community re a gene bank of livestock. Proceeding Conference. Conservation Early Domesticated Animals of the Southern Africa, Pretoria, South Africa.
- Kesley JA, Corl BA, Collier RJ, Bauman DE (2003). The Effect of Breed, Parity, and Stage of Lactation on Conjugated Linoleic Acid (CLA) in MilkFat from Dairy Cows. J. Dairy Sci. 86: 2588-2597.
- Krauss RM, Deckelbaum RJ, Ernst N, Fisher E, Howard BV, Knopp RH (1996). Dietary guidelines for healthy American adults: A statement

for health professionals from the Nutrition Committee, American Heart Association. Circulation, 94: 1795.

- Lindmark-Manson H (2008). Fatty acids in Bovine milk fat. Food Nut. Res. p. 52.
- Mapekula M, Chimonyo M, Dzama K, Mapiye C (2009). Milk production and calf rearing in communal areas of the Eastern Cape Province of South Africa. Trop. Anim. Health Prod. 41: 1475-1485.
- Mapiye C, Chimonyo M, Muchenje V, Dzama K, Marufu MC, Raats JG (2007). Potential for value-addition of Nguni cattle products in the communal areas of South Africa: a review. Afr. J. Agric. Res. 2(10): 488-495.
- Muchenje V, Dzama K, Chimonyo M, Raats JG, Strydom PE (2008a). Meat quality of Nguni, Bonsmara and Angus steers raised on natural pasture in the Eastern Cape, South Africa. Meat Sci. 79: 20-28.
- Muchenje V, Dzama K, Chimonyo M, Raats JG, Strydom PE (2008b). Tick susceptibility and its effects on growth performance and carcass characteristics of Nguni, Bonsmara and Angus steers raised on natural pasture. Animal, 2: 298-304.
- Muchenje V, Dzama K, Chimonyo M, Strydom PE, Hugo A, Raats JG (2009b). Some biochemical aspects pertaining to beef eating quality and consumer health: a review. Food Chem. 112: 279-289.
- Muchenje V, Dzama K, Chimonyo M, Strydom PE, Raats JG (2009a). Relationship between stress responsiveness and meat quality in three cattle breeds. Meat Sci. 81: 653-657.
- Muchenje V, Hugo A, Dzama K, Chimonyo M, Raats JG, Strydom PE (2009c). Cholesterol levels and fatty acid profiles of beef from three cattle breeds raised on natural pasture. J. Food Comp. Anal. 22(4): 354-358.
- National Department of Agriculture (2005). Annual Report. Government Printer, Pretoria.
- Ndlovu T, Chimonyo M, Okoh AI, Muchenje V, Dzama K, Dube S, Raats JG (2009). A comparison of nutritionally-related blood metabolites among Nguni, Bonsmara and Angus steers raised on sweetveld. Vet. J. 179: 273-281.

- Nqeno N, Chimonyo M, Mapiye C, Marufu MC (2009). Ovarian activity, conception and pregnancy patterns of cows in the semiarid communal rangelands in the Eastern Cape Province of South Africa. Anim. Rep. Sci. 118: 140-147.
- Park PW, Goins RE (1994). *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food Sci. 59: 1262-1266.
- Raats JG, Magadlela AM, Fraser GCG, Hugo A (2004). Re-introducing Nguni nucleus herds in 100 communal villages of the Eastern Cape province. A proposed co-operative project between the University of Fort Hare, Agricultural & Development Research Institute (ARDRI) and the Eastern Cape Department of Agriculture and the Kellogg Foundation.
- Ramsay KA (2000). The Nguni and its future in southern Africa, Lecture: Annual Nguni Production Sale, Bartlow Combine, KwaZulu-Natal, 12 Sept., 1999.
- Rukkwamsuk T, Geelen MJH, Kruip TAM, Wensing T (2000). Interrelation of Fatty Acid Composition in Adipose Tissue, Serum, and Liver of Dairy Cows During the Development of Fatty Liver Postpartum. J. Dairy Sci. 83: 52-59.
- SAS (2003). Statistical Analysis System Institute Inc. Users Guide, Carry, NC, USA.
- Sevi A, Taib L, Albenzio M, Muscio A, Annicchiarico G (2000) Effect of parity on milk yield, composition, somatic cell count, renneting parameters and bacteria counts of Comisana ewes. Small Rumin. Res. 37: 99-107.
- Stanton CF, Lawless G, Kjellmer D, Harrington R, Devery KJA, Connolly F, Murphy J (1997). Dietary influences on bovine milk *cis*-9, *trans*-11 conjugated linoleic acid content. J. Food Sci. 62: 1083-1086.