



FATTY ACID PROFILE, THROMBOGENIC AND ATHEROGENIC INDICES IN THE RAW MILK OF COW AND SHEEP BREEDS

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Abstract

Fatty acids, thrombogenic (TI) and atherogenic (AI) indices of the raw milk of cow and sheep breeds were compared. Thirty raw milk sample 15 each from cow and sheep breeds were analysed. Fatty acids were derivatized following the Official Methods of the American Oil Chemists' Society (AOCS) with modifications. The saturated fatty acids present in the raw milk of cow and sheep breeds were butyric, caproic, caprylic, capric, lauric, myristic, palmitic, and stearic acids in which palmitic had the highest percentage composition, while caprylic had the lowest percentage composition. However, the unsaturated fatty acids present in the raw milk of cow and sheep breeds were oleic, linoleic and linolenic respectively. The oleic had the highest percentage composition, while linolenic had the lowest composition. Raw milk from cow breeds contains higher percentage of myristic and palmitic acids, while raw milk from sheep breeds contains higher percentage of butyric, stearic, oleic, and linolenic acids respectively. Fatty acids sums in the raw milk of cow and sheep breeds decreased in the order of SFA > MUFA > PUFA. The ratio of $n-6/n-3$ in the raw milk of cow and sheep breeds were (1.31 – 2.19) and (1.78 – 2.31), similarly the value of AI and TI in the raw milk of cow and sheep in the present study is > 1.00 which is the recommended level for health safety. Raw milk from WFC and BS had highest percentage fatty acid composition and higher value of health indices which indicate that WFC and BS had higher risk of 'lifestyle diseases' such as coronary heart disease and cancer.

Key words: Breeds, Fatty acid, Thrombogenicity, Atherogenicity.

Introduction

Dietary factors were recognized to have a role in changing the percentage fatty acid in the milk of cows (Moloney *et al.*, 2011) and ewes (Chen *et al.*, 2014). Individual fatty acids (FA) produced determines the lipid fraction's health impact (Kuczyoska *et al.*, 2012). Studies have shown that there is a link between the fatty acids composition and the chronic disorders such as cardiovascular diseases and diabetes (Oliver *et al.*, 2009). Nevertheless, research



indicates that only few individual fatty acids have negative consequences on consumer health (Ndubeze *et al.*, 2006). Dietary that is rich in individual fatty acids, like lauric (C12:0), myristic (C14:0) and palmitic acids (C16:0), are connected to an increased risk of coronary heart diseases, obesity and atherosclerosis (Olafadehan and Adewumi 2010). The 'healthy fats' are the unsaturated fatty acids (UFA), due their impact on the level of cholesterol in blood (2006; Olafadehan and Adewumi 2010). Conjugated linoleic acid (CLA) (C18:2cis9trans11), α -linolenic (LNA, 18:3n-3) and oleic acids (C18:1cis9), could be improved in milk through pasture feeding (Ndubeze *et al.*, 2006; Kuczyoska *et al.*, 2012). The health benefits of Conjugated Linoleic acid include the lowering of cholesterol content, anti-carcinogenic, antidiabetic and immunomodulation effects in the blood (Knowles *et al.*, 2006). While n-3 fatty acid has a benefit of preventing heart disease and improved immune response (Gomez-Cortes *et al.*, 2008). The cholesterol content is less in polyunsaturated fatty acids compared to monounsaturated fatty acids (Oliver *et al.*, 2009), while Oleic acid and linolenic acid have antiatherogenic and anticancer properties (Oliver *et al.*, 2009). Apart from its effect on cholesterol level, linoleic acid quality enhances sensitivity to insulin and therefore lowers the incidence of type 2 diabetes (Olafadehan and Adewumi 2010).

The Fatty acids of raw milk is important for the nutritional quality of dairy products. The health lipid indices (e.g. atherogenic and thrombogenic indices) are used in evaluating the nutritional value of milk fat. Raw milk Fatty acids in cow is connected to intrinsic (pregnancy, stage of lactation, breed or genotype) or extrinsic (season, temperature, nutrition) factors. Diets used in the herd plays an important role in determining the variation in milk fatty acid composition, and the variability in fatty acid composition is genetically determined (Adesina, 2013). Researchers have addressed the issue of breed and genotypic effects on milk fatty acids composition (Chilliard *et al.*, 2007; Kuczyoska *et al.*, 2012). The primary fat component of the human diet is saturated fatty acids. Breeds differences from several independent investigations are ambiguous for most of the fatty acid profiles (Muchenje *et al.*, 2009). Few studies evaluated the fatty acids profiles health indices and nutritional quality of raw milk from different breeds under semi-intensive husbandry system (Oliver *et al.*, 2009). Thus, this study was aimed to compare the fatty acids profile and health indices of the raw milk from local cow and sheep breeds raised among Fulani rearers managed under semi-intensive husbandry system in Kano and Jigawa States, Nigeria.

Materials and methods

Milk sampling and Analysis:

Hand-milking was done by the herdsmen in the farm in the morning between 06.00 – 07.30am. Milk samples for analysis were collected in hygienic conditions from breeds of each cow. Raw milk samples were collected from each breed in the morning before grazing into clean, white plastic container of 120cm³ capacities. The nipples were sterilized with ethanol before milking. Samples were transported to the laboratory in an ice cold box for analysis. All analysis was done in triplicate.



Fatty acids determination

Fatty acids were derivatized following the Official Methods of the American Oil Chemists' Society (AOCS, 2005) with modifications. Individual fatty acids were determined by gas chromatography, using the Varian CP 3800 system with a split/splitless injector and a flame-ionization detector (FID). Samples (1 μ l) of fatty acid methyl esters were placed on a CP-Sil 88 capillary column (length: 100 m, inner diameter: 0.25 mm). Fatty acids were identified by comparing their retention times with those of commercially available reference standards purchased from Supelco, Inc. Analyses of samples and reference standards were performed under identical conditions, i.e. carrier gas - helium, injector temperature 260°C, detector temperature 260°C, initial oven temperature 110°C, raised to 249°C.

Statistical Analysis

Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by student t-test using SPSS Version 11.0 software package (SPSS Inc., Chicago Illinois, USA). Significant was defined at $P < 0.05$ using Duncan's multiple range tests. Atherogenic index (AI) and thrombogenic index (TI) were calculated based on the formula by (Ulbricht and Southgate 1991).

Results and Discussion

Fatty acids profile of the raw milk of cow and sheep breeds

The percentage fatty acids composition of the raw milk of cow and sheep breeds is presented in Table 1 - 2. From the results, the saturated fatty acids present in the raw milk of cow and sheep breeds were C_{4:0}(butyric), C_{6:0} (Caproic), C_{8:0} (Caprylic), C_{10:0} (Capric), C_{12:0} (Lauric), C_{14:0} (Myristic), C_{16:0} (Palmitic), and C_{18:0} (Stearic) in which C_{16:0} (Palmitic) had the highest percentage composition, while C_{8:0} (Caprylic) had the lowest percentage composition. However, the unsaturated fatty acids present in the raw milk were C_{18:1} (oleic), C_{18:2} (linoleic) and C_{18:3} (linolenic) respectively, in which C_{18:1} (oleic) had the highest percentage composition, while C_{18:3} (linolenic) had the lowest composition. These results were consistent with the findings of Marounek *et al.*, (2012), the major fatty acids in milk, were C_{16:0} (Palmitic) and C_{18:1} (oleic). Differences in forage may affect milk fatty acid composition as stated by Chillard *et al.*, (2007). Raw milk from cow breeds contains higher percentage of C_{14:0}(myristic) and C_{16:0}(palmitic), while sheep breeds contains higher percentage of C_{4:0} (Butyric), C_{18:0} (Stearic), C_{18:1} (Oleic), and C_{18:3} (linolenic). Fatty acids sums in all the breeds of the selected ruminants decreased in the order of SFA>MUFA>PUFA which is in agreement with the findings of other studies (Signorelli *et al.*, 2008; Sinanoglou *et al.*, 2015).

Table 1 Fatty acids (%) of the raw milk of cow breeds

Fatty acids	RBC	SGC	WFC	FAO/WHO (1985) Standard
Butaric C _{4:0}	2.42±0.05 ^a	2.09±0.04 ^b	2.78±0.05 ^c	
Caproic C _{6:0}	1.70±0.04 ^a	1.51±0.06 ^b	2.11±0.06 ^c	
Caprylic C _{8:0}	1.56±0.05 ^a	0.88±0.03 ^b	1.93±0.05 ^c	



Capric	C _{10:0}	4.49±0.05 ^a	3.81±0.05 ^b	4.82±0.06 ^c	
Lauric	C _{12:0}	3.62±0.05 ^a	2.92±0.04 ^b	4.14±0.05 ^c	
Myristic	C _{14:0}	13.38±0.20 ^a	13.12±0.30 ^b	13.77±0.60 ^b	
Palmitic	C _{16:0}	27.77±1.04 ^a	27.01±1.04 ^b	29.19±1.13 ^c	
Stearic	C _{18:0}	9.49±0.13 ^a	8.52±0.13 ^b	10.07±0.13 ^c	
Oleic	C _{18:1}	13.88±0.23 ^a	12.01±0.19 ^b	14.68±0.19 ^c	
Linoleic	C _{18:2}	1.76±0.09 ^a	1.32±0.03 ^b	2.48±0.07 ^c	2.5 – 9.0
Linolenic	C _{18:3}	1.05±0.03 ^a	0.91±0.04 ^a	1.26±0.09 ^b	0.5 – 2.0
TSFA		61.68±2.08 ^a	55.20±1.97 ^b	66.28±2.30 ^c	10
TUFA		17.69±0.49 ^a	14.34±0.48 ^b	20.02±0.58 ^c	
TFA		79.37±3.00 ^a	69.54±2.72 ^b	86.30±3.82 ^c	20 – 35
MUFA		14.88±0.23 ^a	12.01±0.19 ^b	15.68±0.19 ^c	
PUFA		2.81±0.04 ^a	2.33±0.02 ^b	4.34±0.05 ^c	6.0 – 11.0

RBC = Red Bororo cow, SGC = Sokoto Gudali cow, WFC = White Fulani cow,

Mean±S.D with different alphabet superscripts are significantly different ($P < 0.05$) between breeds.

The fatty acids profile of the raw milk from cow breeds are presented in Table 1. From the results 55.20 – 66.28% of the raw milk fatty acids of cow breeds comprised of total saturated fatty acids (TSFAs), (C_{4:0} to C_{18:0}). The highest TSFA among cow breeds was recorded in WFC (61.68±2.30%) while SGC (55.20±1.97%) had the lowest percentage composition. The result of the present study for TSFA was higher than (47.20 – 51.00%) reported by Nfor *et al.*, (2014) for GUD, WF and RB cow breeds, but lower than (65.38 – 65.67%) reported by Pilarczyk *et al.*, (2015) for HF and S cow breeds. C_{4:0} (Palmitic acid), the major saturated fatty acid, was (27.01 – 29.19%) of total fatty acids, with SGC (27.01±1.04%) having the lowest composition and WFC (29.19±1.13%) had the highest percentage composition. The result of the present study for C_{4:0} was higher than (18.00 – 25.00%) reported by Nfor *et al.*, (2014) for GUD, WF and RB cow breeds, but lower than (32.97 – 33.05%) reported by Pilarczyk *et al.*, (2015) for HF and S cow breeds, while C_{8:0} (Caprylic acid) was the lowest saturated fatty acid and was (0.88 – 1.93%) of total fatty acids, with SGC (0.88±0.03%) having the lowest composition, while WFC (1.93±0.05%) had the highest percentage composition. The result of the present study for C_{8:0} was higher than (0.10 – 0.13%) reported by Nfor *et al.*, (2014) for GUD, WF and RB cow breeds, but lower than (3.24 – 3.44%) reported by Pilarczyk *et al.*, (2015) for HF and S cow breeds.

The total unsaturated fatty acids (C_{18:1} to C_{18:3}) was 14.34 – 20.02% of the raw milk. The highest was recorded in WFC (20.02±0.58%) and SGC (14.34±0.48%) had the lowest percentage composition. The result of the present study for TUFA was higher than (3.76 – 4.66%) reported by Nfor *et al.*, (2014) for GUD, WF and RB cow breeds, but lower than (34.34 – 34.66%) reported by Pilarczyk *et al.*, (2015) for HF and S cow breeds. C_{18:1} was the major unsaturated fatty acid and its percentage composition was (12.01 – 14.68%), with SGC (12.01±0.19%) having the lowest composition and WFC (14.68±0.19%) had the highest percentage composition. The result of the present study for C_{18:1} was higher than (1.85 – 2.00%) reported by Nfor *et al.*, (2014) for GUD, WF and RB cow breeds, but lower than (27.40 – 27.51%) reported by Pilarczyk *et al.*, (2015) for HF and S cow breeds, however, C_{18:3} was the lowest unsaturated fatty acid and its composition was (0.91 – 1.26%) with SGC (0.91±0.04%)



having the lowest composition and WFC ($1.26 \pm 0.09\%$) had the highest percentage composition. The result of the present study for $C_{18:3}$ was lower than ($1.61 - 2.34\%$) reported by Nfor *et al.*, (2014) for GUD, WF and RB cow breeds, but close to ($0.94 - 1.08\%$) reported by Pilarczyk *et al.*, (2015) for HF and S cow breeds.

Table 2 Fatty acids (%) of the raw milk of sheep breeds

Fatty acids		BS	US	YS	FAO/WHO (1985) Standard
Butaric	$C_{4:0}$	3.61 ± 0.06^a	3.13 ± 0.06^b	2.87 ± 0.07^c	
Caproic	$C_{6:0}$	2.79 ± 0.06^a	2.46 ± 0.04^a	2.19 ± 0.05^b	
Caprylic	$C_{8:0}$	2.24 ± 0.03^a	2.11 ± 0.03^a	2.06 ± 0.05^a	
Capric	$C_{10:0}$	5.61 ± 0.04^a	4.31 ± 0.04^b	4.02 ± 0.06^c	
Lauric	$C_{12:0}$	4.68 ± 0.05^a	4.02 ± 0.06^b	3.68 ± 0.05^c	
Myristic	$C_{14:0}$	12.32 ± 0.14^a	11.03 ± 0.13^b	11.34 ± 0.13^c	
Palmitic	$C_{16:0}$	27.11 ± 1.07^a	26.76 ± 1.03^b	24.44 ± 1.04^c	
Stearic	$C_{18:0}$	11.00 ± 0.07^a	10.67 ± 0.50^b	10.45 ± 0.70^b	
Oleic	$C_{18:1}$	16.49 ± 0.60^a	15.79 ± 0.70^b	15.19 ± 0.80^c	
Linoleic	$C_{18:2}$	1.55 ± 0.03^a	1.28 ± 0.04^b	1.01 ± 0.03^c	2.5 - 9.0
Linolenic	$C_{18:3}$	2.04 ± 0.03^a	1.53 ± 0.01^b	1.49 ± 0.02^b	0.5 - 2.0
TSFA		74.89 ± 2.01^a	67.23 ± 2.00^b	66.52 ± 2.01^c	10
TUFA		21.41 ± 0.70^a	19.60 ± 0.80^b	19.21 ± 0.78^c	
TFA		96.30 ± 2.55^a	86.83 ± 2.76^b	85.73 ± 3.15^c	20 - 35
MUFA		16.49 ± 0.60^a	15.79 ± 0.09^b	15.19 ± 0.80^c	
PUFA		4.92 ± 0.04^a	3.11 ± 0.05^b	3.42 ± 0.06^c	6.0 - 11.0

BS = Balami sheep, US = Uda sheep, YS = Yankasa sheep,

Mean \pm S.D with different alphabet superscripts are significantly different ($P < 0.05$) between breeds.

The highest TSFA among the sheep breeds was recorded in BS ($74.89 \pm 2.01\%$) and YS ($66.52 \pm 2.017\%$) had the lowest percentage composition. The result of the present study for TSFA was higher than 64.23 reported by Maria *et al.*, (2013) for sheep milk, but lower than 75.03 ± 0.77 reported by Sinanoglou *et al.*, (2015) for Chios sheep breed. $C_{16:0}$ is the major saturated fatty acid and its composition ranged from ($24.44 - 27.11\%$) with YS ($24.44 \pm 1.04\%$) having the lowest composition and BS ($27.11 \pm 1.07\%$) had the highest percentage composition. The result of the present study for $C_{16:0}$ was higher than ($22.08 - 23.68\%$) reported by Sinanoglou *et al.*, (2015) for Karagouniko and Chios indigenous Greek sheep breeds. However, Aguilar *et al.*, (2014) reported value of ($27.80 - 28.88\%$) higher than the present result. On the other hand $C_{8:0}$ was the lowest saturated fatty acid with values ranging from ($2.06 - 2.24\%$) with YS ($2.06 \pm 0.05\%$) having the lowest composition and BS ($2.24 \pm 0.03\%$) had the highest percentage composition. The result of the present study was higher than 1.87% reported by Maria *et al.*, (2013) for sheep milk, but lower than ($3.77 - 4.15\%$) reported by Sinanoglou *et al.*, (2015) for Karagouniko and Chios indigenous Greek sheep breeds.

($19.21 - 21.41\%$) of the raw milk of sheep breeds comprised of total unsaturated fatty acids. The highest TUFAs among sheep breeds was recorded in BS ($21.41 \pm 0.70\%$), while YS ($19.21 \pm 0.78\%$) had the lowest percentage composition. The result of the present study for



TUFA was higher than (21.79 – 32.07%), (24.96 – 28.23%) and 34.57% reported by Aguilar *et al.*, (2014), Sinanoglou *et al.*, (2015) and Maria *et al.*, (2013) for sheep milk breeds. The major unsaturated fatty acid (C_{18:1}) ranged from (15.19 – 16.49%) with SY (15.91±0.80%) having the lowest composition and BS (16.49±0.60%) had the highest percentage composition. The result of the present study was lower than (19.10 – 21.38%), (16.66 – 18.73%) and 20.18% reported by Aguilar *et al.*, (2014), Sinanoglou *et al.*, (2015) and Maria *et al.*, (2013) for sheep milk breeds. C_{18:3} was the lowest unsaturated fatty acid within the raw milk of sheep breeds and the values ranged from (1.49 – 2.04%) with YS (0.91±1.49%) having the lowest composition and BS (2.04±0.03%) had the highest percentage composition. The result of the present study for C_{18:3} was lower than 0.59 – 1.10% and 0.50 – 0.77% reported by Aguilar *et al.*, (2014) and Maria *et al.*, (2013) for sheep milk from Chile, France and Spain and local sheep milk. The difference in fatty acids content among the breeds might be due to the feed, ingredients in the feed, feeding habit and the microbial activity in the rumen of the ruminants (Parodi, 2014). Mele *et al.*, (2007) and Schennink *et al.*, (2008) attributed differences in milk fatty acids composition among the breeds to varying activity of Delta⁹ – desaturase (Samková *et al.*, 2012).

Table 3 Fatty acids (%) n-6/n-3, AI and TI of the raw milk of cow and sheep breeds

Fatty acids	RBC	SGC	WFC	BS	US	YS	
n-6/n-3	1.68±0.04 ^a	1.31±0.04 ^b	2.19±0.06 ^c	2.31±0.03 ^a	2.02±0.05 ^b	1.78±0.04 ^a	< 4.0
AI	3.59±0.06 ^a	2.90±0.04 ^b	4.21±0.09 ^c	4.66±0.08 ^a	4.35±0.05 ^b	4.26±0.04 ^a	> 1.0
TI	2.65±0.06 ^a	2.03±0.05 ^b	3.14±0.10 ^c	3.96±0.04 ^a	3.43±0.03 ^b	3.14±0.02 ^c	> 1.0

Mean±S.D with different alphabet superscripts are significantly different (P < 0.05) between breeds

The n-6/n-3 ratio, atherogenicity index (AI) and thrombogenicity index (TI) are commonly used to assess the nutritional value and consumer health of intramuscular fat, it is an important determinant for reducing the risk of many chronic diseases (Simopoulos, 2002; Pilarczyk *et al.*, 2015). In general, a ratio of n-6/n-3 of 1.0 – 4.0 is required in the diet to combat lifestyle diseases such as coronary heart diseases and cancers (Simopoulos, 2002). In the present study the range of n-6/n-3 ratios of cow and sheep breeds were (1.31 – 2.19) and (1.78 – 2.31) respectively which were within the recommended level. The range of n-6/n-3 of the present study is lower than (2.76 – 6.41) reported by Aguilar *et al.*, (2014). Similarly the atherogenic index (AI) and thrombogenic index (TI) take into account the effects that single FAs might have on human health and, in practice, on the probability of increasing the incidence of pathogenic phenomena such as atheroma and/or thrombus formation (Pilarczyk *et al.*, 2015). In general, AI and TI value of >1.0 is required in the milk, which improves human health because of the beneficial effect on the cardiovascular system. In the current study the AI of the cow and sheep breeds were (2.90 – 4.21) and (4.26 – 4.66) respectively, while that of TI of the cow and sheep breeds were (2.03 – 3.14) and (3.14 – 3.96) respectively. The values in the present study are > 1.00 which is the recommended level for health safety.



Conclusion

The study showed that the individual fatty composition did not differ significantly among the breeds, but TSFA, TUFA, TFA, MUFA and PUFA differed significantly ($P < 0.05$) among the breeds. Raw milk of WFC and BS had higher fatty acid composition, n-6/n-3 ratios, atherogenicity index (AI) and thrombogenicity index (TI), while raw milk from SGC and YS had lowest individual fatty acid compositions, n-6/n-3 ratios, atherogenicity and thrombogenicity indices which indicate that raw milk of SGC and YS had lower risk of 'lifestyle diseases' such as coronary heart disease and cancer, thus based on the fatty acid profile milk from the raw milk of SGC and YS is the most recommended for human consumption.



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