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# Fatty Acid Composition of Fat Globule Membrane Neutral Lipids from Egyptian Buffalo, Goat and Cow's Milk

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*ABSTRACT*

*Fat globule membrane neutral lipids (FGM-NL) were isolated from Egyptian buffalo, goat and cow's milk, fractionated by TLC and individual fractions were quantitatively determined. Fatty acid (FA) contents of each NL fraction were determined by GLC. Triglycerides (TG) were found to be the main fraction in FGM-NL from all species, followed by free fatty acids (FFA), 1,2 diglycerides (1,2 DG), 1,3 diglycerides (1,3 DG), monoglycerides (MG) and then cholesterol (Ch). No significant differences (at $P \leq 0{\cdot}01$) were observed among the three species. The GLC analysis of FGM-NL fractions showed that C16:0, C18:0 and C18:1 were predominant FAs (18–33%), C14:0 was the intermediate level FA (4–8%). Measurable amounts (1–3%) of C12:0, C15:0, C16:1, C18:2 and C18:3 in addition to other minor FA were also detected with slight species variations. The distributions of short chain FAs ( < C10), saturated and unsaturated longer chain FAs in FGM-NL fractions among the three species were also compared.*

## INTRODUCTION

Fat globule membrane (FGM) is a complex mixture of components accounting for about 1% of the lipid phase in milk. Neutral lipids (NL) account for 72–75% of the total FGM lipids (Kuldip & Tapas, 1982). Distribution of FGM-NL fractions and their fatty acid (FA) composition are of scientific and technological interest and can reveal some interesting



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aspects of the biosynthesis of FA. However, some are of real (essential) nutritional significance.

Amount and composition of FGM-NL fractions are poorly known; few investigators (Chandan *et al.*, 1971; Hladik & Forman, 1968) have examined the detailed composition of FGM-NL from cow's milk. However, comparative aspects of NL composition, in terms of species variations, have not been fully investigated and need further attention. This work compares the distribution and FA composition of FGM-NL fractions from Egyptian buffalo, goat and cow's milk.

## MATERIALS AND METHODS

### Extraction of FGM-NL

Five fresh bulk milk samples (each of two replicates) were collected from buffalo, goat and cow herds of the Faculty of Agriculture, Ain Shams University, during October–December. The FGM material was isolated and its lipid moiety was extracted from the membrane material; lipids, after purification, were separated into phospholipids and NL as described before (Hamzawi & Shahin, 1986).

### Fractionation of NL

The NL were fractionated into individual fractions by TLC on silica gel G according to Kenneth & Sami (1963). Visualization of the separated bands was carried out by spraying with 2·7 dichlorofluoresceine and exposing the chromatoplates to a UV lamp. The separated NL fractions were identified by their $R_f$ value with the aid of reference NL standards chromatographed on the sample plate with the samples. The NL fractions were quantitatively determined by the method of March & Weinstein (1966) and the results obtained were statistically analysed by analysis of variance according to Steel & Torrie (1960). The appropriate silica gel portions containing the respective NL fractions were scrapped and eluted with diethyl ether from the silica gel. Composite samples of the eluates were evaporated to dryness and then transesterified according to DeMan (1964).

### GLC analysis

The FA methyl esters were analysed using a Hewlett–Packard Model 5840 A gas chromatograph as described before (Hamzawi & Shahin, 1986).

# RESULTS AND DISCUSSION

## Distribution of FGM-NL fractions

The TLC analysis revealed monoglycerides (MG), cholesterol (Ch), 1,2 diglycerides (1,2 DG), 1,3 diglycerides (1,3 DG), free fatty acids (FFA) and triglycerides (TG) as the six main fractions of FGM-NL in each of buffalo, goat and cow's milk. Table 1 shows the relative quantitative distribution of these fractions among the three species. It is obvious from Table 1 that TG are the major components of all FGM-NL fractions in all species and significantly higher ($P < 0·01$) in comparison with the other five fractions. On the other hand, differences in TG content between species were insignificant, although average TG levels were observed to be slightly higher in cow FGM-NL (77·5%) followed by goat (75·7%) and then buffalo (74·2%). Cholesterol is the smallest of all NL fractions; it is slightly higher in buffalo (1·86%), followed by goat (1·32%) and then cow (1·80%).

Table 1 shows that differences in MG in milk FGM-NL from the three species were insignificant although the trend was slightly higher in buffalo (6·37%) followed by goat (5·82%) and then cow (5·28%). Also, the levels of DG (1,2 + 1,3 DG) in milk FGM-NL were found to be the same in the three species, values being 9·94, 9·55 and 9·72% for buffalo, goat and cow's milk, respectively. The FFA fractions are also not significantly different in the FGM-NL of all three species with a slightly higher value in goat (7·66%), followed by buffalo (6·60%) and then cow (6·29%).

Our results are in agreement with Beri *et al.* (1984) who found that TG constituted the major NL fraction, followed by DG, FFA, MG and Ch. They reported that FGM-NL fractions were slightly higher in buffalo than in cow, TG (52·7 *vs* 45·5%) and DG (7·4 *vs* 5·5%); they found also that FFA and MG were similar in both buffalo and cow FGM-NL. Also, the present

**TABLE 1**
Distribution of Milk FGM-NL Fractions from Various Species[a]

| *FGM-NL fractions* | *Buffalo* | *Goat* | *Cow* |
|---|---|---|---|
| | *Wt. % of the total* | | |
| Monoglycerides | 6·37 | 5·82 | 5·28 |
| Cholesterol | 1·86 | 1·32 | 1·20 |
| 1,2 diglycerides | 5·33 | 5·29 | 5·38 |
| 1,3 diglycerides | 4·61 | 4·26 | 4·34 |
| Free fatty acids | 6·60 | 7·66 | 6·29 |
| Triglycerides | 74·2 | 75·7 | 77·5 |

[a] Average of five samples.

**TABLE 2**
Fatty Acid Composition of Mono- and Diglycerides of Milk FGM from Various Species

| *Fatty acids* | *Monoglycerides* | | | *1,2 diglycerides* | | | *1,3 diglycerides* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Buffalo* | *Goat* | *Cow* | *Buffalo* | *Goat* | *Cow* | *Buffalo* | *Goat* | *Cow* |
| | | | | % of the total | | | | | |
| C4:0 | — | — | — | — | 1·39 | — | 0·96 | 0·61 | — |
| C6:0 | 1·49 | 1·46 | 1·26 | 0·69 | 4·11 | 1·63 | 0·54 | 0·87 | 0·83 |
| C8:0 | 0·85 | 1·99 | 1·47 | 0·74 | 0·26 | 1·28 | 1·25 | 0·66 | 0·82 |
| C10:0 | 3·77 | 2·07 | 1·80 | 1·85 | 2·22 | 2·20 | 0·78 | 0·61 | 0·83 |
| C12:0 | 2·25 | 2·59 | 3·13 | 0·98 | 1·17 | 1·37 | 1·56 | 1·39 | 1·77 |
| C12:1 | 0·20 | 0·56 | — | — | 0·24 | 0·28 | — | 0·31 | 0·26 |
| C14:0 | 8·31 | 5·85 | 8·85 | 4·30 | 6·27 | 7·20 | 4·92 | 4·90 | 6·12 |
| C14:1 | 1·73 | 1·57 | 0·27 | 0·62 | 0·89 | 1·20 | 1·21 | 1·35 | 1·17 |
| C15:0 | 1·43 | 1·03 | 0·83 | — | 1·19 | 1·33 | 1·20 | 1·12 | 1·33 |
| C16:0 | 31·8 | 27·7 | 17·4 | 28·9 | 28·9 | 30·3 | 27·8 | 29·1 | 31·0 |
| C16:1 | 3·45 | 3·88 | 3·53 | 1·32 | 3·67 | 3·82 | 4·21 | 3·13 | 4·24 |
| C17:0 | — | 1·34 | 1·72 | — | 2·18 | 2·26 | 2·51 | 2·69 | 1·90 |
| C18:0 | 23·4 | 25·2 | 23·4 | 30·1 | 25·3 | 23·9 | 28·1 | 30·9 | 25·3 |
| C18:1 | 18·8 | 22·0 | 30·3 | 27·4 | 19·9 | 20·4 | 22·6 | 19·9 | 22·0 |
| C18:2 | 1·86 | 2·01 | 3·10 | 0·60 | 0·87 | 0·73 | 0·43 | 0·83 | 1·03 |
| C18:3 | 0·70 | 0·29 | 1·27 | 1·55 | 1·00 | 1·53 | 0·63 | 1·12 | 1·02 |
| C20:0 | — | 0·48 | 0·73 | 1·04 | 0·45 | 0·53 | 1·34 | 0·51 | 0·41 |
| Total short chain (<C10): | | | | | | | | | |
| | 6·11 | 5·52 | 5·53 | 3·27 | 7·98 | 5·11 | 3·52 | 2·75 | 2·48 |
| Total saturated long chain (>C10): | | | | | | | | | |
| | 67·1 | 64·1 | 56·1 | 65·3 | 65·4 | 67·0 | 67·5 | 70·6 | 67·8 |
| Total unsaturated long chain (>C10): | | | | | | | | | |
| | 26·8 | 30·3 | 38·4 | 31·5 | 26·6 | 27·9 | 29·0 | 26·7 | 29·7 |

results are in good agreement with those of Hofi *et al.* (1977), who found that buffalo and cow's milk FGM-NL consisted of: MG (3·1 and 3·1%); 1,2 DG (4·7 and 5·2%); 1,3 DG (2·9 and 4·0%); TG (75·4 and 74·3%); FFA (6·0 and 6·1%) and Ch + ChE (7·0 and 6·7%), respectively. The results in this study fall in the range given by Huang & Kuksis (1967) who reported that cow's FGM-NL contained 71–83% TG, 4·3–10% DG, 8·7–15·7% FFA and 1·2–8% Ch. On the other hand, Anderson & Cheeseman (1973) reported a lower value for TG ($19{\cdot}0 \pm 4{\cdot}2\%$), DG ($3{\cdot}3 \pm 1{\cdot}0\%$) and the same value for MG ($6{\cdot}5 \pm 1{\cdot}3\%$) from bovine milk FGM material isolated with the aid of sodium deoxycholate during early lactation. Chandan *et al.* (1971) found that TG accounts for 68·5%, DG + Ch for 6·7%, MG for 3·32% and FFA + pigments for 4·5% in cow's FGM-NL. Unfortunately, there were no available data in the cited literature for goat's FGM-NL fractions to compare.

## Fatty acid composition of FGM-NL fractions from various species

### *Monoglycerides*

Table 2 shows the FA composition of FGM-MG from buffalo, goat and cow's milk. It is obvious from the results that FGM-MG from each of buffalo, goat and cow's milk had C16:0, C18:0 and C18:1 as the predominant FA with some species variations. C16:0 was the most predominant FA in both buffalo and goat's MG (31·8 and 27·7%), followed by C18:0 (23·4 and 25·2%) and then C18:1 (18·8 and 22·0%), respectively, while C18:1 was the most predominant FA in cow's MG (30·3%), followed by C18:0 (23·4%) and then C16:0 (17·4%). It is also seen that C14:0 is present at intermediate levels (5·85–8·85%) in all the three species with a slightly lower level in the case of goat's MG. Among the other FAs, C10:0, C12:0, C16:1 and C18:2 were detected in measurable amounts in all the three FGM-MG fractions with slight species variations. Values were 3·77, 2·25, 3·45 and 1·86% for buffalo's MG; 2·07, 2·59, 3·88 and 2·01% for goat's MG and 1·80, 3·13, 3·53 and 3·10% for cow's MG, in the same order. The other FAs were distributed in nearly similar profiles in the three species, except that C17:0 and C20:0 were not detectable in the buffalo FGM-MG. It could be observed that cow's MG was characterized by a lower proportion of total saturated long chain FAs (56·1%), whereas buffalo's MG had a higher level (67·1%). The total short chain FAs were almost at the same level (5·52–6·11%) in all three mammals' FGM-MG.

These results are in agreement with those of Chandan *et al.* (1971) for cow's FGM-MG. They found that C18:1 and C16:0 were the predominant FAs, values being 38·5 and 17·5%; they reported a lower value of C18:0 (6·2%) and a higher value of C14:0 (10·5%). They obtained similar profiles for the other FAs, as C6:0, C8:0, C10:0, C12:0, C18:2 and C18:3 acids constituted 1·6, 1·7, 3·8, 4·2, 3·1 and 3·1%, respectively. Unfortunately there are no available data in the cited literature for FA composition of FGM-MG in both buffalo and goat's milk.

*Diglycerides*

The DGs were separated into two fractions, i.e. 1,2 and 1,3 DG. Table 2 indicates that C18:0 was the major FA in buffalo FGM-1,2 DG (30·1) whereas C16:0 was the major one in both cow (30·3%) and goat (28·9%). Similar profiles were attained for FA distribution in both goat and cow 1,2 DG, except for the presence of C14:0 in goat. On the other hand, C4:0, C15:0 and C17:0 were not detected in the case of buffalo FGM-1,2 DG. C14:0 was in intermediate amounts with a slightly higher level in cow (7·20%), followed by goat (6·27%) and then buffalo (4·30%). Equal portions of C10:0, C12:1, C18:2 and C18:3, were observed in the FGM-1,2 DG fraction of all three species. It is seen from Table 2, also, that goat 1,2 DG was characterized by the highest level of total short chain FAs (7·98%), followed by cow (5·11%) and then buffalo (3·27%), whereas buffalo 1,2 DG possessed the higher level of total unsaturated longer chain FAs (31·5%). Total saturated long chain FAs were almost in the same proportion in all three mammals' FGM-1,2 DG.

With regard to 1,3 DG, buffalo, goat and cow FGM attained the same profile except for undetectable amounts of C4:0 in the case of cow and C12:1 in the case of buffalo 1,3 DG. C16:0 was the most predominant FA in 1,3 DG of cow (31·0%) followed by C18:0 (25·3%) and C18:1 (22·0%), while C18:0 was the most predominant in both goat (30·9%) and buffalo (28·1%). C14:0 and C16:1 were present at intermediate levels (3–6%) in all species with slightly higher levels in cow (6·12 and 4·24%), followed by buffalo (4·92 and 4·21%) and then goat (4·90 and 3·13%), respectively. Table 2 indicates that total short chain FAs were slightly higher in buffalo FGM-1,3 DG (3·52%), whereas it possessed nearly the same value for both goat and cow (2·75 and 2·48%, respectively). On the other hand, goat FGM-1,3 DG were characterized by higher levels of total saturated longer chain FAS and lower levels of total unsaturated ones, values being 70·6 and 26·7%, whereas those of buffalo and cow have nearly the same level, values being 67·5 and 29·0% for buffalo and 67·8 and 29·7% for cow 1,3 DG, respectively.

The results are in general agreement with those quoted in the literature for FA composition of total DG. Chandan *et al.* (1971), reported that C16:0, C18:1 and C18:0 were the predominant FAs in bovine FGM-DG, values being 32·6, 20·3 and 17·6%, respectively. They found that other FAs, i.e. C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C16:1, C17:0, C18:2 and C18:3

constituted 1·8, 1·2, 2·7, 3·0, 8·6, 2·3, 4·5, 1·9, 1·7, and 1·6%, in the same order. However, similar values for C16:0 (33·3%) and total unsaturated FAs (28·1%) were reported by Hladik and Forman (1968), who found that cow FGM-DG contained lower levels of C18:1 (11·7%) and C18:0 (9·0%) and higher levels of C14:0 (9·7%) and C16:1 (5·3%). No published data regarding FA composition of FGM-DG from either buffalo or goat milk were available.

*Free fatty acids*

Table 3 shows the quantitative FA distribution of the FFA fraction in FGM-NL from buffalo, goat and cow milk. It is seen from the Table that FA profiles of the buffalo and goat FFA fraction were comparable except

**TABLE 3**

Fatty Acid Composition of Free Fatty Acids and Triglycerides of Milk FGM from Various Species

| *Fatty acids* | *Free fatty acids* | | | *Triglycerides* | | |
|---|---|---|---|---|---|---|
| | *Buffalo* | *Goat* | *Cow* | *Buffalo* | *Goat* | *Cow* |
| | | | % of the total | | | |
| C4:0 | — | — | 1·42 | — | — | — |
| C6:0 | — | — | 0·40 | 0·42 | — | 0·28 |
| C8:0 | — | — | 0·36 | 0·59 | — | 0·38 |
| C10:0 | 0·89 | 0·60 | 0·72 | 1·02 | 0·45 | 0·23 |
| C12:0 | 1·70 | 1·50 | 1·83 | 1·42 | 1·00 | 2·99 |
| C12:1 | — | — | — | 0·26 | — | 0·20 |
| C14:0 | 7·03 | 3·93 | 4·86 | 7·69 | 4·32 | 8·47 |
| C14:1 | 0·65 | 0·87 | 0·77 | 1·79 | 1·34 | 1·02 |
| C15:0 | 0·86 | 0·59 | 0·97 | 1·96 | 1·54 | 1·28 |
| C16:0 | 31·0 | 30·6 | 28·5 | 33·7 | 26·3 | 30·1 |
| C16:1 | 3·21 | 3·23 | 3·00 | 1·20 | 3·29 | 3·19 |
| C17:0 | 3·14 | 2·34 | 2·49 | 1·44 | 2·49 | 0·89 |
| C18:0 | 30·3 | 30·4 | 31·5 | 23·1 | 31·6 | 24·2 |
| C18:1 | 18·8 | 23·0 | 20·0 | 19·4 | 20·7 | 21·2 |
| C18:2 | 1·00 | 1·34 | 1·32 | 2·27 | 2·55 | 2·00 |
| C18:3 | 1·02 | 1·69 | 0·29 | 1·55 | 1·72 | 1·22 |
| C20:0 | 0·49 | — | 1·55 | 1·46 | 1·91 | 2·30 |
| C22:0 | — | — | — | 0·74 | 0·85 | 0·80 |
| Total short chain (<C10): | 0·89 | 0·60 | 2·90 | 2·03 | 0·45 | 0·89 |
| Total saturated long chain (>C10): | 74·5 | 69·3 | 71·7 | 71·6 | 70·0 | 70·3 |
| Total unsaturated long chain (>C10): | 24·6 | 30·1 | 25·4 | 26·4 | 29·6 | 28·8 |

that C20:0 were not detected in the goat FFA. It is noticed, also, that C16:0, C18:0 and C18:1 were the major FFA in all FGM-FFA fractions from the three species, values being 31·0, 30·3 and 18·8% for buffalo, 30·6, 30·4 and 23·0% for goat and 28·5, 31·5 and 20·0% for cow, respectively. C14:0 was present at a relatively higher level for buffalo FGM-FFA fraction (7·03%), followed by cow (4·86%) and then goat (3·93%). It is evident from the results in Table 3 that the cow FGM-FFA fraction contained measurable levels of short chain FAs, values being 1·42, 0·40 and 0·36% for C4:0, C6:0 and C8:0 acids, which are not detected in either buffalo or goat milk. The other FA, i.e. C12:0, C14:1, C15:0, C16:1, C17:0, C18:2 and C18:3 were detected in comparable amounts in each of the FFA fractions from the three species. Table 3 shows that the buffalo FFA fraction was characterized by the highest level of total saturated long chain FA (74·5%), whereas goat FFA fraction had the highest level of total unsaturated long chain FA (30·1) and the lowest level of total short chain FA (0·60%) and cow FFA possessed the highest level of total short chain FA (2·90%).

Hladik and Forman (1968) found that the FFA fraction consisted mainly of C12:0 (19·4%), C16:0 (17·9%), C14:0 (9·7%), C18:1 (9·1%), C10:0 (7·7%) and C18:0 (5·4%) in addition to other minor FA. There were no other available data concerning FA composition of FGM-FFA fractions from either buffalo or goat milk to compare.

*Triglycerides*

The FA distribution of FGM-TG fractions from buffalo, goat and cow milk (Table 3) showed similar profiles. It is seen from the Table that C16:0, C18:0 and C18:1 were the most predominant FAs in FGM-TG from each of the three species, with some species variations, values being 33·7, 23·1 and 19·4% for buffalo, 30·1, 24·2 and 21·2% for cow and 26·3, 31·6 and 20·7% for goat TG, respectively. C14:0 was present in intermediate amounts with a relatively higher level in cow (8·47%), followed by buffalo (7·69%) and then goat (4·32%). It is observed also that FGM-TG fractions from all species contained measurable amounts of long chain FA, i.e. C18:2, C18:3, C20:0 and C22:0: Values were 2·27, 1·55, 1·46 and 0·74% for buffalo, 2·55, 1·72, 1·91 and 0·85% for goat and 2·00, 1·22, 2·30 and 0·80% for cow FGM-TG, respectively. On the other hand, buffalo FGM-TG contained the highest level of total saturated long chain FAs (71·6%) and the lowest level of total unsaturated ones (26·4%), whereas goat FGM-TG contained the lowest level of total short chain FAs (0·45%).

The obtained results are in good agreement with Hladik and Forman (1968) who showed that C16:0, C18:1, C18:0 and C14:0 were the main FA in FGM-TG from cow's milk, values being 33·6, 16·6, 9·6 and 12·0%, respectively. Bracco *et al.* (1972) reported that FGM-TG contained 30·1%

(C16:0), 26·7% (C18:1), 14·5% (C18:0) and 8·6% (C14:0). Also, Chandan *et al.* (1971) found that C16:0 (54·0%), C18:0 (23·5%), C18:1 (17·7%) and C14:0 (13·3%) were the major FAs in FGM-TG from cow milk, with a pronounced high level of C16:0. Kuldip & Tapas (1982) found that FGM-TG from buffalo contained mainly C16:0, C18:1, C14:0 and C18:0 in levels of 39·0, 25·8, 13·7 and 13·0%, respectively, in addition to C12:0 (3·10%), C16:1 (2·03%), C20:4 (1·72%) and C10:0 (1·63%).

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