EFFECT OF MATERNAL DIETARY SUPPLEMENTATION ON LACTATIONAL AMENORRHEA AND NUTRITIONAL STATUS OF THEIR INFANTS.

BY

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ABSTRACT

Effects of maternal nutritional status and maternal dietary supplementation, on the growth of suckling infants, breast-feeding patterns, Prolactin concentration, return of menstruation and ovulation were studied in 162 marginally malnourished mother- infant pairs. Subjects were randomised into two study groups. One group of 83 mothers received 40g Australian high protein, high-energy biscuits and served as supplemented subjects. The second group of 79 mothers did not receive any nutritional supplement and served as controls. The subjects were followed up monthly until their second menstruation. Anthroponietric measurements, information on breast feeding patterns and infant's breast milk intake were recorded at each visit. In addition blood samples (5mls) were obtained at least one and a half hours before a breast feeding episode and thirty minutes after the commencement of a breast feeding episode for prolactin estimation. Aliquots of the blood sample were used for haemoglobin and albumin estimations. The subjects were visited at home at least three times a week to collect early morning urine samples for pregnanediol-3-alpha glucuronide and estrone-3-glucuronide determinations and to ensure compliance with dietary supplements.

There were no significant differences in the frequency of daytime and night time breast feeding, and mean duration of breast feeding episodes in the

two groups of mothers through-out the follow up period (P>0.05). A similar growth pattern was recorded for infants of the two groups of mothers. Significant differences were observed in some anthropometric measurements between the two groups of mothers, within six months of commencement of supplementation. Body mass index increased from 20.2 to 21 at the end of the sixth month in the supplemented subjects and it was reduced from 20.5 to 20.2 in the control subjects. The increase in Body mass index of the supplemented mothers not was significant (p>0.05).

There was no significant difference between milk output and daily energy expenditure of both groups. The overall prevalence of breast-feeding was high (98-69%) in the first 4 months of life but it reduced to less than 50% by the 7th month. Most of the mothers gave water to their babies in addition to breast milk. None of the infants was wet nursed or showed preference for the left breast. Only 2% of infants of the supplemented mothers and 3% of infants of the control mothers showed preference for the right breast.

There were no significant differences between the basal and suckling-induced Prolactin concentrations in the two groups of mothers studied, thus showing that supplementation of the mothers' diet had no effect on the concentration of the blood Prolactin levels. There was no significant difference between the duration of lactational amenorrhoea of the two groups of mothers.

Basal Prolactin concentration and suckling induced Prolactin concentrations declined in parallel to suckling activity with time postpartum.

The return of fertility (ovarian cyclic activity) postpartum was monitored by the onset of follicular development and ovulation in the first and second menstruation in the two group of mothers. The urinary concentration of pregnanediol-3- alpha glucuronide and Estrone-3- glucuconide was used to determine the level of ovarian cyclic activity. The result showed that even when menstruation has occurred, there were still a lot of irregularities in the follicular development and ovulation of the lactating women.

The results of this study indicate that dietary supplementation of the marginally malnourished mother did not affect the growth of the infants, breast-feeding patterns and the duration of lactational amenorrhoea of the mothers.

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CERTIFICATION

I certify that this work was carried out by Mrs Omobola Abioye Ogundahunsi in the Department of Human Nutrition, College of Medicine, University of Ibadan and The Obafemi Awolowo College of Health sciences, Ogun State University.

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STEROID NOMENCLATURE

The steroid nomenclature given below is based on the revised rules for steroid nomenclature published by IUPAC commission on Biochemical Nomenclature in 1969.

The following is a list of the steroids used in this script. Both trivial and the corresponding systematic names are given, while abbreviations are indicated in brackets.

Trivial Name	Systematic Name
Oestriol (E_3)	1,3,5(10)-Oestra-triene-3,16α,17β-triol
Oestrone (E_1)	3 hydroxy-1,3,5(10)-oestra-triene-17-one
Oestradiol (E_2)	1,3,5(10)-Oestra-triene 3, 17β diol
Progesterone (P^4)	4-Pregnene-3,20-dione
Pregnanediol (P, diol)	5β-pregnane-3α,20α-diol

INTRODUCTION

There are conflicting reports on the role of maternal nutritional status in lactation performance, infant growth and health of the lactating mothers. A decrease in the growth of infants was reported with exclusively breast fed infants in circumstances of maternal undernutrition in less developed countries.(Waterlow,Ashworth and Griffiths 1980). Gopalan (1958) showed 26% increase in volume of breast milk secreted by poorly nourished Indian women following a 62% increase in daily protein intake. Bassir (1975) obtained similar results in Western Nigeria when he used a vegetable-protein supplement (30g of soya flour daily).Edozien, Rahim-khan and Waslien (1976) showed that protein supplementation increased milk production and weight gain in the baby without a change in the protein content of the milk among some Nigerian women. However a study in India, in which inadequately nourished mothers were fed with milk biscuits, showed a rise in serum albumin (Rajalakshmi 1971). The adequacy of human milk is not only reflected by volume produced or by the composition, but also by assessment of physical growth, and good health (Jelliffe and Jelliffe 1979). Anthropometric assessment is a reliable means of determining the adequacy of nutrition However, difficulties in interpreting growth curves in relation to the prevalence, selection of reference standards thought to be the most appropriate

for the particular group, and the significance of infections including diarrhoea may limit the use of anthropometry. This is more common with the babies of poorly nourished mothers in poor, economically developing countries. Consequently, this study was designed in a longitudinal manner to monitor the growth of infants of marginally malnourished mothers instead of severely malnourished mothers.

Prolonged lactational amenorrhea is a common phenomenon in the poor, less developed nations of the world (Lunn and Prentice, 1983). In the absence of adequate sex education, lactational Amenorrhea provides the only restriction on the number of children most women in these countries have. Consequently, a clear understanding of the mechanism involved in controlling anovulation during this period is essential in order to exploit the phenomenon as a natural method of contraception. There is, however, a controversial view on the role of undernutrition. The negative effect of maternal malnutrition on a woman's reproductive capacity is underscored by the fact that menstrual cycles in non-lactating women ceased at times of famine and in anorexia nervosa (Smith 1947). In addition, prolonged postpartum amenorrhoea was confined almost exclusively to the poorest parts of the world (Crisp and Stonehill 1971). In addition, menstrual cycles in non-lactating women are interrupted during famine and in anorexia nervosa (Smith 1947). Although there is evidence of direct correlation between severe malnutrition and fertility,

it has been difficult to demonstrate a similar reduction in fertility in response to mild to moderate malnutrition.

Supplementation of dietary intake of lactating mothers may inhibit ovulation (Lunn, Prentice and Austin 1980; Delvoye, Delogne-Desnoeck and Robyn 1976). However, the relationship between reduction in fertility, pattern of infant suckling and maternal nutritional indices is not well defined (Hennart, Hofvander, Vis and Robyn 1985). Increased infant suckling may inhibit ovulation by increasing prolactin concentration (Gross Eastman, Bowen and McEldutt, 1979). An increased level of prolactin initiates a oegative feed back inhibition of hormones involved in ovulation through the GnRH pulse generator (Mcneilly, Tay and Glassier 1994b). Although the role of prolactin in the regulation of ovarian function is well documented, the exact mechanism by which prolactin affects the duration of postpartum amenorrhea is not known (Mcneilly 1994(a), Lunn 1994). Suckling induced prolactin concentrations affect return of menstruation, ovulation and growth of suckling infants. A suckling frequency of more than five minutes per day and more than ten minutes per feed was shown to maintain complete suppression of ovarian activity (Lunn Prentice, Austin and Whitehead 1984). The threshold required, however, to suppress ovarian activity appears to differ between populations (Gray,Campell, Apelo, Eskam,Zacus,Ramos,Gebret and Labbok 1990,Kennedy, 1990) These population / ethnic variations underscore the need to evaluate the

relationship between nutritional status and lactational amenorrhoea among the indigenous population. It is plausible that other factors may modulate ovarian function in addition to prolactin. (Lunn 1994)

The effect of dietary supplementation in undernourished women on the duration of postpartum amenorrhea is not clearly defined. Reduction in the duration of lactational amenorrhea and inter pregnancy interval varies with contents of supplementation (protein and energy or energy alone) and amount of supplement (Delgado 1978). While Delgado (1978) were reporting changes as a result of dietary supplementation, Adair, Politt and Mueller. (1984) who supplemented the diet of marginally undernourished lactating women over a period of four months found no significant difference in milk yield, maternal weight or skinfold thickness.

Studies on the relationship between Nutrition. Lactation performance and postpartum amenorrhea are few in Nigeria. These studies focussed on limited aspects of the relationship. Bassir (1975) studied the nutritional aspect of breastmilk of Nigerian women, Edozien (1976) studied the effect of deficiency of protein on the breastmilk quantity and Omololu(1976) studied the significance of breastfeeding in Nigeria. Igbedioh (1994) investigated the influence of mother's occupation and education on breastfeeding and weaning in Makurdi Nigeria.

AIMS AND OBJECTIVES

The study described in this thesis was conducted to investigate other aspects of the effect of food supplementation on

1. Maternal and child nutritional status
1. Breastfeeding patterns (frequency of suckling, duration of suckling and introduction of supplementary feeds)
1. Basal and suckling induced prolactin concentrations in relation to lactational amenorrhoea
1. Duration of lactational infertility in marginally malnourished women.

CHAPTER ONE

LITERATURE REVIEW

1.1.1 Maternal Nutrition, Lactation and Infant Health.

Maternal nutrition, lactation and infant health are related factors. During lactation a mother requires additional food to meet the nutritional needs of the growing child and herself. In the less developed countries, breast feeding markedly reduces child malnutrition and infection, enhancing survival during the early months of life (Jelliffe and Jelliffe 1971). However, the presence of maternal malnutrition during the lactation period may severely reduce milk production and adversely affect the infant's nutrition and development (Hamilton 1984). In addition, the mother's nutrition may deteriorate further. Breast feeding could lead to excessive maternal weight loss postpartum if the woman cannot balance the energy requirements of lactation and her other roles are not complimented with additional nutritional intake, decreased energy expenditure or metabolic changes (Ebrahihim, 1976). Infant birth weight and maternal nutritional stores postpartum are dependent on the mothers' fat or lean tissue reserves at term (Kravosec 1990). Well nourished women are able to cope with the increased nutritional demands with fat stores laid down during pregnancy as well as increased calorie consumption or decreased energy expenditure during lactation (Kravosec 1990). Lactation is

not directly influenced by dietary deprivation although lactation performance based on quantity of milk produced and duration of lactation may be subject to dietary influences (WHO 1982). Behavioural patterns of maternal -infant interactions i.e., the frequency, duration and intensity of suckling influenced lactational performance more than dietary influences. (WHO 1981) Paradoxically these patterns may be an indirect product of maternal or infant nutritional status. For example, fatigue due to anaemia may limit responsiveness of mother to infant expression of need, or vigour of suckling may be muted due to low birth weight or protein energy undernutrition (WHO 1982).

In developing countries most mothers including those who gain little weight during pregnancy and have less than adequate calories postnatally, secrete sufficient milk to support the neonate. The limitations imposed by maternal nutritional status occur in the post natal period but in most instances not before at least 3 - 4 months (Whitehead, Paul, Black, and Wiles 1981).

Collaborative studies in two developed countries (Scotland and The Netherlands) and three developing countries (Thailand, The Gambia, and The Philippines) support the theory that weight gain during pregnancy affects the weight of babies produced and the lactation performance of the mother. The countries in which the women gained the least weight and fat were the same

ones where women produced the babies with the lowest adjusted birth weight (Thailand, The Philippines, and especially The Gambia). Several issues were raised by these observations. For example, there is a need to define the

1. Effect of maternal nutrition on lactation,
1. Effect of lactation on infant survival,
1. Effect of maternal nutrition and lactation on fertility.

1.1.2 Effect Of Maternal Nutrition on Initiation and Duration of Lactation

There is evidence suggesting that lactation is not significantly influenced by maternal nutritional status except under conditions of extreme food shortage. Chronically undernourished women in the developing world are able to initiate lactation just as well as, if not more successfully than well nourished women in affluent industrialised nations (Rajalakshmi 1971). Lactation is almost universal in many poor rural societies with less than 5% of women being unable to lactate (WHO1981). The association between failure to lactate and poor maternal nutrition is not conclusive.

Determining the relation between maternal nutrition and the duration of breast feeding is complicated , by the fact that the length of time a mother chooses to breast feed her infant depends on a variety of external factors in addition to nutrition. These factors include frequency, intensity, duration of

nipple stimulation that influence the let down reflex. Consequently, prolonged lactation has been recorded among women who by usual indirect measures of nutritional status should be most malnourished. For example undernourished Ethiopian women receiving 1300 kcal of energy daily were able to provide adequate breast-feeding for their infants (WHO 1985).

1.1.3 Effect of Maternal Dietary Intake and Nutritional Status On Breast Milk Composition

There is a wide variation in the influence of maternal nutritional status and dietary intake on the composition of breast milk. Some studies have demonstrated that the energy, protein, fat and lactose contents of breast milk are subject to limited variations in the nutritional status or dietary intake of the mother. The energy content of breast milk produced by Gambian women consuming 1700Kcal/day was 72Kcal /100ml. This is very similar to the energy content of 69Kcal/100ml found in the milk of British women (Whitehead 1982). Furthermore, when the lactating mothers in the Gambia had an intake of 1100 - 1200 Kcal/day the energy value of their milk decreased by only 10% (Whitehead 1982).

Studies conducted amongst adequately nourished Gambian women, economically advantaged and disadvantaged Ethiopian women, and Swedes showed similar concentrations of protein in breast milk despite clear differences in the dietary intake and nutritional status of these women

(Lonnerdal, forsum and Hambreus 1976). A lower concentration of protein was however observed in the milk of malnourished mothers (Hanafy, Moisey, Seddick and Habit 1972). Effect of high protein (20% of total energy intake) and low protein (8% of total energy intake) diets on the quantity of breast milk determined in Swedish women showed that high-protein diet was associated with a higher concentration of total nitrogen, true protein, and non-protein nitrogen in the breast milk. No difference was observed in the concentrations of lactoferrin, lactalbumin, or serum albumin. The protein and lactose concentrations in breast milk of Nigerian women however remained unchanged after dietary supplementation with protein (Edozien 1976). These evidences underscore the existence of racial difference in the effect of diet on protein concentration of breast milk.

Results from studies conducted in African women show that fat content of breast milk is subject to limited dietary control. Breast milk produced by Caucasian British women who obtain about 40% of their dietary food energy from fats, contain similar amounts of fat as Gambian women who obtain 10% or less of their dietary food energy from fat (Whitehead *et al* 1981). The relative amounts of breastmilk fatty acids in both groups of women is however influenced by the diet (Hambreus and Sjobin 1978; Insull(1959).

The mother's diet and nutritional status modulate the concentration of water-soluble vitamins in breast milk. The concentration of riboflavin, vitamin C. thiamine, folic acid, vitamin B6 and pantothenic acid in breast milk is closely correlated with the mother's dietary intake, and the concentrations of these vitamins in her plasma (Whitehead *et al* 1981, Bates, Bates, and Whithworth 1982, Prentice 1980). A low intake of water - soluble vitamin results in a decreased concentration of water soluble vitamins in breast milk (Belavady, Gopalan and Ramakrishnan 1959, Deodhar 1960). It has been demonstrated that supplementing the mother's diet with vitamins, particularly water-soluble vitamins, increases the vitamin concentration in breast milk (Belavady and Gopalan 1960, Karmarkar,Rajalakshini and Ramakrishnan1963, Kon and Mowson 1950.).

The concentration of fat-soluble vitamins in breast milk is also affected by maternal nutritional status. A significantly lower concentration of vitamin A and β-carotene was found in the breast milk of economically disadvantaged Ethiopian mothers compared with breast milk of well nourished mothers of the same race (Lonnerdal, Forsum and Hambreus 1976). Fortification of sugar with vitamin A resulted in an increased concentration of vitamin A in breast milk in Guatemalan women (Arroyave 1979).

Dietary intake however does not influence the mineral and trace elements concentrations in breast milk. It has been demonstrated that similarly, potassium concentrations in breast milk are independent of dietary intake (Iyengar1982). There are no significant differences in the sodium or potassium concentrations of breast milk obtained from women on a low salt diet and those on normal diet (Defilippi 1981). Supplementation of maternal diet with iron and manganese does not increase the iron or manganese content of breast milk (Underwood 1977).

1.1.4 Influence Of Maternal Nutritional Status and Diet On Breast Milk Quantity.

The relationship between breast milk output and the maternal nutritional status is complicated by the influence of psychological and sociological factors. For example, fear of weight loss or wrong perception of nutritional status may inhibit the let - down reflex (Jelliffe *et al* 1979) The mother's ability to acquire food and what is considered to be the desirable body weight for a woman in her particular society also influence milk output (WHO 1985) Maternal nutrition may thus influence breast milk volume either directly or indirectly (WHO 1985). Studies of the effect of maternal diet supplementation as it affects breast milk production have yielded conflicting results because the food supplement may replace part of the diet instead of

complementing it, and the length of dietary supplementation may be too short (WHO 1985)

Supplements of 300 Kcal / day given from the 45th day of gestation until weaning increased milk volume and resulted in a measurable positive impact on child growth, however, the breast milk became diluted (Chavez and Martinez 1980). Similarly, postpartum protein supplementation of 20 - 50g of protein per day in Nigerian women produced increases in infant intake and maternal secretion capacity (Edozien 1976). The mothers secreted more milk than the infants consumed before diet supplementation and after commencement of diet supplementation, the babies milk consumption increased. The babies showed a significant improvement in growth (height and weight) compared with the babies in a control group of mothers without dietary supplementation. The reason for this post supplementation increase in milk production is however not clearly defined.

A study conducted in the Gambia produced different results. Nursing mothers diets supplemented with over 700 Kcal / day for 12 months (The mean maternal intake was 2291 Kcal / day) showed an initial improvement in maternal body weight and subcutaneous fat stores. However there was no increase in the intake of breast milk by the infant (Prentice 1981). This is not consistent with increases shown in breast milk yield with diet supplementation

of the nursing mother (Belavady Acbix and Whitehead 1980, Gopalan 1958). While well-nourished motheis were able to secrete significantly more milk than infants were able to ingest, this was not the case for malnourished mothers (Khinn Maung Naing 1980). Others have also found that malnourished women produced 22% less milk than the well nourished ones. There was no correlation between the matemal weight / height ratio or skinfold thickness and the quantity of breast milk, except for severely malnourished women (Hanafy *et al* 1972, Bailey 1965).

Several investigatois have reported that the average daily intake of breast milk by infants in developed countries is in the range of 600-800 ml / day rather than 850 ml / day (Whitehead 1982). In the United States of America, the mean expressed breast milk volumes in mothers of premature babies during the first couple of weeks postpartum ranged between 1098 and 1673 ml / day (WHO 1985).

1.1.5 Maternal Nutrition and "Lactational Infertility"

Lactational infertility is a period of lactation when the menstrual cycle is suspended. It is one of the natural factors controlling conception intervals (Lunn et al. (1983). It may be a control system for the number of children born by many women throughout the developing world. Although severe

malnutrition undoubtedly does reduce fertility, there is no conclusive evidence of a similar effect of mild to moderate malnutrition. It has been proposed that the lack of suckling induced inhibition of fertile cycles in the industrialised nations of the west is due to the good nutritional status of the women (Frisch 1988).

Duration of lactational infertility was compared with maternal weight, wealth and communities, these indices were found to be inversely correlated with duration of lactational amenorhea (Chowdhury 1978, Bongaart and Delgado 1979, Prema Naidu, Neelakumari and Ramalakshimi 1981, Huffman, Chowdhury, Akins, Charkraborty and Simpson 1980). These studies were however criticised because the indices used (weight, wealth and communities) to assess maternal nutrition were not the accepted nutritional indices.

Much of the evidence from developing countries suggests that supplementation of the diet of undernourished breast feeding women during pregnancy or lactation caused a more rapid return of fertility (Lunn *et al* 1980, 1981). These findings have been related to changes in basal prolactin levels. However they have not been correlated with changes that might have occurred in infant suckling behaviour and maternal breast feeding pattern. A relationship between these factors and the length of lactational amenorrhoea has been documented (Mcneilly 1984). A suckling frequency of more than five

times per day and more than ten minutes per feed was found to maintain a complete suppression of ovarian activity (Lunn *et al* 1980).

Better nourished women experience a shorter period of lactational infertility than their poorer counterparts even when they were fully breast feeding. Well nourished women resume ovulatory menstrual cycles within 3 months post-patum, while poorly nourished women in developing countries usually do not resume ovulatory menstrual cycles for a year or more postpartum (Frisch 1988)

Lactational hyperprolactinaemia and the return of menstruation during lactation were not directly related to the nutritional status of the mother. Serum prolactin levels of urban lactating women with good nutritional status were significantly higher compared with rural women in Zaire (Hennart and Vis 1980). A "CRITICAL BODY COMPOSITION HYPOTHESIS" OF OVULATION has been proposed to explain the relationship. This hypothesis assumes that a minimum threshold of weight for height exists below which ovulation cannot occur (Frisch 1985). High body fat (mass) and high weight are associated with short periods of lactational amenorrhea (Prema *et al* 1981)

Results from studies on the effect of food supplementation to undernourished women on the duration of lactational amenorrhea are conflicting. A reduction in the duration of lactational amenorhea and inter

pregnancy interval (1 to 2 months maximum) was demonstrated when the diets of undernourished women was supplemented (Delgado 1958). It has however been demonstrated that the effect varies depending on whether the supplementation contained both protein and energy or energy alone and with the amount of supplement (Chavez and Martinez (1973). A reduction of six months was demonstrated in the inter pregnancy interval when a group of Mexican women received diet supplementation during both pregnancy and lactation (Chavez and Martinez 1973). In both studies, it was difficult to evaluate whether the effect was solely a result of improved maternal nutrition since the infants were receiving some of the supplement.

Lunn has conducted extensive studies on the effect of maternal malnutrition and lactational infertility (Lunn *et al* 1980, 1983a). In those studies, a supplement of approximately 720Kcal / day was provided to pregnant and lactating women, and no extra supplements were given to the weaning infants. Supplementation especially if received during pregnancy in addition to the postpartum period resulted in a reduction of 21 weeks in the return of fertility. The resumption of ovarian activity was monitored by plasma concentration of prolactin, oestradiol and progesterone concentrations, but the resumption of menstruation was not documented. The reliability of use of plasma prolactin values as a direct indication of the return of fertility is not well established. Although an improvement in maternal diet appeared to result

in only a small reduction in suckling frequency, no estimates of total daily suckling duration, night time suckling duration or frequency were made. Suckling activity is closely reflected by changes in serum prolactin concentrations (Mcneilly 1980). Consequently changes in serum prolactin may well reflect changes in suckling activity (Mcneilly 1980).

Most of these supplementation studies are suggesting that dietary supplementation affects the duration of lactational infertility by altering the quality and quantity of milk available to the infant and thereby act via changes in suckling. As has been mentioned earlier evidence for alterations in milk yield or constituents because of dietary supplementation is conflicting.

Experiments conducted in non primate mammals on a poor plane of maternal nutrition showed a marked increase in suckling frequency among the calves of red deer compared with that ofcalves of well fed hinds. (Lowden, Mcneilly and Milne 1983). More frequent suckling was a strategy forced on the calves because the rate of milk secretion was halved as a result of poor nutrition. Plasma prolactin levels were much higher among the poorly fed hinds.

Higher suckling frequency (Delgado 1958) and longer suckling duration (Huffman *et al* 1980) have been observed in poorly nourished children compared with better nourished infants in studies conducted in humans. In a more recent study of Australian women breast-feeding for an extended period,

there was no correlation between maternal nutritional status and the duration of amenorrhea. Neither the time of first supplement introduction to the baby nor the amount of supplement given was an accurate predictor of the return of ovulation or menstruation (Patticia, James, Marilyn and Roger 1991).

Although there was a correlation between lactational amenorrhoea and maternal triceps skinfold thickness three months postpartum, the correlation was not significant and in the overall view it is the infant not maternal supplementation that influences length of postpartum amenorrhoea (Kurck and Rasmassen 1993). Two possible mechanisms explaining the relationship between maternal nutritional status and length of postpartum amenorrhoea have been suggested (Kurck *et al* 1993). The first possible mechanism is that women with poor nutritional status may experience greater inhibition of the ovulatory hormones from the same amount of suckling as do women with good nutritional status and thus experience longer amenorrhoea. The second possible mechanism is that children of mothers with poor maternal nutritional status suckle more to get an adequate amount of breast milk (Lunn *et al* 1984; Loudon *et al* 1983). The increased suckling increases inhibition of the ovulatory hormones, and lengthens amenorrhoea. This probably occurred because the children with higher weight gain consumed more breast milk, suckle more and provide greater inhibition to their mother's ovulatory

hormones, whether or not the infants of malnourished mothers suckled more for equal weight gain could not be explained from the data (Kurck *et al* 1993).

Although maternal energy intake from supplement was not associated with length of amenorrhoea, better maternal nutritional status was associated with a small reduction in length of amenorrhoea (Kurck *et al* 1993). The difference was so small that, even if a woman experienced a large improvement in her nutritional status, she would not have time to bear an additional child during her reproductive years. Thus, the desirable outcomes from maternal supplementation, such as increased birth weight and improved health and nutritional status of women, are not outweighed by a small undesirable reduction in postpartum lactational infecundability (Kurck *et al* 1993).

There are flaws in most of the demographic studies which form the basis for this conclusion on the relationship between nutrition and lactational amenorrhoea (Popkin,Gurkey, Akin, Adair, and Udry 1993). These flaws include

- Poor measurement of the desired indices of nutritional status.
- Use of inadequate (cross-sectional) methods to analyse a dynamic relationship.
- Lack of time-varying data on nutritional status for adequate sample of women

- Lack of attention to key confounding factors such as infant-feeding patterns or mother's diet and activity patterns
- Failure to account for the statistical problems of unobserved heterogeneity and endogeneity of nutritional variables.

Popkin *et al* (1993) demonstrated that poor nutritional status has a significant effect on the duration of postpartum amenorrhoea in contrast to results from other studies suggesting that nutrition does not matter. Results of this study show that nutrition plays an important role in within-population variation in fertility (Popkin *et al* 1993). The effect of Body Mass Index (BMI) on Postpartum amenorrhoea however, manifest only at extremely low BMI. Thus suggesting that it is chronic malnutrition that affects postpartum amenorrhoea and not borderline malnutrition (Menken 1981).

The effects of dietary fat on the return of postpartum amenorrhoea are striking (Popkin *et al* 1993). There is clear evidence of an effect of variations in fat intake on birth spacing. Lower fat intake has also been demonstrated to be associated both with a longer menstrual cycle and with longer menses (Jones, Judd, Taylor, Campbell and Nair 1987).

Participants of the 9th Nestle Nutrition workshop on "Maternal Nutrition and Lactational infertility" were able to deduce the following as

being the possible associations between malnutrition and prolonged post partum amenorrhoea.

1. The association is only because poor mothers in developing countries breast feed longer than well-to-do mothers. These poor mothers tend to be malnourished.

2. Malnourished women undertake activities that change the nature of their breast feeding, and these are during the harvest season. For instance, they may work in the fields more during the day and suckle the child more at night. Night suckling may have a greater anovulatory effect than suckling during the day.

3. Malnourished mothers give inadequate supplementary food to their infants at a time when supplementary food is necessary to meet the older infants nutritional requirements. The infants consequently suckle more energetically at a time when normally they should be less dependent on breast milk.

4. Maternal malnutrition results in lower milk production, so that the babies suckle for longer periods to obtain adequate quantity of milk. This increased nipple stimulation prolongs postpartum amenorrhoea.

5. Maternal malnutrition directly postpones ovulation even though suckling is the same. For instance the sensitivity of the suppressive effect of suckling on the Gonadotrophin releasing hormone (GnRH) pulse generator may be increased (Habicht 1986)No evidence exists to date, however, for this mechanism.

1.1.6 Endocrine Control of Lactational Infertility

During pregnancy, pulsatile release of gonadotrophin releasing hormone (GnRH) is inhibited. This inhibition results in a reduction in pituitary content of luteinising hormone (LH) to around 1% of normal at term (De Lastra Llados 1977). Plasma concentrations of follicle stimulating hormone (FSH) in breast feeding women increase to within normal early follicular phase levels by 4 to 8 weeks postpartum (Tay, Glassier and Mcneilly 1992). It is this increase in FSH, which is probably responsible for the induction and continued production of the waves of follicle development observed by ultrasound during lactational amenorrhoea (Mcneilly *et al* 1994b). In the absence of adequate pulsatile LH stimulation these follicles will either remain inactive, or produce only small amounts of oestrogen.

Plasma concentrations of LH increase from undetectable levels around day seven postpartum to low normal levels by 3 to 4 weeks postpartum (Kremer 1991, Tay *et al* 1992). Studies on the pulsatile patterns of LH secretion over 24hr periods during the resumption of ovarian activity have shown that pulsatile release of LH can occur by 4 weeks postpartum in breast feeding women but the frequency remains low and variable (Tay *et al* 1992). In other studies very low amplitude LH pulses were released at a normal frequency (Nunley Urban and Kitchen 1991). However overall results indicate

that LH pulses and the consequent pulsatile release of GnRH from the hypothalamus is not completely inhibited throughout lactation. Consequently when FSH stimulates follicle growth to occur, pulsatile LH release may be occurring and this would allow the production of oestradiol from a proportion of follicles. However, the amount of oestradiol produced will vary considerably since the frequency of LH pulses is very variable. As lactation progresses and suckling declines, the frequency of pulsatile LH secretion increases to near the frequency occurring in the normal follicular phase and sustained oestradiol secretion will occur (Glassier Mcneilly and Howie 1984). Although a normal increase in plasma oestradiol may occur during breastfeeding, the increase may not trigger the release of preovulatory surge of LH. Normal luteal phase function resumes only when suckling has reduced to the point where there is no longer an inhibition of both the normal pattern of pulsatile LH secretion and generation of preovulatory LH surge. (McNeilly, 1993). This process has been confirmed when the replacement of a normal pattern of GnRH release by pulsatile infusion pump in fully breast feeding amenorrheic women at 6 weeks postpartum resulted in development of normal estrogenic follicles as seen by ultrasound (Glassier, Mcneilly and Baird 1986).However, inadequate luteal function in the majority of these women related to a poor preovulatory LH surge.

The link between the suckling stimulus activating nerve terminals in the nipple and the disruption of the pattern of release of GnRH from GnRH neurones in the hypothalamus remains unknown (McNeilly 1994a). Feed back effects of high plasma concentration of prolactin have been suggested as a possible mechanism. The suckling pattern inhibits ovulation by increasing the basal levels of circulating prolactin (Delvoye,and Delogne-Desnoeckk 1976, Gross *et al* 1979). Suckling of at least six or more times daily and including night feeds have been found to inhibit ovulation (Hennart *et al* 1980). One study from the developed world shows that infertility can be extended among well nourished women who breastfeed on demand compared to their counterparts who breast feed on schedule (Kipley and Kipley 1972). An increase in prolactin level following an improvement in maternal nutritional status with subsequent increase in duration of lactational infertility has been demonstrated (Lunn *et al* 1984). However, not all studies corroborate these findings (Mcneilly 1994a, Tay *et al* 1993). For example, when breast feeding women were treated with the dopamine antagonist metoclopramide, there was a large release of prolactin. However there were no effects on FSH or pulsatile release of LH. Although there is no doubt that prolactin is required for milk production, the potential role for prolactin in causing the infertility associated with suckling remains very unclear (Mcneilly 1994a, Tay *et al* 1993). An increased release of prolactin in response to suckling over a 24-hour period was

obsetved in women with prolonged lactational amenorrhoea (Diaz Seron Ferre and Cardenas 1989, 1991). The correlation between duration of hyperprolactinaemia and duration of lactational amenorrhea suggests a role for prolactin, however it is more likely that since the plasma concentration of prolactin depends on the frequency and duration of suckling, the hyperprolactinaemia reflects suckling activity and that the true relationship is between high suckling activity and prolonged lactational amenorrhoea (Tay *et al* 1992). Other factors in addition to hyperprolactinaemia are involved in the control of fertility during lactation (Mcneilly 1994(a), Hennart *et al* 1985). Diaz *et al* (1991) investigated the early difference in the endocrine profile of long and short lactational amenorrhoea in 48 women from the first postpartum month until the recovery of ovulation and in a cross sectional study. prolactin (PRL), luteinising hormone (LH), follicle stimulating hormone (FSH), oestradiol, progesterone, cortisol, and dehydroepiandrosterone sulphate were measured. In that study a smaller PRL increase was detected in response to suckling in nursing women who ovulated within 6 months postpartum compared to those who did not. These results suggest some probable sources of variability in the duration of lactational amenorrhoea in their population. The greater PRL response to suckling associated with longer amenorrhea was suggested to be due to higher sensitivity of the breast - hypothalamus-pituitary system or a stronger suckling stimulus in the group. Prolactin in the early

postpartum period may transiently increase milk production, but that chronic hyperprolactinaemia has no effect on lactational performance (Barguno Del, Cruz and Figuras1987), suggesting that prolactin response to suckling may not be essential for maintenance of milk secretion. Basal prolactin levels and neurogenic reflexes were suggested to be instrumental to maintaining established lactation (Glassier *et al* 1984). Both basal concentration of prolactin response and the magnitude of the prolactin response to suckling decrease with time post partum.(Glassier *et al* 1984).

1.1.7 Progesterone

Progesterone is the most important progestin in humans. It is synthesized in the ovary, testis and adrenal from circulating cholesterol. Large amounts are also synthesised and released by the placenta during pregnancy (Katzung and Trevor 1992) Progesterone is responsible for the alveolobular development of the secretory apparatus in the breast. It also causes the maturation and secretory changes in the endometrium that are seen following ovulation.

The estimation of progesterone in plasma is a recent introduction and studies of progesterone metabolism are generally made on the inactive excretion product, pregnanediol.

The following steroids which appear in this thesis are named according to the approved system of steroid nomeclature according to the rules suggested by the International Union of Pure and Applied Chemists (IUPAC) 1969.

1.1.8 Oestrogens:

The principal human oestrogens are oestradiol, oestrone and oestriol. Apart from their actions on the sexual organs and activities, the oestrogens are protein anabolisers. Oestrogens are excreted in the urine principally after conjugation with glucuronic acid in the liver and in cirrhosis there is increased excretion of free oestrogens with diminished conjugation. The main use of oestrogens assay is the possible detection of abnormal pregnancy during the monitoring of foeto-placental function. Low values are found in primary and secondry amenorrhoea. In the successful treatment of amenorrhoea, generally for infertility, by human chorionic gonadotrophin or by clomiphene, there is a rise in the urinary oestrogen excretion and this must be monitored for the control of therapy.

CHAPTER 2

SUBJECTS, MATERIALS AND METHODS

2.1.1 Location of the study:

The study was conducted in Sagamu Local Government Area, Ogun State, Nigeria. The subjects were drawn from lactating women attending the post natal clinics of the Ogun State University Teaching Hospital, Private Clinics and Primary Health Centres in the Local Government Area.

2.1.2 Ethical approval

Ethical consent was obtained from the Ogun State University Ethical Committee. Ethical approval for the study was obtained prior to commencement of the study.

2.1.3 Criterin for subject selection.

Subjects were selected for the studies based on the following criteria.

1. *The body mass index (BMI) (weight / height2) which has been approved by the FAO and WHO 1986 as an index of nutritional status was used to determine the nutritional status of the mothers. A cut off point of 18.5 for men and 20 for women has been reported in the literature as the lower cut off points for people with chronic undernutrition (Waterlow et al 1989). This is rounded off to 20 for both seses. These values can not be extrapolated to the developing countries. Based on a recent FAO data from developing countries, a cut off point of 18.5 was found to be a reasonable lower limit in both sexes for developing countries (Waterlow et al 1989)*

2. *The average weight of lactating women in this environment was also determined, this was found to be 55kg. In view of these findings, women*

that have a BMI of 20.5 and below i. e the undernourished and moderately undernourished were recruited for the study.

In addition to these selection criteria, the mother and the infant also satisfied the following inclusion criteria before they were enrolled for the study

2.1.4 Inclusion Criterin

Infant:

1. Healthy at time of entry into the study (no ill health requiring hospitalisation i. e thriving child)
2. singleton

Mother:

1. Aged 20-37 years at the time of delivery (Not a teenager)
2. Parous i. e has had between one and three children prior to the index child, this will ensure that the mother has had experience on child rearing
3. Previously breast-fed at least one child
4. The index child was born by a vaginal delivery at 35-37 weeks gestation or beyond, to ensure that nothing interferes with the natural method of child birth
5. Willing and able to participate in the study and abide by the protocol
6. Accessible for follow up
7. Does not intend to use hormonal contraceptives, so that this will not interfere with the level of hormones to be measured

2.1.5 Exclusion Criteria

Women were not admitted into the study if any of the following criteria apply

1. The mother is suckling another infant at the same time, as this may affect the level of induced prolactin as a result of sucking
2. The infant is also being breast fed by someone else.
3. The intention to be involved in any process that would entail seperation of mother and baby for more than 8 hours during the study period

2.1.6 Admission Procedure

On admission, mother-infant pairs were screened to ensure that they satisfy the inclusion criteria set out above. Suitable and consenting mothers and their infant were admitted before the seventh day postpartum (day 0 = day of delivery) but not during the first 72 hours after delivery. This is to enable the mother and child to settle down. In addition, the following pieces of information were recorded on the admission forms

- Date of entry into the study
- Personal data of both parents
- Date of birth
- Ethnic group
- Education
- Usual occupation
- Marital relationship
- Socio-economic situation
- Medical history
- Serious illness
- Hospitalisation
- Surgery
- Gynaecological history, including menstrual cycle date, prior to conception of the present child
- Contraceptive use
- Postpartum period to date
- Obstetric history
- Physical data of mother and child
- Last pregnancy
- Mother's dietary pattern
- Last delivery
- In addition the nutritional status of the subjects were assessed based on
- Weight, height/length, mid arm circumference, chest circumference, head circumference
- Skinfold thickness at the biceps and triceps
- Haemoglobin concentrations
- Serum albumin

■ Dietary intake of the mother by weighing and recall methods
The details of the information collected at admission is shown in (APPENDIX I)

Pre-informed consents of the study subjects were obtained before the commencement of the study.

2.1.7 Subject Allocation And Description Of Dietary Supplement.

A total of 162 lactating subjects were recruited for the studies. Subjects were randomised into two study groups using a table of random numbers. On enrolment, each subject picked a sealed envelope containing a number corresponding to one of either study group. One study group of 79 mothers did not receive any dietary supplements and served as control. The second group of 83 mothers received dietary supplements in form of biscuits and served as the supplemented group. The biscuit was developed and produced in Australia based on a formulation recommended by the Commonwealth Scientific and Industrial Research Organisation. The nutrient composition and the percentage of recommended dietary intake provided by the biscuits is presented in Table 2.1. Essentially, the biscuit provides approximately 10% RDA for energy and 15% RDA for protein for lactating women. The biscuit has been successfully used as supplementary feeding of nutritionally disadvantaged groups, such as refugee groups, famine victims and in undernourished individuals for more than a decade (Buchanan and Townsend 1969). The biscuit is lactose free and is suitable for use in a population with a high prevalence of lactose intolerance such as Nigerians (Olatunbosun et al 1971).

Table 2.1 Composition of the Australia High Protein Biscuit (per 100grams weight)

Energy	450kcal	Protein	20g	Carbohydrate	50g
Fat	20g	Iron	25mg	Calcium	125mg
Iodine	125ug	Vitamin A	1000ug	Vitamin B12	7.5mg
Vitamin B2	1mg	Niacin	27.5mg	Vitamin C	62.5mg
Folate	1.25mg				

* Arnotts Biscuits Ltd., Homebush, Australia.

A group of 162 lactating mothers was studied. On the day of admission to the study, blood samples (5 ml) were collected for prolactin estimation at least 60-90 minutes after a suckling episode (between 4.00pm-7.00pm). A second sample was collected 30 minutes after the commencement of a new suckling episode. The samples were obtained to provide information on the effect of suckling on prolactin concentration. The blood samples were obtained in the evening on account of the effect of diurnal variation on prolactin levels in the blood.

All the subjects were educated on the method of completing infant-feeding record charts. Each subject was expected to record daily details of infant feeding in the infants daily record charts (APPENDIX 3) and infants detailed record chart (APPENDIX 4).

2.2 REVIEW OF METHODOLOGY

2.2.1 Activity pattern.

Activity pattern of the mothers was evaluated during the first 3 months of admission. Evaluation was based on the method described by James and Schofield (1990) with a predicted basal energy need of an adult woman as 8.7[weight (kg)] + 829 (FAO/WHO/UNU Report, 1985).

Activities of the lactating mothers were recorded through out the day and the time spent on each activity was also recorded on typical work load assessment form (Appendix 6). The activity pattern was computed using the following procedure

1. Calculation of total energy allowance for the different activities.
2. Estimation of BMR for body weight using predictive equation for an adult woman (8.7W (kg) + 829) where W is the weight of the woman.
3. Estimation of BMR per hour by dividing value calculated in 2 above by 24
4. Calculation of the energy cost of each activity per hour by multiplying the integrated energy index for that activity by the BMR expressed in Kcal and
5. Multiplication of this total energy cost for each activity by the hours spent on each activity.
6. Summing up the different activity expenditures to give the total daily energy cost, allowing all the residual day time to have an integrated energy index of 1.4.

An example of this calculation is presented below:

If a woman weighs 50 kg her BMR will be 8.7 X 50 + 829 =1264 kcal. This is equivalent to approximately 53 kcal per hour

Table 2.2 Activity Pattern of supplemented and Control Mothers

ACTIVITY	TIME (hrs)	INTEGRATED ENERGY INDEX	TOTAL ENERGY COST kcal (BMR/hrx IEI x Time)
In bed	8	1	424
Occupational activities	7	3	1113
Household tasks	5	2.7	715.5
Other discretionary activities	1	3.3	174.9
Residual time needs	3	1.4	222.6
Total	24		2650

The total energy cost is 2650 kcal. This can also be expressed as a ratio to BMR i e Physical activity level you divide this value by the value of BMR in 1 above. This value is equal to 2.1.

2.2.2 Assessment of Milk Volume.

The test weighing method of infants or test feeding method of measuring the infant breastmilk intake was adapted and used in this work because of its simplicity (WHO 1985) Records of infants number and duration of infants feeding episodes during the day and night were recorded by the mothers on forms referred to as daily infant feeding chart (APPENDIX 3) and detailed infant feeding chart (APPENDIX 4) Test weighing of infants milk intake for 5 minutes was measured and the total breastmilk intake for 24 hours was computed from this measurement using the infants feeding chart i e weight of baby before intake and weight of baby after breast milk intake. The only problem here is with babies that take frequent feeds. This gives a small weight difference and so a 9 volts digital sensitive scale providing a sensitivity of 0.01kg was used for the measurements.

2.3 Follow- Up Procedure

All the subjects were visited at least 3 times a week. During each visit the subjects were interviewed and the record charts were examined in order to

- assess compliance with the dietary supplement intake
- Ensure proper completion of the record chart
- see details of infant health and nutritional intake being recorded on the charts

In addition, early morning urine sample was collected for pregnanediol -3- and estrone-3-glucoronides estimation.

All the subjects were also seen at the research clinic at monthly intervals, during these visits blood samples were obtained and subsequently analysed to determine the following haematological and biochemical parameters.

- Haemoglobin concentration
- Serum albumin
- Prolactin concentration

The following anthropometric measurements were obtained at the monthly visit to assess the nutritional status of the mother and the growth of the baby. All measurements were recorded on nutritional assessment forms (Appendix 5)

MOTHER	CHILD
1. Weight	1. Weight
2. Skinfold	2. Head circumference
3. Mid-arm circumference	3. Mid-arm circumference
	4. Length
	5. Chest circumference

Information on the breastfeeding patterns, episodes of illnesses, types of supplements taken, status of menstrual cycle, sexual intercourse were recorded on follow up forms designed for the project (Appendix 2)

2.3.1 Anthropometric Measurement Of Infants

The following anthropometric measurements were obtained as described in order to assess growth and health of infants.

Weight The infants were weighed using a 9-volt battery operated digital electronic scale. The scale provided readings to the nearest 0.01kg.

Length The crown to heal length of all infants was measured using the Pedo-baby set which has a fixed plastic end and a movable foot end with a steel tape measure attached from one end to the other.

Head-Circumference The child's head was steadied and the greatest circumference was measured by placing the tape round the fore-head just superior to the supra-orbital ridges and round the head to the maximum occipital prominence. Measurements were made to the nearest 0.1cm.

Chest Circumference Using tape, measurement of the chest circumference at the level of the nipples in mid inspiration was made. Measurements were made to the nearest 0.1cm

Mid-Upper Arm Circumference (Muac) Measurements of MUAC are used in the assessment of nutritional status of children. This was done by allowing the left arm to hang freely and the circumference of its mid point, halfway between the olecranon and the acromium measured.

Growth Reference Curves.

The nutritional status of the infants was computed by using EPI-NUT module of the Epi-info (vesion 6) (World Health Organisation/ Centre for Disease Control (CDC) 1995) The analysis performed by the program are based on growth reference curves developed by the National Centre For Health

Statistics (NCHS) and CDC (Dibley et al 1987). The following nutritional indices were calculated.

HAP	Height for Age Percentile
HAZ	Height for Age Z score
HAM	Height for Age percent of Median
WAP	Weight for Age Percentile
WAZ	Weight for Age Z score
WAM	Weight for Age percent of Median
WHP	weight for Height percentile
WHZ	Weight for height Z score
WHM	Weight for Height percent of Median

SKINFOLD THICKNESS

The measurement of fat fold thickness was done using reliable callipers. The measurement was obtained after the following procedures

1. The subject bends her arm at the elbow and laying the hand across the stomach (if she is right handed, the left arm was measured and *vice versa*) in order to find the midpoint of the arm.
2. The shoulder was palpated to locate the acromial process and the olecranon process at the tip of the elbow.
3. A measuring tape was used to measure the mid-point of the acromial and the olecranon process, the point marked with a pen.
4. The fat fold measurement was made by letting her arm hung loosely to the side, and a grasp of fold of skin and subcutaneous fat between the thumb and fore finger slightly above the mid point mark of the skin was pulled away from the underlying muscle and the callipers was placed over the fat fold mark. The measurement was read to the nearest 1.0mm in two to three seconds. The measurement was read thrice and the average of the reading was recorded.

Dietary intake

The 24 hour recall and weighing was adapted for this study. The 24 hour recall is commonly used in nutrition surveys to obtain estimates of the typical food intakes. (Whitney 1990) Consequently, the actual food taken in one of the 3 days was measured by staying in the house of the subjects.

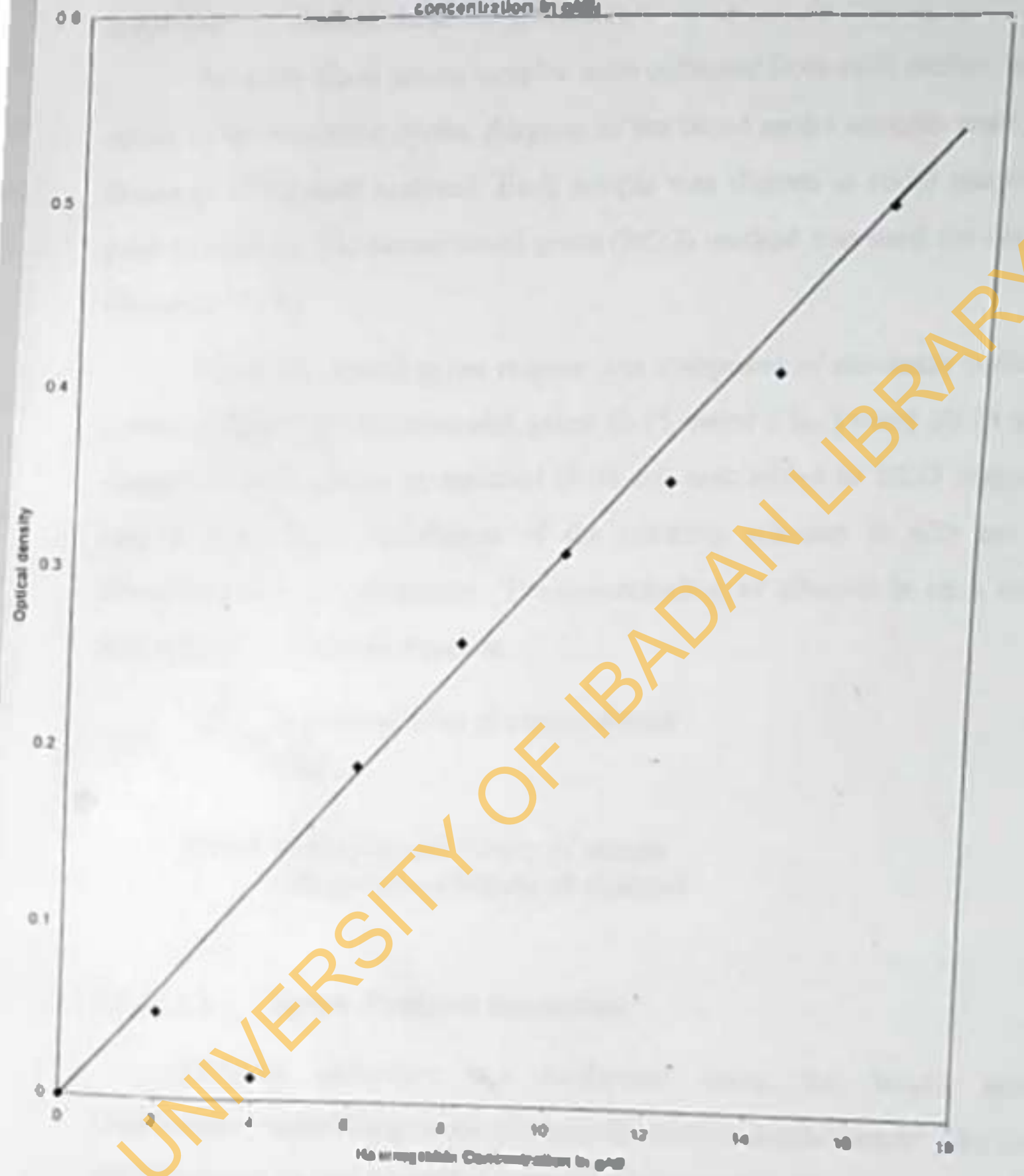
2.3.2 LABORATORY INVESTIGATIONS

2.3.2(a) *Hemoglobin Estimation*

The cyanmthaemoglobin method was used to determine hemoglobin concentration. The method is based on the conversion of hemoglobin to stable cyan-methaemoglobin by potassium ferricyanide in drabkins solution. Drabkins reagent contains 1gm of sodium bicarbonate. ($NaHCO_3$) 200mg potassium ferricyanide ($K_3Fe(CN)$) and 50mg potassium cyanide (KCN) dissolved in 1000ml deionised water.The absorbance of the solution is measured in a photo electric colorimeter. The protocol used for hemoglobin determination is as follow.

Blood sample (20ul) was added to 4ml of drabkins solution. The tube containing the solution was covered with a rubber bung and inverted several times. The optical density of the solution was determined using a photoelectric colorimeter at a wavelength of 540nm. Serial dilutions of cyan-methaemoglobin solution were used to prepare a standard curve. Concentration of haemoglobin in each sample was determined from the standard curve.

FIGURE 2:1 standard graph relating optical density reading to haemoglobin concentration in g/dl



2.3.2 (b) *Serum Albumin Estimation*

Monthly blood serum samples were collected from each mother until the return of the menstrual cycles. Aliquots of the blood serum samples were stored frozen (- 15°C) until analysed. Each sample was thawed at room temperature prior to analysis. The bromocresol green (BCG) method was used for the assay (Doumas, 1971).

The Bromocresol green reagent was composed of succinate buffer (95 mmol / l) (pH 3.8), Bromocresol green (0.15 mmol / l), Tween 20 (9 g / l). Aliquot of each sample or standard (0.02 ml) was added to BCG reagent (3 mls) in test tubes. Absorbance of the resulting solution at 620 nm was determined using a colorimeter. The concentration of albumin in each sample was calculated using this equation.

$$\frac{OD_s}{OD_{std}} \times \text{concentration of standard used}$$

Where OD_s is Optical density of sample
OD_{std} is Optical density of standard

2.3.2 (c) Serum Prolactin Estimation

Prolactin estimation was performed using the World Health Organization reagent Programme protocol for enzyme immunoassay. The assay was developed for use in routine estimation of prolactin. The assay consists of three main stages:

1. Incubation of sample with a bead coated with anti prolactin antibody for 30 minutes. Prolactin binds to the antibody, and other serum components are removed by means of a magnetic separation, including a wash step using the method described below.

Reaction of the antibody bound prolactin with an enzyme conjugate for 2 hours, excess enzyme label was removed by magnetic separation including two wash steps.

An enzyme / substrate reaction to measure the amount of antibody enzyme conjugate and hence prolactin bound. The reaction produces a colour change (yellow to pink) and in One hour. The reaction is terminated by the addition of a stopping reagent. The absorbance of the reaction mixture was determined using a Serono spetrophotometer at 492 and 550 nm.

olactin Assay Reagents

Magnetic Antibody (coated bead)	*Provided as a suspension diluted with assay buffer before use.*
Prolactin standards (lyophilised horse serum)	*Standard 1 = 0 mIU/L prolactin* *standard 2 = 100 mIU/L prolactin* *standard 3 = 220 mIU/L prolactin* *standard 4 = 530 mIU/L prolactin* *standard 5 = 1110 mIU/L prolactin* *standard 6 = 2800 mIU/L prolactin* *standard 7 = 7500 mIU/L prolactin*
Enzyme-labelled antibody	*31 times concentrate solution*
Substrate	*Phenolphthalein monophosphate powder (dissolved in substrate buffer before us)*
Assay Buffer(2X concentrate)	*0.05m phosphate buffer pH 7.4, containing magnesium, sodium and zinc chloride ,bovine and Maurine serum proteins , a surfactant and 0.1% sodium Azide .Diluted with 2litres of freshly prepared distilled water.*
Wash solution(4X concentrate)	*Tris/Hcl buffer pH (7.4) ,containing Magnesium and zinc chlorides , a surfactant and 0.1% sodium azide .This was diluted with 4litres of distilled water before use.*
Substrate buffer(2X concentrate)	*Diethanolamine /Hcl buffer, containing magnesium and zinc chlorides ,and 0.02% sodium azide. Diluted with 2 litres of distilled water before use.*
Stop Buffer(4x concentrate)	*10 times the concentrate of sodium hydroxide and a chelating agent in glycine buffer. Diluted with 4 litres of distilled water before use.*

Assay Procedure.

1. 100µl of standards or samples in duplicate were dispersed into tubes 1-100
2. 100µl washed magnetic antibody beads added to tubes 1-100 by using a repeating 100µl multidose pipette.

Summary of tube contents.

Tubes 1-14 *Standard tubes(including zero standard)*
100µl standard and 100µl washed magnetic antibody

Tubes 15-100 *Unknown samples including two sets of QC samples, one at the beginning and one at the end of the assay.*
100 µl sample and 100µl washed magnetic antibody.

Tube 101 *Substrate blank (not used until colour development stage and contains only substrate solution and stop buffer.*

First incubation.(immuno-extraction of prolactin)

1. Tubes 1-100 were vortex mixed for five minutes.
2. The tubes were covered with scalon plastic film and transferred to a water bath at 37°C (30 minutes).

First wash step.

1. The assay tubes were removed from the water bath
2. 500µl of wash buffer was added to tubes 1-100 and vortex mix gently for 5 seconds
3. The tubes were allowed to stand on a magnetic separator for 5 minutes.
4. Supernatant liquid was decanted from all tubes by inverting the separator first and then on an adsorbent paper to drain, and remove remaining droplets in tubes by gently tapping the tubes in the separator on the filter paper.
5. Separator was restored to an upright position.

6. 300µl of diluted labelled antibody solution was added to all the tubes

Second incubation (reaction with labelled antibody)

1. All the tubes (1-100) were vortex mixed
2. The tubes were covered and transferred to a water bath at 37 °C (two hours)

Second wash step (double wash)

1. Tubes were removed from the water bath
2. 500µl of wash buffer was added to tubes 1-100 and vortexed for 5seconds
3. The tubes were left in a magnetic separator for five minutes
4. The supernatant liquid was removed from all the tubes by inverting the separator. This was then inverted over an absorbent paper to drain, and remove remaining droplets in the tubes by gently tapping the tubes in the separator on the paper
5. The separator was returned to an upright position
6. Steps 2-5 were repeated

Colour Development

1. 500µl of substrate solution was added to all tubes including the substrate blank (1-101)
2. Tubes 1-101 were vortexed
3. The tubes were covered and incubated for 1 hour at (37°) in a water bath
4. The tubes were removed from the water bath
5. 1ml of stop buffer was added to all tubes (1-101) in the same sequence that the substrate buffer was added

6. The tubes were left on a magnetic separator for at least 10 minutes to allow the sediment to settle down to give a clear solution.

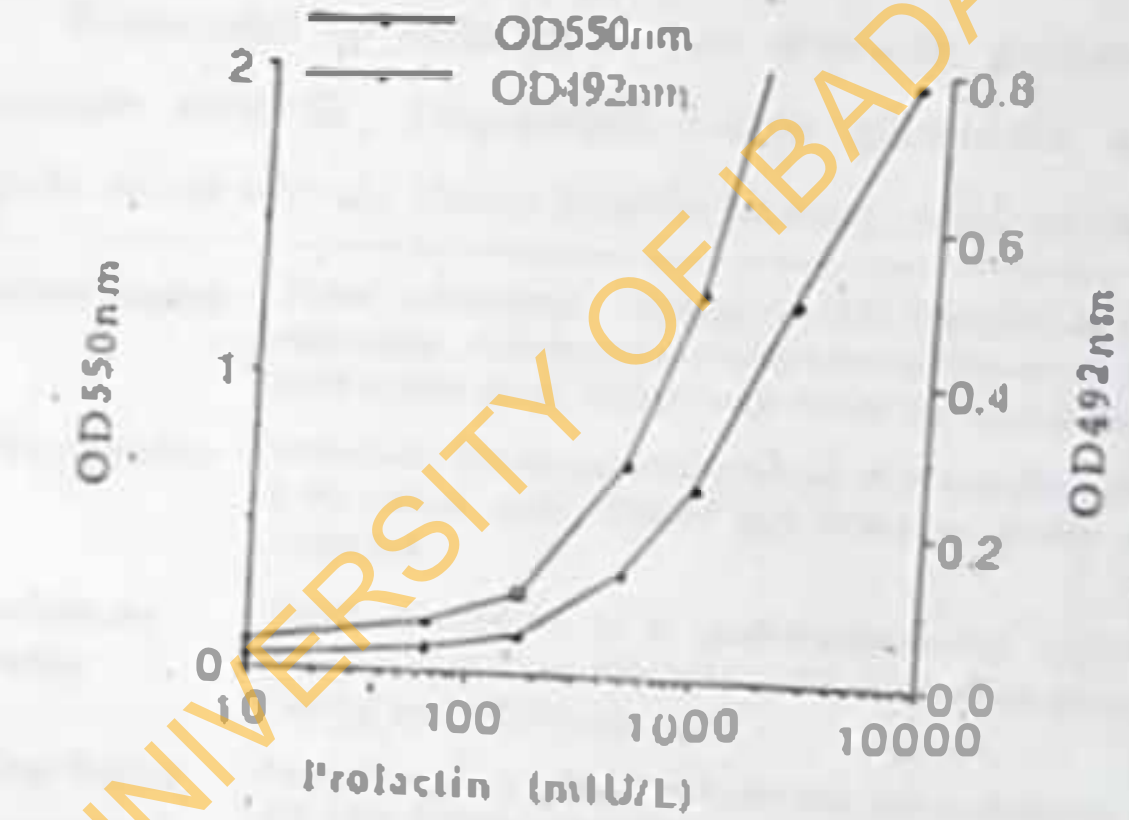
Colour measurement.

The optical density of all tubes was measured within 24 hours of completion of the assay using a serono serozyme photometer. Result was analysed using the WHO immunoassay Data Processing Program.

TYPICAL PROLACTIN ENZYME IMMUNOASSAY STANDARD CURVE

The graph below shows a prolactin enzyme immuno assay calibration curve of optical density 550nm versus prolactin concentration. This is used for the interpolation of most test sample results. Measured optical density values greater than 2 for standards or samples were incorrect and were not used to calculate results. Samples with optical density 550nm values greater than 2 were re-measured at 492nm for calculation of results from a calibration curve of optical density 492nm versus prolactin concentration (also shown in graph).

Fig 2.2 Typical Prolactin Enzyme Immunoassay Standard Curve.



2.3.3 Urinary Estimations

Aliquots (5ml) of early morning urine sample were collected from the mothers once a week until the return of menstruation and stored frozen until analysed. Prior to storage thiomersal (0.1%) was added as a bacteriostat to the samples.

2.3.3 (a) Urinary Pregnanediol-3-alpha Glucuronide Estimation

Urinary Pregnanediol 3 alpha glucuronide was quantified by enzyme immunoassay (Ahsan *et al* 1993) The assay uses a second antibody immobilised on a magnetic solid phase for separation of free from antibody bound hormone.

Assay Reagents.

Pregnanediol -3- alpha glucuronide standards, pregnanediol-3 alpha glucuronide antiserum. Pregnanediol 3-alpha glucuronide enzyme label, Magnetic second antibody. Quality controls for assay, substrate reagent.

- **Assay buffer** — *100ml concentrate contains 0.1M Tris/Hcl buffer (pH 7.4) containing magnesium sodium and zinc chlorides, bovine serum proteins, a surfactant and 0.1% sodium Azide. Diluted with 400ml of freshly prepared distilled water.*
- **Wash buffer** — *100ml concentrate containing Magnesium and zinc chlorides, a surfactant and 0.1% sodium azide. Diluted with 900ml of freshly prepared distilled water before use.*
- **Substrate buffer** — *100ml concentrate of a diethanolamine/Hcl buffer (pH 9.6) containing Magnesium and zinc chlorides and 0.25g sodium azide. Diluted with 400ml of distilled water before use.*
- **Stop buffer** — *50ml concentrate of Sodium hydroxide and a chelating agent in glycine buffer, (pH 10.4) Diluted with 450ml of distilled water before use.*

Preparation of standard Distilled water (2 mls) was added to the standards and mixed thoroughly. The following concentrations were obtained.

standard 1	18.1nmol/L
standard 2	34.5nmol/L
standard 3	71.6nmol/L
standard 4	133.6nmol/L
standard 5	266.0nmol/L
standard 6	525.6nmol/L

Preparation of sample.

Samples were thawed at room temperature and diluted 1in 100 with distilled water. Aliquot (25µl) of each urine sample was added to 2475µl of assay buffer and vortexed. Aliquots of the mixture (500µl) were dispensed into duplicate assay tubes. The assay tubes (100) were arranged as detailed below

Tubes 1-2	Non specific Binding tubes (NSB tubes)
Tubes 3-4,93-94.	Zero Antigen tubes
Tubes 5-20	Standard Tubes
Tubes 21-26,95-100	3 Internal Quality Control Tubes (IQC)
Tubes 26-92	Diluted urine samples

1. 500ul of standard, buffer or diluted urine sample in duplicate were dispersed into tubes 1-100
2. 100ul of antibody (To NSB tubes 100ul assay buffer and not antibody) and 100ul of enzyme label was added to all the tubes. All tubes were vortexed
4. The antigen-antibody reaction was accomplished in a refrigerator overnight or alternatively at 37°C for 2hours

Immunomagnetic separation

1. At the end of this period, assay tubes were removed from the refrigerator or water bath
2. The supernatant was removed from the 2nd antibody and this was replaced with 10ml of assay buffer, and the solution was gently mixed to suspend the particles. The second antibody (100µl) was then added to tubes 1-100 and left for one hour at room temperature

Separation and wash step

1. 1ml of wash buffer was added to tubes 1-100 and was briefly vortexed
2. The tubes were staked on a magnetic separator for 5-10minutes.

3. The supernatant was removed from all tubes by inverting the seperator. The inverted separator was then put on an absorbent paper to remove and drain remaining droplets in the tubes by gently tapping the tubes

4. The separator was put back in an upright position and steps 1-4 was repeated

Colour Development

1. 0.5ml of substrate solution was put into tubes (1-100) and to tube 101 for use as substrate blank.
2. The tubes were vortexed
3. The tubes were incubated for 1hour at 37°. The magnetic particles settled down at the bottom of the tubes
4. The tubes were removed from the water bath.
5. 1ml of stop buffer was added to all the tubes (1-101) tube 101 is blank
6. The tubes were left on a magnetic separator for at least 10 minutes to sediment all the particles, producing a clear solution for colour measurement.
7. The optical density (OD) of the supernatant was determined

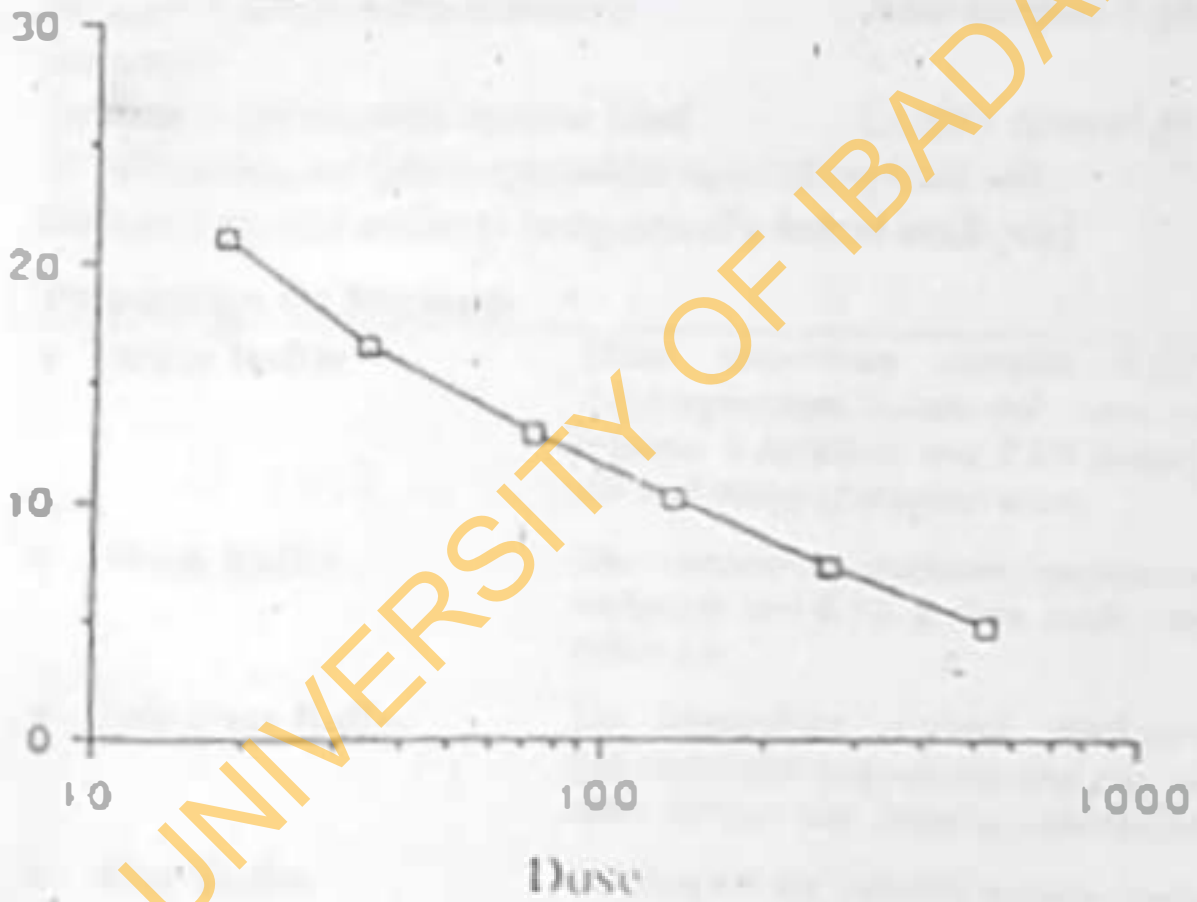
Colour measurement

The OD of the supernatant was determined within 24hours of completion of the assay using a serono serozyme photometer. OD_{450} greater than 2 were unreliable, OD_{492} was used in quantifying such samples.

Calculation of Results

Results was analysed using the WHO immunoassay Data Processing Program. The program plots a dose response curve with the standards. The concentration of the samples were determined from the standard curve.

Fig 2.3 Typical Pregnanediol-3- alpha Glucuronide standard curve



2.3.3 (b) Estimation Of Urinary Estrone-3-Glucuronide.

The assay method used is designed to estimate esttrone-3-glucuroninde in human urine. The assay is an enzyme immunoassay which uses a second antibody immobilised on a magnetic solid bead for separation of free from antibody-bound analyte (Alisan *et al* 1993). The main application of assays for estrone-3-glucuronide in routine clinical practice is in conjunction with pregnanediol-3-alpha glucuronide assays for the assessment of ovarian function. The ratio of the two metabolites is used to distinguish between ovulatory and anovulatory cycles.

Reagents

Estrone-3-glucuronide standards — Anti-estrone-3-glucuronide antiserum

Estrone-3glucuronide enzyme label — Quality control pools assay

Substrate reagent (phenolphthalein monophosphate salt.

Magnetic second antibody (magnetically linked antibody)

Preparation Of Reagents.

- **Assay Buffer** — *100ml concentrate contains 0.1M TrisHCl buffer (pH 7.5),Magnessium,Sodium,and zinc chlorides, Bovine serum proteins, a sufactant, and 0.1% sodium azide .Diluted at time of use with 100ml of distilled water.*
- **Wash Buffer** — *The concentrate contains magnesium and zinc chlorides,a surfactant and 0.1% sodium azide. Diluted with 1litre of water before use*
- **Substrate Buffer** — *The concentrate contains diethanolamine/HCl buffer (pH 9.6),containing magnessium and zinc chlorides and 0.2% sodium azide. Diluted with 500ml of water before use.*
- **Stop Buffer** — *The concentrate contains sodium Hydroxide and a chelating in glycine buffer (pH 10.4).50ml of this concentrate was diluted with 450ml of distilled water before use.*
- **Antiserum** — *10 ml of assay buffer was added to one bottle of lyophilised antiserum and this was mixed thoroughly.*
- **Estrone-3-glucuronide** — *100ul of this was diluted with 9.9ml of assay buffer just before the assay.*
- **Samples** — *1:100 dilution of the samples were made just before the assay.*

- **Standards**

2ml of distilled water was added to the standard and mixed to give a the following concentration.

standard 1	*0.33nmol/L*
standard 2	*0.61nmol/L*
standard 3	*1.20nmol/L*
standard 4	*2.30nmol/L*
standard 5	*4.62nmol/L*
standard 6	*8.25nmol/L*

Assay Procedure

The 100 assay tubes was arranged as follows.

Tubes 1-2	Non specific binding tubes.
Tubes 3-4, 93-94	Zero antigen tubes
Tubes 5-20	Standard tubes
Tubes 21-26,95-100	3 Internal quality control samples (IQC samples)
Tubes 26-92	Prediluted urine samples

1. 500ul of standard, buffer or prediluted urine sample in duplicate was put into tubes 1-100
2. 100ul of antibody (To NSB tube 100ul buffer was added) and 100ul of enzyme label was added to all tubes
3. The reaction was allowed to proceed in the refrigerator overnight or at 37^{0} for 2 hours

Immmomagnetic separation.

1. The assay tubes were removed from the refrigerator or water bath
2. 100ul second antibody reagent was added to tubes 1-100
3. The tubes were left for one hour at room temperature

Separation and wash step

1. 1ml of wash buffer was added to tubes 1-100 and vortexed
2. The tubes were left on a magnetic separator for 5 minutes

3. The supernatant liquid was removed from all the tubes by inverting the separator. The separator was inverted over an absorbent paper to drain and remove remaining droplets in tubes by gently tapping the tubes.
4. The separator was put back in an upright position.
5. steps 1-4 was repeated.

Colour Development.

1. 0.5ml of substrate solution was added to all tubes (100) and tube 101.
2. All the tubes were vortexed.
3. The tubes were incubated for 1hour at 37°.
4. 1ml of stop solution was added to all the tubes (1-101).
5. The tubes were left on a magnetic separator for at least 10 minutes to sediment all the particles.

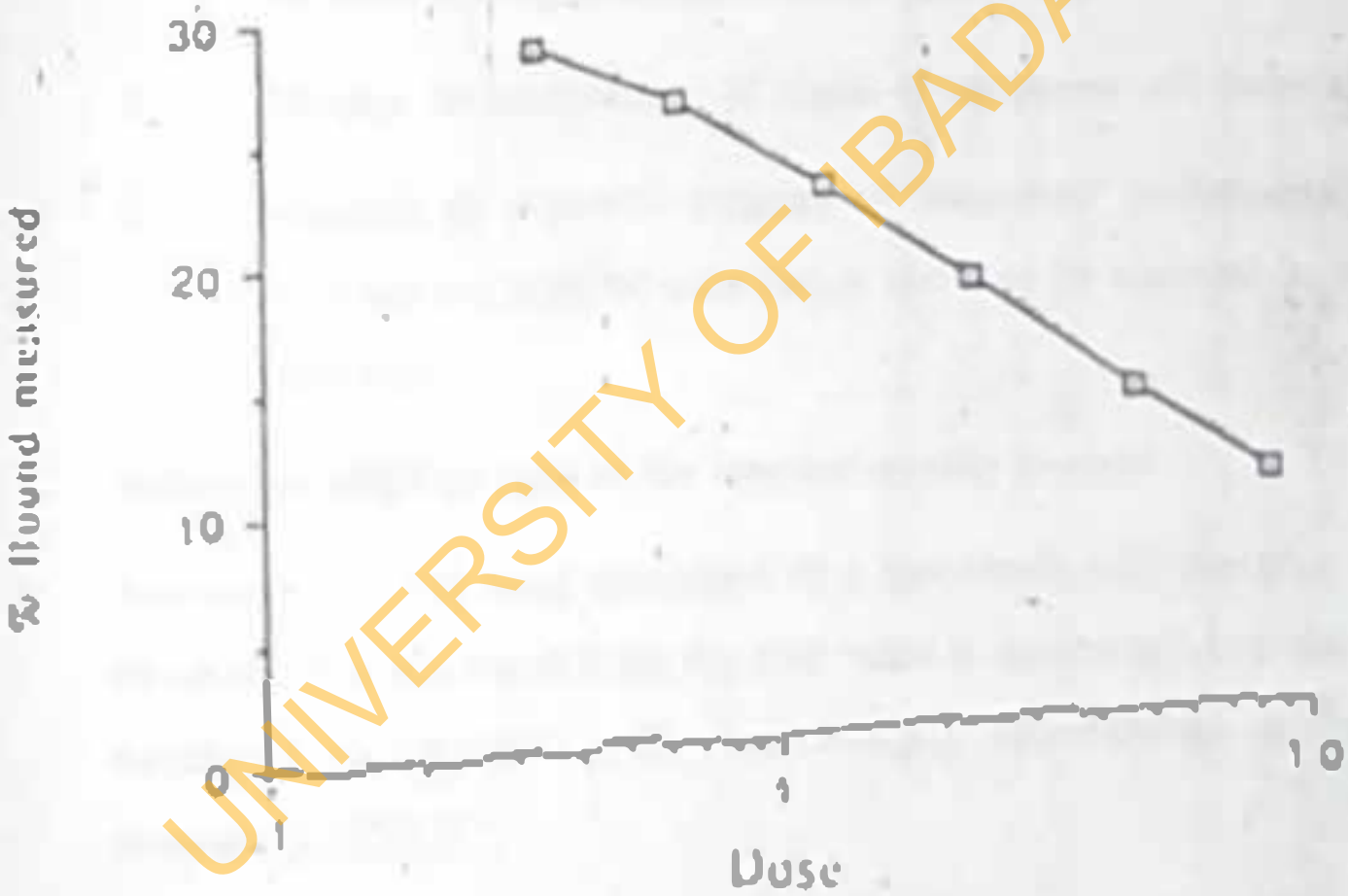
Colour measurement.

The OD of the supernatant was determined within 24hours of completion of the assay, using a secomo sarazyme photometer. OD_{450} greater than 2 were unreliable. OD_{492} was used in quantifying such samples.

Calculation of results

Results were analysed using the WHO immunoassay Data Processing Program. The program plots a dose response curve with the standards. The concentration of the samples determined from the standard curve.

Fig 2.4 Typical Estrone -3- Glucuronide standard curve



2.3.3(c) ASSAY VALIDATION FOR THE HORMONE ASSAYS

The internal quality controls were included in the assay to

1. Detect trends and alterations in results from control sample so that early, effective remedial action can be taken before serious loss of precision occurs
2. Permit valid judgement to be made on the bias of results
3. Provide a continuous record of the precision of assay results.
4. Provide an objective basis for rejection of the measurements of individual unknown samples or the entire assay runs, if the precision and /or accuracy requirements have not been fulfilled.
5. Monitor the performance of pieces of equipment and materials.
6. Maintain an objective measure of laboratory performance over time resulting in a body of information that may be assessed by independent observers.

Definition of terms used in the internal quality control

Accuracy: The exact agreement of a test result with the true value. The deviation of a test result from the true value is inaccuracy and this is what is monitored by Internal quality control(IQC) and external quality control procedures (EQC).

Assigned value: A value established for a control sample. The value may be established by taking the mean of the result from different laboratories (This is the value used in the WHO EQA scheme that was used for this study) or by using the mean results from a number of assay runs. Assigned values are used because it is very difficult to determine the reliability of the true value for

steroid hormones or many peptide hormones. The assigned values are some times called target values or consensus values.

Bias: The deviation of the test result from an assigned value. Bias is usually expressed as a percentage which may be positive or negative.

Replicates: Aliquots from the same sample each is processed as an individual sample.

Precision: Agreement between replicates measurements. It is a measure of reproducibility of the result. Imprecision is most commonly expressed as within -run variation, between run variation and drift.

Within run variation: An index of the imprecision that occurs within every single assay run. Sources of imprecision include pipeting errors and errors on the end- point signal.

Between run variation: An index of imprecision that demonstrates the variability of results from one assay run to another. In other to be able to assess the between run variation the same control samples were assayed in consecutive assay runs.

Drift: An index of imprecision that describes the instability of assay conditions during the assay process. Drift is time related. It is characterised by a clear cut difference between two measurements of a control a sample in one assay run. The control sample was placed in three positions in a series of assay tubes at the beginning, middle and at the end.

Preparations of ordinary control samples

The residues of individual plasma samples were stored frozen at -20°. The samples were thawed when the total volume of the samples amounts to 500ml or more. The samples were mixed together, filtered and dispensed (0.5-

1.0ml) into vials. The vials were sealed and stored frozen as the quality control samples. These QC samples are classified according to the physiological state of the donor i.e non pregnant, non-lactating, or lactating mothers.

In addition to these quality control samples , other control samples with assigned values were obtained from the WHO Collaborating Centre for Immuno assay, Hammersmith Hospital London.

Procedures for quality control , rejection and remedies

The within run variation was measured as a within duplicate variation by assaying the samples in duplicates and measuring the drift in the assay.

Variation within individual unknown samples

Within run variation of individual unknown sample is an index of the precision in measurement of individual samples. A coefficient of variation (CV) for each sample assayed in duplicate was calculated and used as the basis for acceptance or rejection of an assay run. The percentage coefficient of variation (CV) was calculated based on this relationship

$$\%CV = \frac{71.1}{M}$$

where d = is the difference between the two results
M = the mean of the duplicates.

The CV was based on the result of the duplicate and not on the primary data. If the CV of a sample / duplicate exceeds 15%, the mean was considered unreliable and the sample was re assayed.

Variation within individual assay runs

The within run imprecision in individual assay run was assessed by the following parameters

1. *Number of rejected duplicates, and the mean of non rejected duplicates CV's . If the percentage of the rejected sample per assay exceeds 20% and /or the mean CV of the non rejected samples is higher than 7 % the deterioration on precision is apparent and the assay is rejected*

2. *The presence of drift indicates the instability of assay conditions during the assay process. To monitor the drift three sets of internal quality control(IQC) and external control(EQC) samples was included in duplicates at three different points in the assay batch. The difference in the means of the three sets of duplicates was used as a measure of the drift within an assay run. If the mean of the second or third duplicate set of the IQC and EQC samples is larger or smaller by 15% than the mean of the first duplicate, indicates an instability in the assay. The unreliable assay was consequently disregarded.*

Between run variation and control charts:

This is the measure of the reproducibility of results. In order to be able to assess the between run variation the same control samples were assayed in every consecutive assay run. The batches of controls in duplicates were done in each assay run. Each batch of control differs in the concentration of the analytes, representing the low, medium and high concentration patient samples. The reproducibility of the measurements of each control sample was monitored using the following steps

Step 1: Mean values of the measurements of control samples were recorded after the 9th measuremen and the median is found

Step 2: This median is used in the consecutive runs. After each run the results are checked to determine if it falls within the range given by the median (+/- 30% of the median).

Step 3 After at least 20 successful non rejected runs, mean and standard deviation of all results were calculated for each control sample. These two parameters were used to plot a control chart for each control sample. The calculated standard deviation (SD) is used for the construction of warning limits and rejection limits in the control charts.

The warning and rejection limits in the consecutive runs were computed as follow:

$$\text{WARNING LIMITS} = M \pm 2SD$$

$$\text{REJECTION LIMITS} = M \pm 3SD$$

Where M is the mean. The same SD was also used to express the between run variation numerically in the form of the between run coefficient of variation (CV)

$$CV\ (\%) = 100\ SD/M$$

BIAS

The bias in the assay run was also measured during the study. Bias in an assay is measured as a difference from the assigned value. Thus

$$\text{bias}\ (\%) = 100\ \frac{a - A}{A}$$

where A is the assigned value and a is the measured value.

The bias is either negative or positive percentage depending on which value, a or A is larger. A bias chart was plotted with dates of the assay runs on the x axis and the bias on the y-axis.

Rejection. The assay was rejected when the bias was more than +/-20%.

2.4 Criteria For Discontinuation

The study of individual subjects was discontinued before completion if one or more of the following apply.

1. Serious illness or death of the mother or the infant.
2. Illness necessitating the initiation of prescribed drugs with continuation beyond two weeks.
3. Non compliance with study protocol.
4. Mother's personal reasons.
5. Initiation of hormonal contraceptives.

Participation in the study terminated at any of the following end points.

1. The occurrence of two episodes of vaginal bleeding which are perceived to have been normal menstruation.
2. Pregnancy confirmed by biochemical and clinical examination.

CHAPTER 3

RESULTS

Baseline data collected at admission show the following socio-economic pararneters of the mother at admission The age distribution of mothers at admission is presented in Table 3.1 The two groups of mothers who participated in the study were between the ages of 20-34years. Table 3.2 shows the marital status of the mothers at admission. The mothers in the two groups were married and living with their husbands. They were not single parents, separated or widowed mothers

The percentage of mothers who had 6 years of education in both groups (primary education only) was high. This is presented in Table 3.3. Such a low level of education is usually associated with poverty, unemployment and low purchasing power. The same factors may predispose to under nutrition.

TABLE 3.1: Age Distribution Of The Supplemented And Control Mothers At Admission

	% DISTRIBUTION	
Age range (years)	Control subjects	Supplemented Subjects
20-24	33	42
25-29	40	39
30-34	26	19
35-39	1	0

CHAPTER 3

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25-29	40	39
30-34	26	19
35-39	1	0

TABLE 3.2 Marital Status Of The Supplemented And Control Mothers At Admission

	% DISTRIBUTION	
	Control subjects	Supplemented Subjects
Married	100	98
Common Law	0	2
Single	0	0
Separated	0	0
Widowed	0	0

TABLE 3 3: Total No Of Years Of Education Of The Supplemented And Control Mothers

Years of Education	1-6	7-12	≥ 13
	% Distribution	% Distribution	% Distribution
Control	83	17	0
Supplemented	88	11	1

The mothers who live in Sagamu town were classified as urban dwellers. Rural dwellers lived in other towns in Remo land (Ipara-Remo, Isara Remo, Ode-Iemo, Iperu, Ogere). These are smaller towns surrounding Sagamu. About 90% of the mothers that were recruited for the study came from Sagamu (Table 3.4). About 80% of mothers in the two groups fall in the lower socio-economic class (Table 3.4). There was a low incidence of history of any serious illnesses in the two groups of mothers studied (Table 3.5).

TABLE 3.4 The Percentage Distribution Of Socio-Economic Indexes Of The Supplemented And Control Mothers at Admission

	% of women		% Distribution of socio-economic class		
	Urban	Rural	Upper	Middle	Lower
Supplemented	89	11	2	16	82
Control	90	10	0	21	79

TABLE 3 5: History Of Serious Illness/ Breast Or Obstetric/Gynaecology surgery/Mothers With Inverted Nipples Of The Supplemented And Control Mothers

	% of women with serious illness		% of women breast/obstetric/ gynaecology surgery		% of women with inverted nipples	
	Yes	No	Yes	No	Yes	No
Supplemented	0	100	4	96	4	96
Control	3	97	4	96	3	97

A high percentage of mothers in the two groups did not have any history of breast or obstetric / gynaecology surgery (Table 3 5). Less than 5% of mothers in both groups had inverted nipples (Table 3.5). All the women studied had at least one child before enrolment (Table 3 6). Similarly the mothers in the two groups have between 1 and 2 living children (Table 3 6)

Table 3.6 Previous live Birth And living children Of The Supplemented And Control Mothers at Admission

No of Children	1	2	3	≥4	Mean	S.D
Supplemented %	47	33	18	2	1.76	0.84
Control %	42	32	25	1	1.85	0.84

Number of living children

No of Children	1	2	3	≥4	Mean	S.D
Supplemented %	52	34	12	2	1.65	0.79
Control %	41	41	17	1	1.81	0.77

The breastfeeding patterns of the mothers are presented in Tables 3.7 Ninety nine percent of mothers in the supplemented group and 100% of mothers in the control group breastfed their previous children including the last child (Table 3.7)The average duration of exclusive breastfeeding of the last child was 3 months in the two groups (Table 3.7) The mothers in both groups breastfed their children for 10-12 months before weaning (Table 3.7).

TABLE 3.7 Breastfeeding Pattern Of Children Of The Supplemented And Control Mothers That Previously Breastfed

% Distribution of children previously breastfed						
No of children	1	2	3	4	Mean	S.D
Supplemented	49	33	16	2	1.71	0.82
Control	41	41	17	1	1.81	0.77
Duration Of Full Breastfeeding Of Last Child(Exclusive)						
Months	3	3-6	7-9	9-12	>12	Mean	S.D
Subplemented	87	13	0	0	0	1.41	0.96
Control	84	16	0	0	0	1.71	1.32
Duration Of Breastfeeding Of Last Child						
Months	3	3-6	7-9	10-12	>12	Mean	S.D
Supplemented	4	15	15	23	43	12.04	5.43
Control	1	26	15	22	36	10.83	4.91

The mean duration of lactational amenorrhoea while breastfeeding the last child was 8.48 months for the supplemented mothers and 7.33months for the control.(Table 3.8). The alcohol, Tobacco and protein consumption pattern of the mothers enrolled in the study are present in Tables 3.9. Fish is the most often consumed protein, while poultry meat is least consumed amongst the mother enrolled for the study. During follow up the protein consumption pattern was verified by means of questionnaire/interview. The weekly protein consumption pattern during the follow-up are present in tables 3.10 - 3.12.

TABLE 3.8: Duration Of Lactational Amenorrhea Of The Supplemented And Control Mothers While Breastfeeding The Last Child

Months	3	3-6	7-9	10-12	>12	Mean	S.D
supplemented	16	32	13	17	22	8.48	6.01
Control	25	29	13	20	13	7.33	5.68

TABLE 3.9: Alcohol, Tobacco And Protein Consumption Of The Supplemented And Control Lactating Mothers

	% Smoking	% Drinking
Supplemented	2	7
Control	0	10

Weekly Consumption Of Red Meat, Poultry And Fish

	Red Meat	Poultry	Fish
Supplemented	87%	24%	96%
Control	95%	29%	96%

TABLE 3.10: Percent Weekly Consumption Of Poultry Meat Of The Supplemented And Control Mothers

Months Postpartum	Frequency											
	Supplemented						Control					
	0	1	2	3	4	5	6	1	2	3	4	>5
1	90.8	1.5	6.1	1.5	0	0	96.6	100	8.3	1.7	0	3.4
2	89.5	7.0	1.5	0	1.5	0	78.2	12.7	3.6	1.8	0	3.6
3	94.3	5.7	0	0	0	0	88.9	4.4	0	2.2	0	4.4
4	87.8	8.2	0	2.0	2.0	0	81.0	14.3	0	2.4	0	2.4
5	84.6	5.1	0	7.7	2.6	0	85.7	11.4	0	0	0	2.9
6	90.0	14.0	0	2.9	2.9	0	84.4	12.5	0	0	0	3.1
7	83.3	100	3.3	3.3	0	0	76.2	9.5	4.8	0	9.5	0
8	87.5	8.3	0	4.2	0	0	94.7	5.3	0	0	0	0
9	84.2	10.5	5.3	0	0	0	92.9	7.1	0	0	0	0
10	81.3	12.5	0	0	0	6.3	90.9	0	0	0	9.1	0
11	93.3	6.7	0	0	0	0	81.8	18.2	0	0	0	0
12	91.3	7.9	0	0	0	0	100	0	0	0	0	0

The Percentage of women who do not eat poultry meat at all was very high.

TABLE 3.11 Percent Weekly Consumption Of Red Meat Of The Supplemented And Control Mothers

Months Post Partum	Frequency					
	Supplemented			Control		
	0-2	3-5	6-8	0-2	3-5	6-8+
1	24.6	50.8	24.6	15.0	50.0	35
2	21.1	57.9	21.1	16.4	58.2	25.4
3	15.1	61.2	20.8	24.4	51.1	24.5
4	18.4	77.6	24.5	19.0	61.9	19.0
5	17.9	59.0	23.1	25.7	54.3	20.0
6	14.3	68.6	17.1	21.9	59.4	18.8
7	20.0	63.3	16.6	28.6	52.4	19.0
8	25.0	54.2	20.8	38.9	44.4	16.7
9	15.8	63.2	21.1	35.7	42.9	21.4
10	31.3	50.0	18.8	18.2	45.6	18.2
11	40	33.3	26.7	36.4	27.3	36.4
12	46.2	38.5	15.4	42.9	0	57.1

Women consume red meat about 3-5times in a week. On the whole people consume red meat more than poultry meat

TABLE 3.12: Percent Weekly Consumption Of Fish Of The Supplemented And Control Mothers

Months Postpartum	Frequency							
	Experimental				Control			
	0-2	3-5	6-8	>8	0-2	3-5	6-8	>8
1	1.5	24.6	60.0	13.8	5.0	28.3	56.7	10
2	5.3	21.5	66.7	7.0	0	27.3	63.6	9.1
3	0	20.8	67.9	11.3	4.4	28.9	62.2	4.4
4	2.0	35.6	55.1	10.2	4.8	31.0	59.5	4.8
5	0	28.2	61.5	10.3	0	25.7	65.7	8.6
6	2.9	34.3	54.3	8.6	0	31.3	65.6	3.1
7	0	26.7	66.7	6.7	0	42.9	52.4	4.8
8	0	20.8	75.0	4.2	5.3	15.8	68.4	10.5
9	0	31.2	63.2	5.3	0	21.4	64.3	14.3
10	0	18.8	75.0	6.3	0	0	72.7	27.3
11	0	13.3	73.3	13.3	0	18.2	63.6	18.2

Mothers in the two groups consume fish about 6-8 times a week. Mothers in the two study groups consume more fish, followed by red meat and very little poultry meat.

Other patterns of nutritional /dietary intake of the mothers are presented in Tables 3:13,3:14,3:15,3:16. Before supplementation the mean protein intake of all the mothers was 51.1 ±.17.0 gms per day. Daily energy and fat intake was 1940.8 + 429 Kcal/day and 21.9 + 7.0gram/day respectively (Table 3.13). The average intake of 1940 Kcal is below 2,700 Kcal, which is the recommended energy intake for lactating mother. Similarly, the protein of 51gms falls below 65.5gms of the recommended Protein intake for lactating women (RDA 1989). Less than 20% of the mothers in both groups took dietary supplements at any time during the period of lactation, Majority of those who did, did so at the first few months postpartum particularly in the 1st month postpartum(Table 3:14).

The supplements most often taken during lactation is cereal (Corn gruel also called pap). There is the belief in the environment that this causes increase in milk output of lactating mothers. Some mothers consume it with powdered or liquid milk. The distribution of supplement consumption is presented in Table 3.16. The mothers in the two groups have similar energy expenditure (Table 3.15) This is expected since the women live in the same environment, and they engage in similar work.

TABLE 3.13 Dietary Intake Prior To Supplementation Of The Supplemented And Control Mothers

	Protein (g) / Day	Energy (Kcal) / Day	Fat (g) / Day
Mean (Std deviation)	51.1(17.0)	1940.8(429)	21.9(7.0)
Minimum	33.6	1423.6	7.2
Maximum	79.2	2723.0	29.4

Values are expressed as mean ± standard deviation.

TABLE 3.14: Percentage Of The Supplemented And Control Mothers Taking Dietary Supplements.

Months Postpartum	Supplemented	Control
1	16	11.6
2	5.2	5.5
3	1.9	2.2
4	0	2.4
5	0	0
6	0	0

Table3 15 Activity Pattern Of The Supplemented And Control Mothers By Months Postpartum.

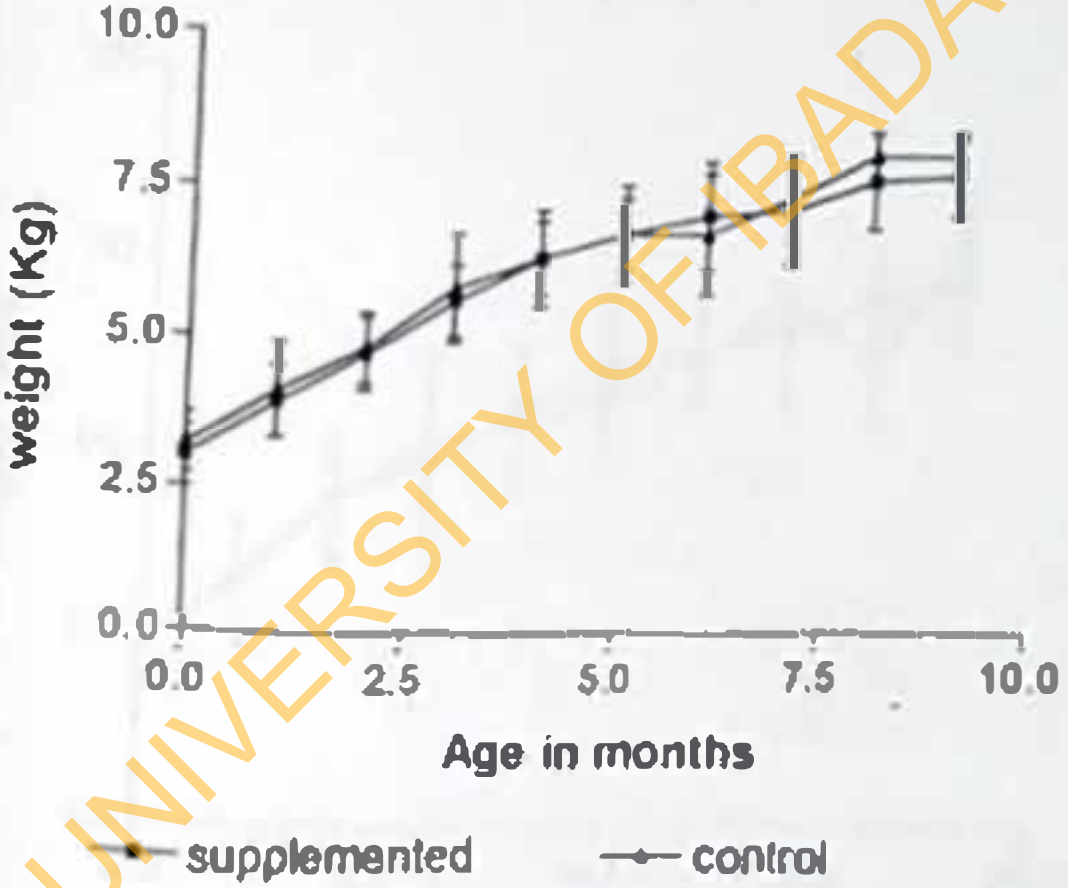
		MONTHS POSTPARTUM		
		0	1	2
ENERGY EXPENDITURE (Kcal)	SUPPLEMENTED	2138	2193	2183
	CONTROL	2137	2158	2218
Students T Test		P<0.05	P<0.05	P<0.05

TABLE 3.16 Percentage Of The Supplemented And Control Mothers Taking The Listed Supplements During Period Of Lactation

Type Of Supplement	Months Postpartum							
	Supplemented				Control			
	1	2	3	>3	1	2	3	>3
MINERALS	0	0	0	0	0	0	0	0
VITAMINS	0	0	0	0	14	0	0	0
CORN SOYA MILK	0	0	0	0	0	0	0	0
POWDERED MILK	44	0	0	0	14	14	14	14
LIQUID MILK	33	0	0	0	40	33	0	0
VEGETABLE OIL	0	0	0	0	14	0	0	0
ANIMAL MILK	0	0	0	0	14	0	0	0
CEREAL	56	33	11	0	57	29	14	0
SUGAR	11	0	0	0	14	0	0	14
YEAST	0	0	0	0	0	0	0	0
ALCOHOL	0	0	0	0	0	0	0	0
FISH	12.5	0	0	0	0	0	0	0
MEAT	12.5	0	0	0	0	0	0	0

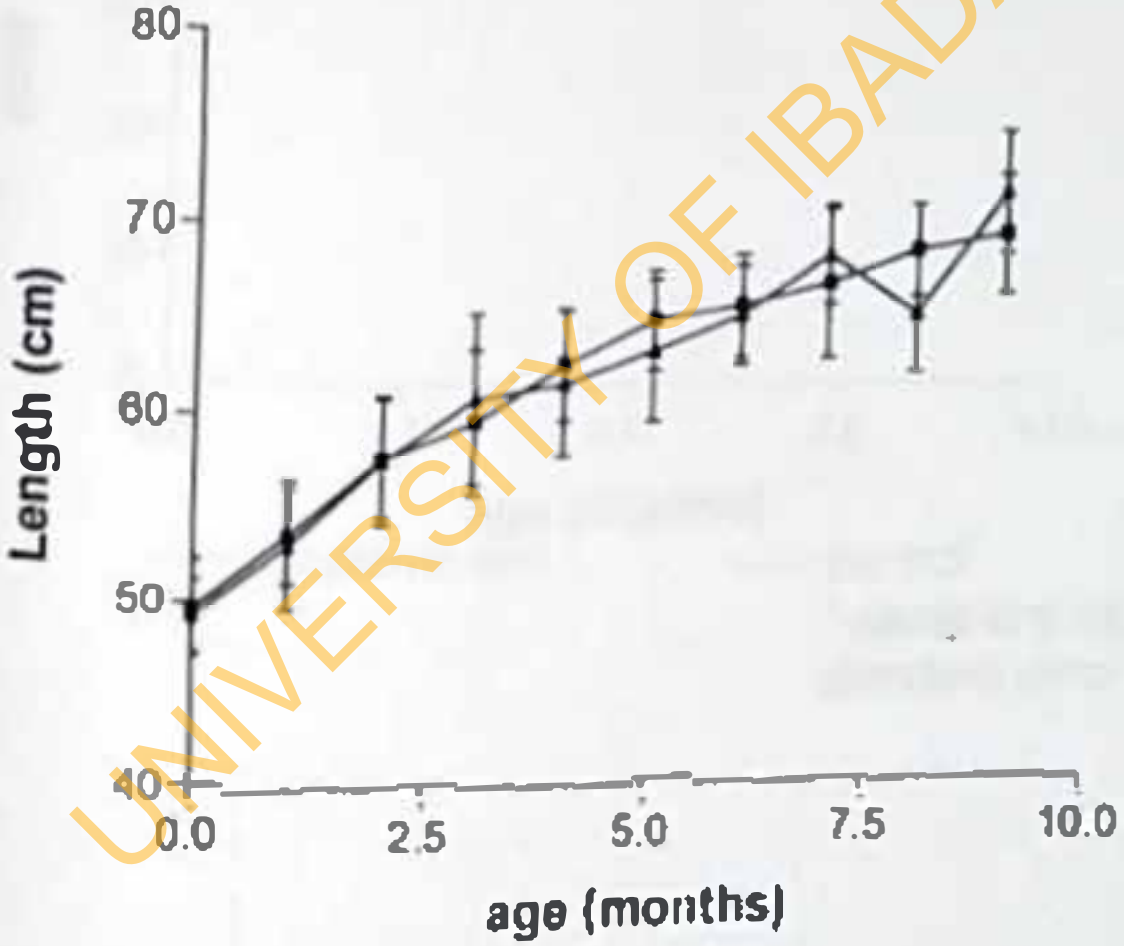
The follow up parameter/ anthropometric measurements of the infants are presented in Table 3:17 and Figure 3:1-3:5. Figure 3 1 show the weight distribution of the infants at different months postpartum. Infants in the two groups had similar weight throughout the 9 months of follow up. There was no significant difference in the weight of the two groups of infants. Figure 3.2 show the length of infants in the two groups. These were similar throughout the follow up period. The differences seen at the 8th and 9th month were not significant. Fig 3.3 show the chest circumference of the infants during 9-month follow up. There was no significant difference in the chest circumference of the two groups of infants throughout the follow up period. Similarly, there were no significant differences in the head circumference of infants of the two groups of mothers (Fig 3.4). The data and the graph of upper mid arm circumference of the infant at the different months postpartum are presented in Table 3:29 and figure 3.5. This parameter was similar in both groups of infants.

Figure 3.1
Mean weights of infants of supplemented and control mothers during the nine months postpartum



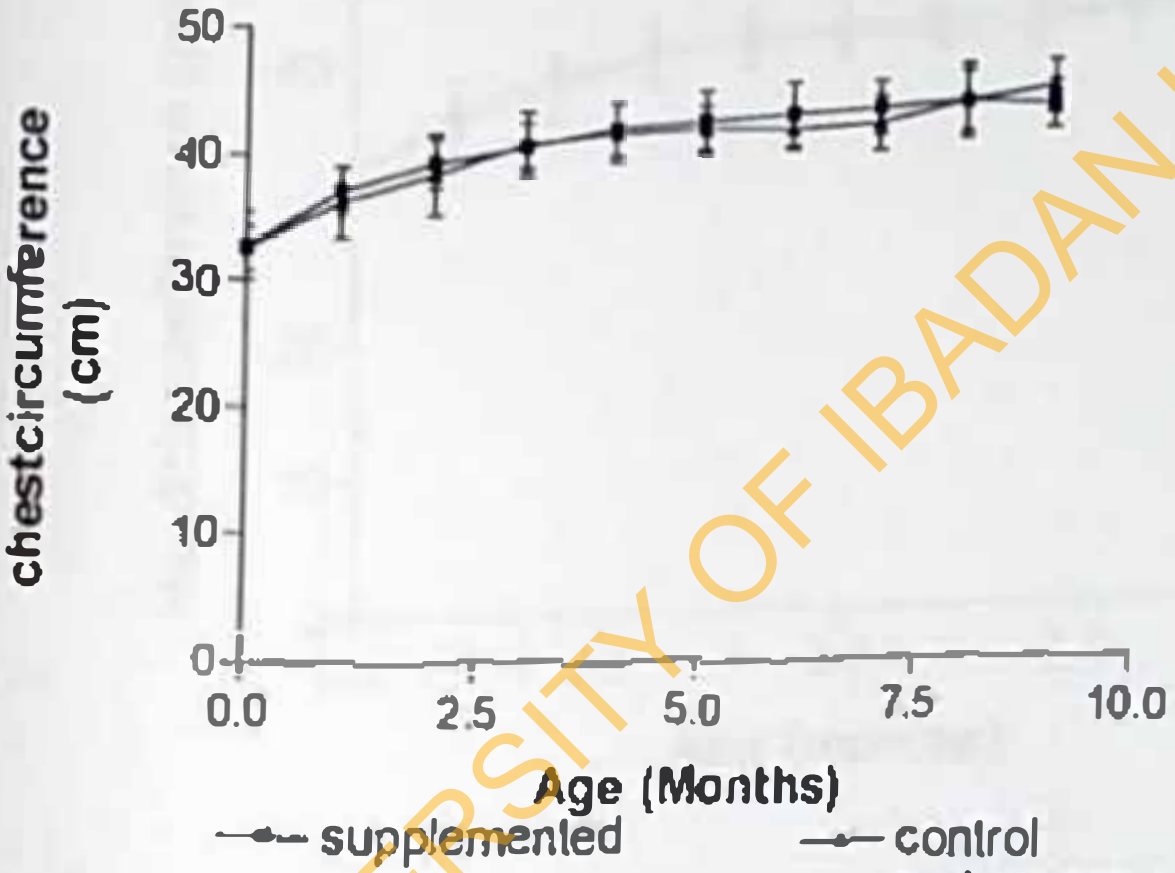
Values are expressed as means and standard error of mean as error bars

Figure 3.2
Mean lengths of infants of supplemented and control mothers and months postpartum



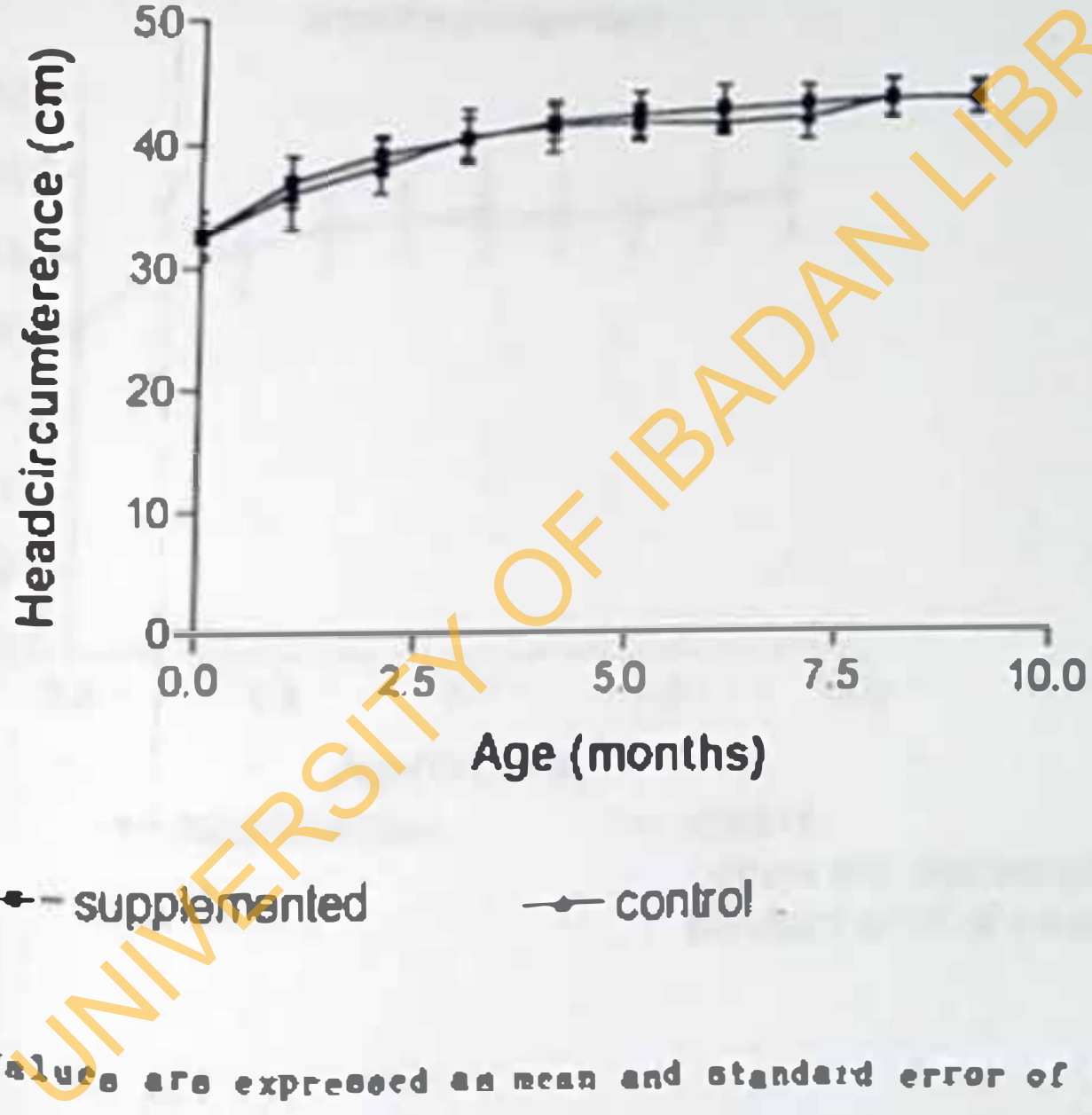
*values are expressed as means and standard error of mean as error bars

Figure 3.3
Mean chest circumference of infants of supplemented and control mother and months postpartum



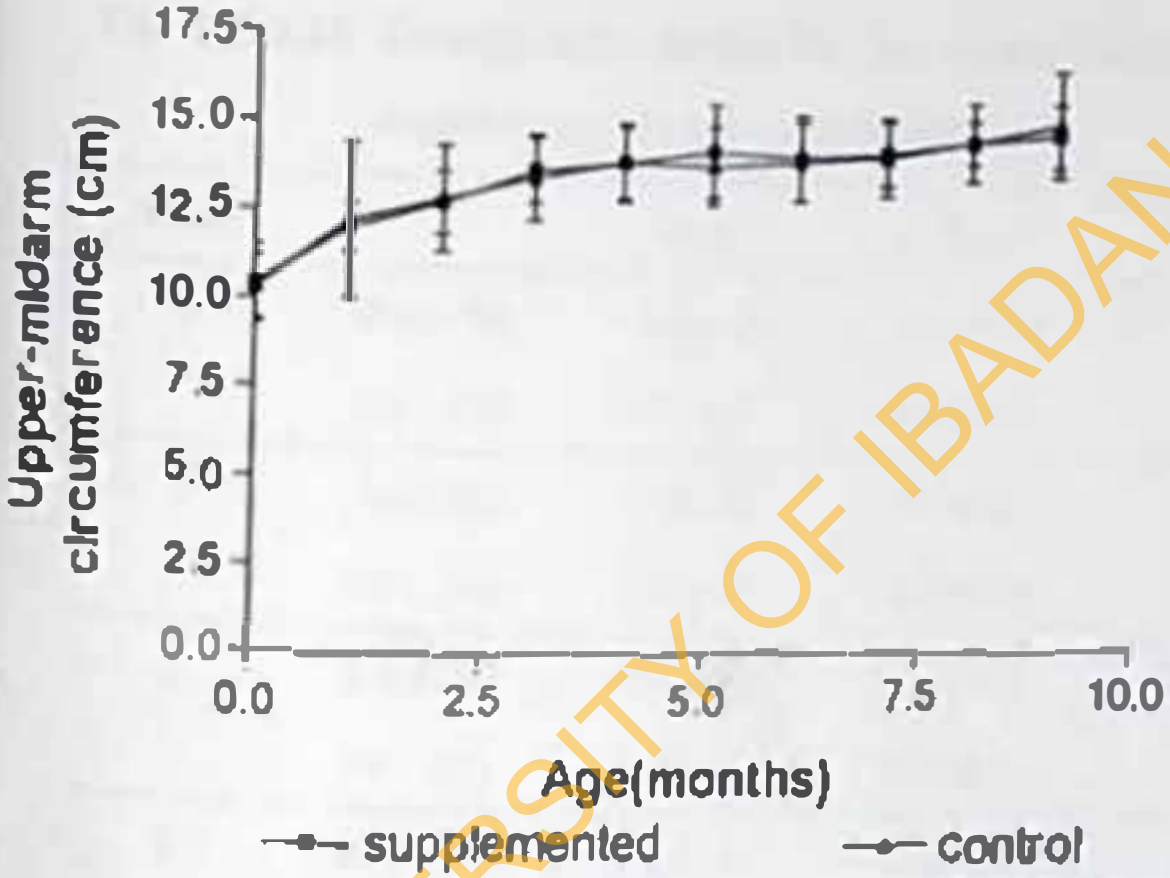
*values are expressed as means and standard error of mean as error bars

Figure 3.4
Mean head circumference of supplemented and control mothers and months postpartum



Values are expressed as mean and standard error of mean as error bars.

Figure 3.5
Mean upper mid arm-circumference of supplemented and control mothers and months postpartum



*values are expressed as means and standard error of mean as error bar

Analyses of comparative anthropometric measurements of the infants are presented in (Tables 3.18 to 3.20) The comparative height for age z score of -2SD nnd +2SD are the cut off values for normal children. Children to the left of -2SD are classified as "short", those to the right of +2SD as "Tall" and those between the two cut-off value as normal. The distribution of height for age measurement shows that the infants have normal heights but they tended more to -2SD, which shows they have marginal stunting.(Table 3.18)

TABLE 3.18 Comparative height for age measurements of infants of the supplemented and control Mothers

Month		HAZ	HAP	HAM
0	Suppl (79)	-0.66(0.1)	33.32(3.0)	95.84(1.3)
	Ctr (75)	-0.46(0.1)	36.91(3.3)	97.94(0.6)
1	Suppl (63)	-0.53(0.2)	35.1(4.3)	97.66(0.8)
	Ctr (54)	-0.53(0.2)	36.04(3.8)	97.69(0.7)
2	Suppl (46)	-0.44(0.2)	38.69(5.0)	98.10(1.0)
	Ctr (37)	-0.66(0.2)	35.61(5.3)	94.56(2.7)
3	Suppl (49)	-0.81(0.2)	32.00(4.4)	96.54(0.81)
	Ctr (35)	-0.89(0.2)	29.33(4.8)	96.23(0.9)
4	Suppl (40)	-0.75(0.3)	36.48(5.4)	96.85(1.1)
	Ctr (30)	-0.87(0.2)	28.13(5.0)	96.10(0.9)
5	Suppl (25)	*-0.49(0.2)	39.05(5.8)	79.99(1.0)
	Ctr (21)	*-1.12(0.2)	21.82(4.3)	95.41(0.6)
6	Suppl (18)	-0.69(0.3)	33.79(5.1)	97.25(0.1)
	Ctr (15)	-0.55(0.2)	35.35(6.9)	97.76(1.0)

SUPPL. Experimental subjects - mothers received dietary supplement
CTR Control subjects - mothers did not receive any dietary supplement
Values are expressed as mean (standard error of mean)
* difference in means are statistically significant

Comparative weight for height z score of -2SD and +2SD are cut off values. Children to the left of -2SD are classified as wasted and +2SD as robust. The distribution of weight for height scores presented in Table 3.19 tended to the side of +2SD except at admission and first month postpartum when the weight for height score was negative. The data show that although the children were marginally wasted at admission, in the subsequent months postpartum they recovered and were normal in both groups during the follow-up period.

TABLE 3:19: Comparative Weight for Height measurements of Infants Of The Supplemented And Control Mothers

Month		# of Subjects	WHZ	WHP	WHM
0	SUPPL	61	-1.03(0.1)	20.24(2.7)	80.33(3.4)
	CTR	52	-1.21(0.1)	19.04(2.9)	85.99(1.6)
1	SUPPL	59	-0.23(0.2)	44.76(4.4)	98.27(2.3)
	CTR	54	0.28(0.2)	41.73(3.4)	96.99(1.8)
2	SUPPL	46	0.33(0.2)	58.39(4.6)	105.81(2.8)
	CTR	39	0.61(0.2)	61.48(4.7)	108.07(3.1)
3	SUPPL	39	*0.69(0.2)	*62.76(3.9)	*109.62(2.9)
	CTR	35	*0.30(0.2)	*54.60(3.9)	*102.46(2.9)
4	SUPPL	40	0.56(0.3)	*56.92(5.2)	108.46(3.6)
	CTR	30	0.75(0.3)	*66.06(5.0)	110.39(3.4)
5	SUPPL	25	*0.08(0.2)	*50.15(6.3)	*101.84(2.7)
	CTR	22	*0.59(0.3)	*64.06(6.2)	*107.85(3.4)
6	SUPPL	18	0.27(0.2)	42.19(6.6)	97.82(2.5)
	CTR	15	0.44(0.3)	39.63(7.8)	95.95(2.6)

SUPPL Experimental subjects - mothers received dietary supplement
CTR Control subjects - mothers did not receive any dietary supplement
Values are expressed as mean (standard error of mean) * difference in means are statistically significant

Comparative weight for age shows the degree of underweight in infants. The cut off point for normal values are –2 sd and +2sd . The mean weight for age z score of the infants through out the follow up period was negative ,but not up to –2sd (Table 3:20). Thus suggesting that the children have normal weight but tended more to the side of underweight than overweight.

TABLE 3 20: Comparative Weight for Age measurements of infants Of The Supplemented And Control Mothers

Month		# of Subjects	WAZ	WAP	WAM
0	SUPPL	79	-0.93(0.1)	23.87(2.4)	86.85(1.4)
	CTR	75	-0.75(0.1)	22.67(2.4)	86.40(1.3)
1	SUPPL	63	-0.44(0.1)	36.52(3.2)	93.01(1.7)
	CTR	54	-0.48(0.1)	34.63(2.9)	92.46(1.5)
2	SUPPL	46	-0.12(0.1)	46.30(3.7)	97.90(1.8)
	CTR	39	-0.06(0.1)	47.75(4.49)	98.89(2.2)
3	SUPPL	49	-0.12(0.1)	*45.02(4.0)	*98.11(2.1)
	CTR	35	-0.48(0.2)	*37.77(4.4)	*92.67(2.5)
4	SUPPL	40	-0.15(0.14)	41.36(4.3)	97.45(2.0)
	CTR	30	-0.16(0.12)	45.30(4.2)	97.64(1.8)
5	SUPPL	25	-0.28(0.15)	39.00(5.1)	96.21(2.0)
	CTR	22	-0.32(0.19)	41.28(5.8)	95.69(2.5)
6	SUPPL	18	-0.72(0.20)	29.70(5.5)	91.17(2.5)
	CTR	15	-0.75(0.24)	28.81(7.2)	90.85(3.0)

SUPPL Experimental subjects - mothers received dietary supplement
CTR Control subjects - mothers did not receive any dietary supplement.
Values are expressed as mean (standard error of mean)
* difference in means are statistically significant.

Mean daily breast milk output data for the mothers enrolled in the study are presented in Table 3:21. The daily breastmilk output (g/24hr) was similar in the two groups of mothers except during the first month postpartum in which the difference in the milk output between the two groups of mothers was significant.

TABLE 3:21: Mean Daily Breast Milk Output (g/24hr) Of The Supplemented And Control Mothers By Months Post partum.

Months postpartum	Supplemented	Control
1	680(410)	1120(760)*
2	750(480)	870(620)
3	1110(1500)	820(480)
4	840(530)	960(640)
5	990(560)	1110(670)
6	720(270)	850(570)
7	660(390)	650(540)
8	960(720)	1240(1230)

Values are expressed as mean of breast milk output (g/24hr) and standard deviation. *Significant p value.

The breastfeeding pattern of the mothers including frequency and duration of breastfeeding episodes are presented in Tables 3:22 and 3:23 The practice of wet nursing was not observed among the women studied. Similarly, breast engorgement, blocked ducts and cracked nipples were not common in the study group. The mothers were not in the habit of using paciliers/comforters for their babies, and less than 4% of the infants showed preference for a particular breast. Babies were mostly fed on demand throughout the follow up period.(Table 3:22) however, The practice of breast expression by hand or pump was not common in the study groups (Table 3:23). The percentage of mothers working outside the home increases with time postpartum is shown in Table 3:23 The mothers were able to breastfeed the babies on demand while working because most of them do petty trading inside or near their homes (Table 3:23)

TABLE 3:22: Percentage Distribution Of The Supplemented And Control Mothers Who Expressed Breastmilk by Hand or Pump and Mothers Who Breastfeed On Demand During The Day.

	% of mothers who expressed milk		% of mothers who breastfed on demand during the day	
Months Postpartum	Supplemented	Control	Supplemented	Control
1	0	2	98	98
2	0	0	96	93
3	0	0	89	89
4	2	0	92	88
5	0	0	90	89
6	0	2	86	94
7	0	0	97	91
8	0	0	92	84
9	0	0	100	86
10	-	-	100	83
11	-	-	98	73

TABLE 3:23 Percent Distribution Of The Supplemented And Control Mothers Working Outside the Home and Mothers breastfeeding Babies on Demand While Working.

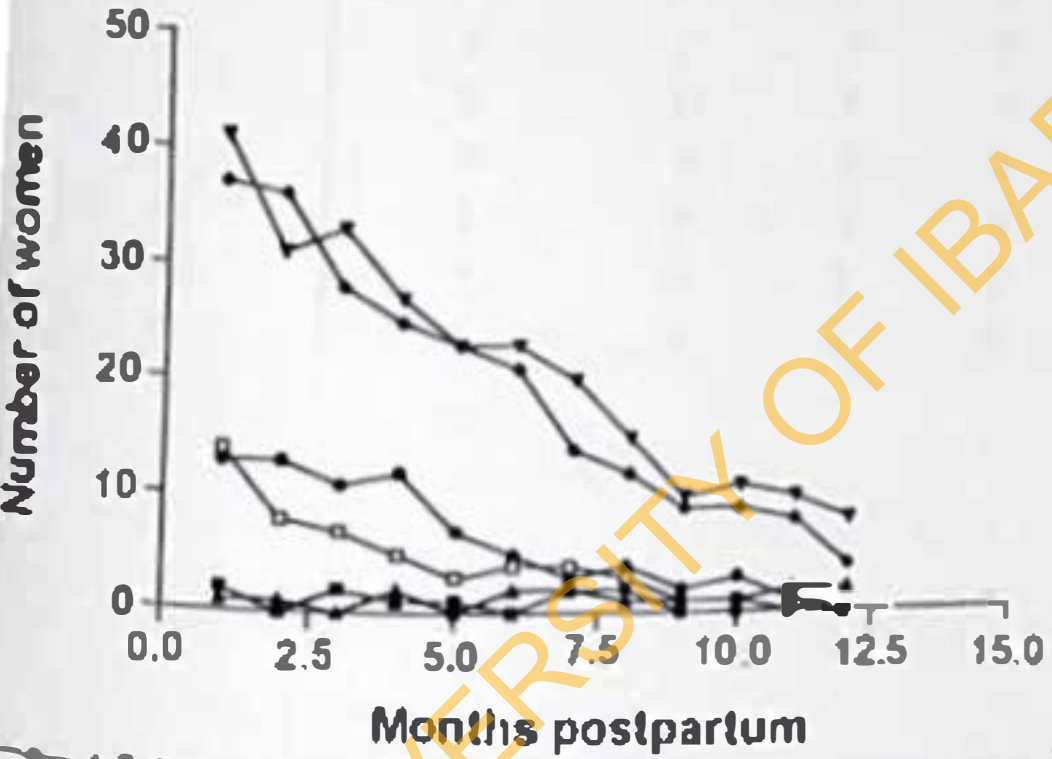
	% of mothers working outside home		% of mothers breastfeeding babies on demand while working	
Months Postpartum	Supplemented	Control	Supplemented	Control
1	2	5	100	67
2	19	16	90	67
3	42	43	83	70
4	53	53	85	70
5	56	62	83	75
6	60	67	75	73
7	55	61	76	69
8	58	63	73	67
9	70	60	71	63
10	75	67	83	63
11	73	50	90	50
12	79	86	90	67

Mothers who breastfed their infants 6-10 times a day were more than those who breastfeed their infant 11-15 times and 1-5times a day. The number of mothers who breastfed between 6-10 times a day and 11-15 times a day decreased with time postpartum(Table 3.24, Figure 3.6). Mothers breastfeeding 5-7 times during the day are more , followed by mothers breastfeeding 8-10 times and 2-4 times (Table 3.25, Figure 3.7). The number of women breastfeeding for a duration of 9-12minutes during the day are more in the two groups of women (Table 3.26, Figure 3.8). Night time breastfeeding was common in the two groups of mothers. Most of the women breastfed 1-4 times in the night (Table 3.27, Figure 3.9).

TABLE 3:24: Frequency of Daily Breastfeeding Episodes Of The Supplemented And Control Mothers

Months postpartum	Frequency					
	Supplemented			Control		
	1-5	6-10	11-15	1-5	6-10	11-15
1	2	41	13	1	37	14
2	0	31	13	1	36	8
3	2	33	11	0	28	7
4	1	27	12	2	25	5
5	1	23	7	0	23	3
6	0	21	5	2	21	4
7	2	20	3	2	14	4
8	2	15	4	1	12	3
9	0	10	2	1	9	1
10	0	11	3	1	9	1
11	0	10	1	2	8	0
12	0	8	0	2	4	0

Figure 3.6
Frequency of daily breastfeeding episodes of supplemented and control mothers and months postpartum



*1-5, 6-10, 11-15 are frequencies

TABLE 3:25: Frequency Of Daytime (6:am - 9.59 pm.) Breastfeeding Episodes Of The Supplemented And Control Mothers

Months Postpartum	Frequency							
	Supplemented				Control			
	2-4	5-7	8-10	11+	2-4	5-7	8-10	11+
1	5	34	19	0	3	34	13	0
2	7	28	10	1	4	30	11	0
3	8	29	10	0	3	29	5	0
4	2	26	12	0	4	23	5	0
5	2	20	9	0	3	20	3	0
6	4	21	3	0	6	18	3	0
7	6	15	5	0	3	13	5	0
8	3	13	4	0	2	11	3	0
9	0	9	3	0	1	11	1	0
10	1	9	1	0	2	9	0	0
11	2	7	2	0	2	9	0	0
12	1	6	1	0	2	4	0	0

Figure 3.7
Frequency of daytime breastfeeding episodes in the supplemented and control mothers and months postpartum

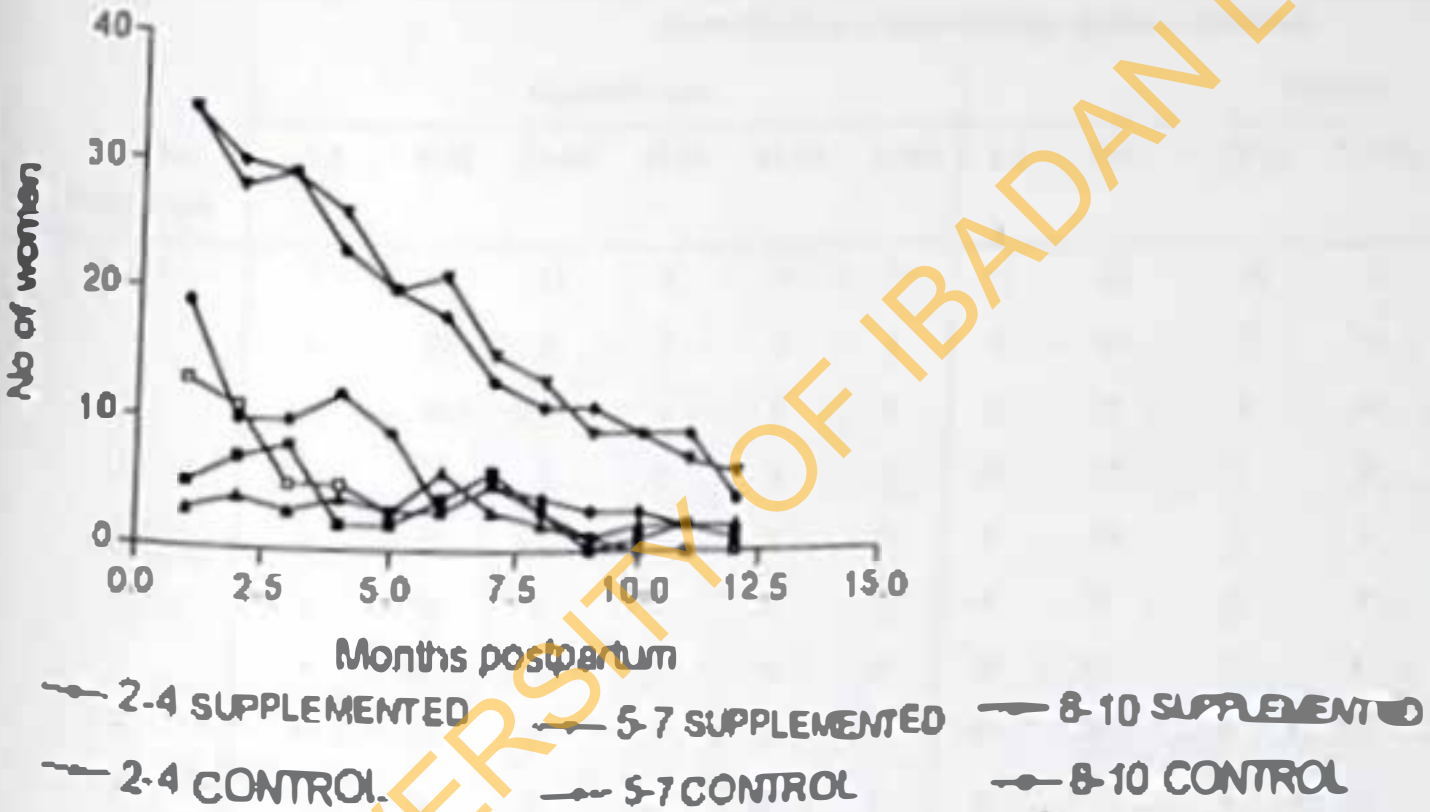


TABLE 3.26: Mean Duration Of Daytime Breastfeeding Episodes Of The Supplemented And Control Mothers And Months Postpartum

Months Postpartum	Mean Duration of Breast feeding Episodes (Minutes)											
	Supplemented						Control					
	4-8	9-12	13-16	17-20	21-24	24-28	4-8	9-12	13-16	17-20	21-24	24-28
1	7	33	12	3	0	1	11	22	13	3	0	1
2	6	27	9	3	0	0	5	29	7	3	0	1
3	5	30	8	3	0	0	2	23	8	3	0	1
4	9	23	5	2	0	0	6	15	7	3	0	1
5	5	18	8	0	0	0	3	14	5	1	0	2
6	4	20	3	1	0	0	4	13	6	0	1	3
7	3	18	4	0	0	0	3	11	4	1	1	1
8	4	15	2	0	0	0	3	10	1	1	0	1
9	2	9	1	0	0	0	2	7	1	0	0	1
10	3	7	3	1	0	0	1	9	0	0	0	1
11	1	7	1	0	0	0	1	6	1	0	0	1
12	0	5	1	0	0	0	2	2	1	0	0	0

Figure 3.8

Mean duration of daytime breastfeeding episodes in the supplemented and control mothers and months postpartum

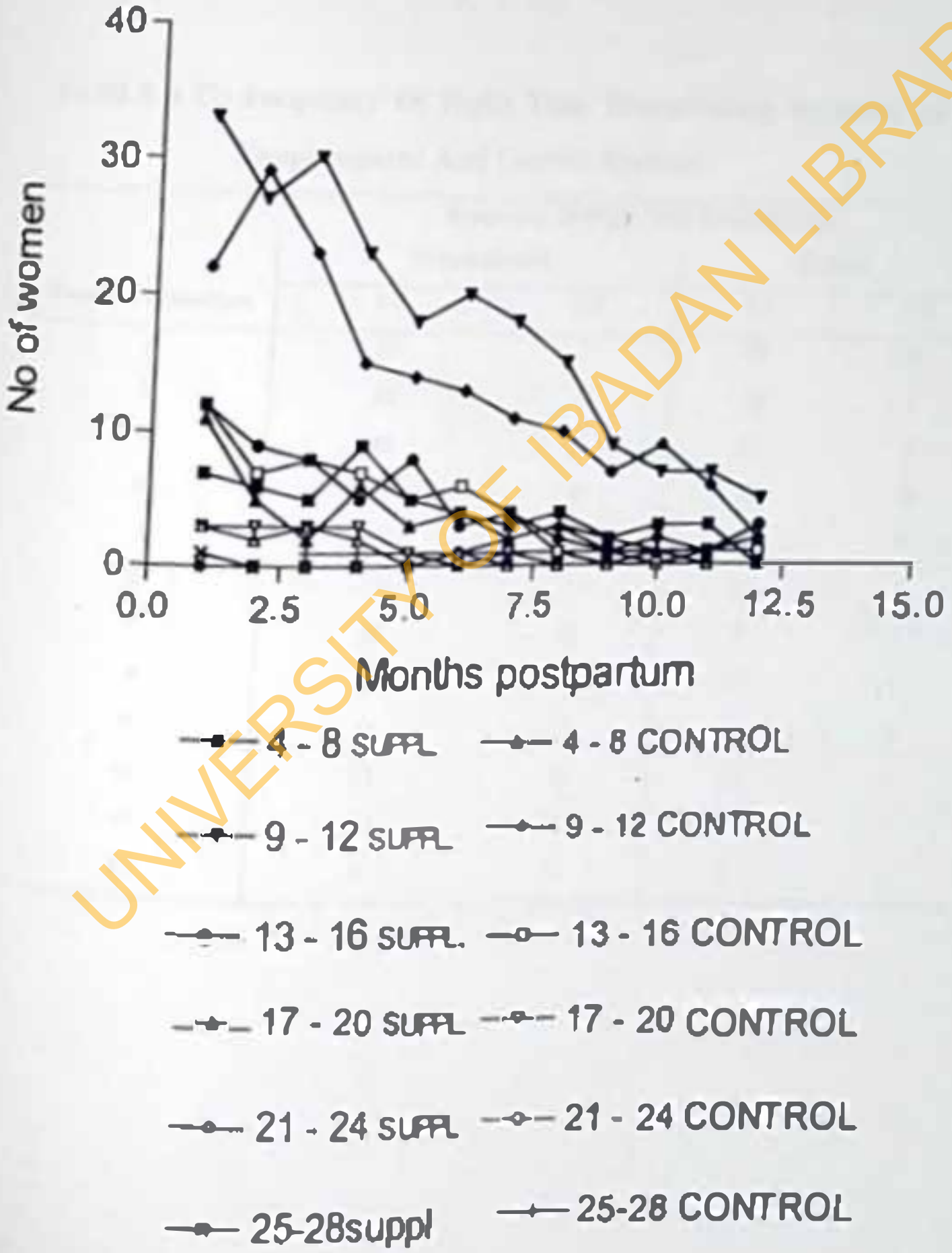
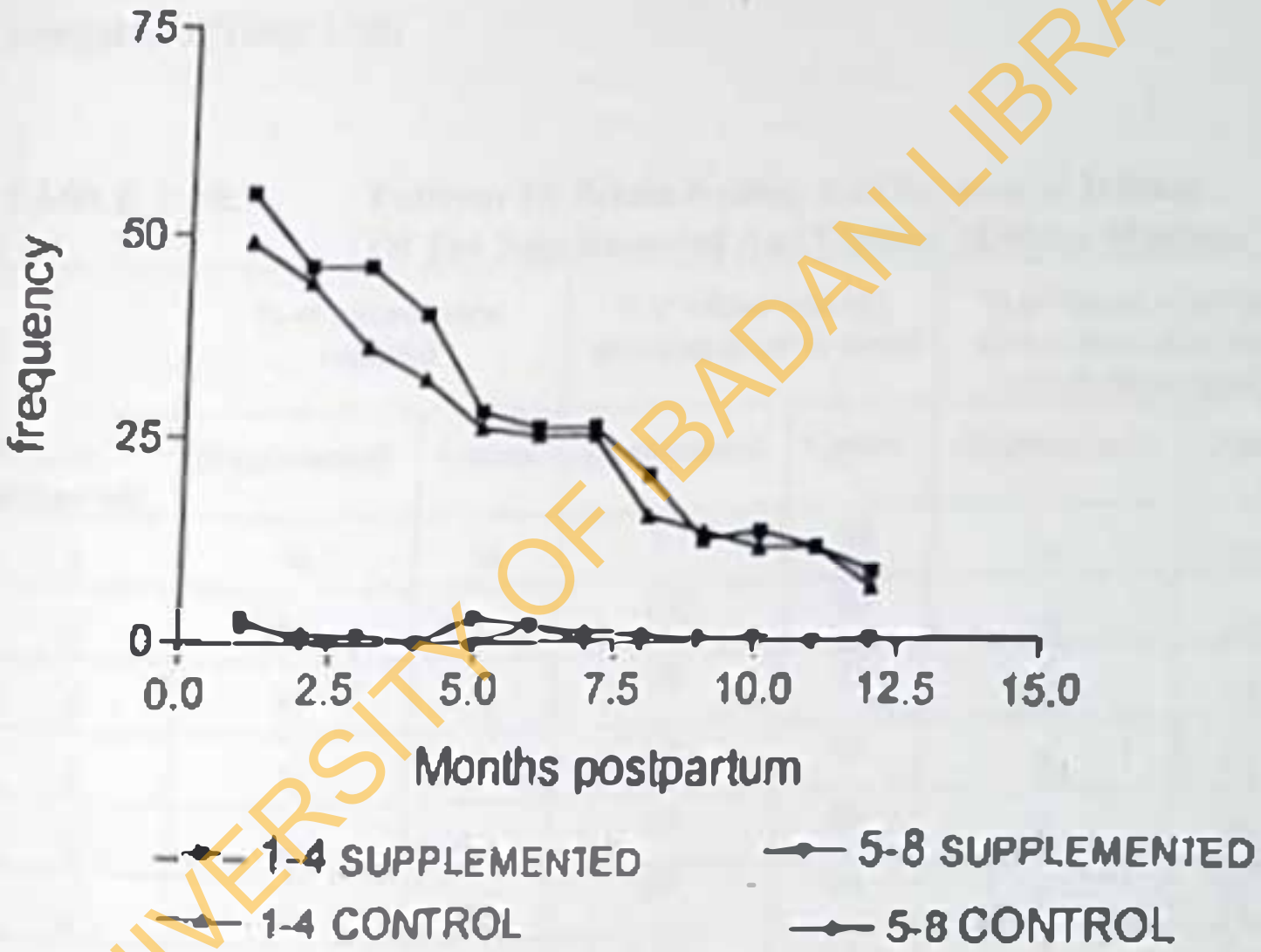


TABLE 3 27: Frequency Of Night Time Breastfeeding Episodes Of The Supplemented And Control Mothers

	Frequency Of Night Time Breastfeeding			
	Supplemented		Control	
Months Postpartum	1-4	5-8	1-4	5-8
1	55	3	49	2
2	46	0	44	1
3	46	1	36	1
4	40	0	32	0
5	28	3	26	0
6	26	2	25	2
7	26	0	2	1
8	20	0	15	1
9	12	0	13	0
10	13	0	11	0
11	11	0	11	0
12	8	0	6	0

Figure 3.9

Frequency of night time breastfeeding episodes in the supplemented and control mothers and months postpartum



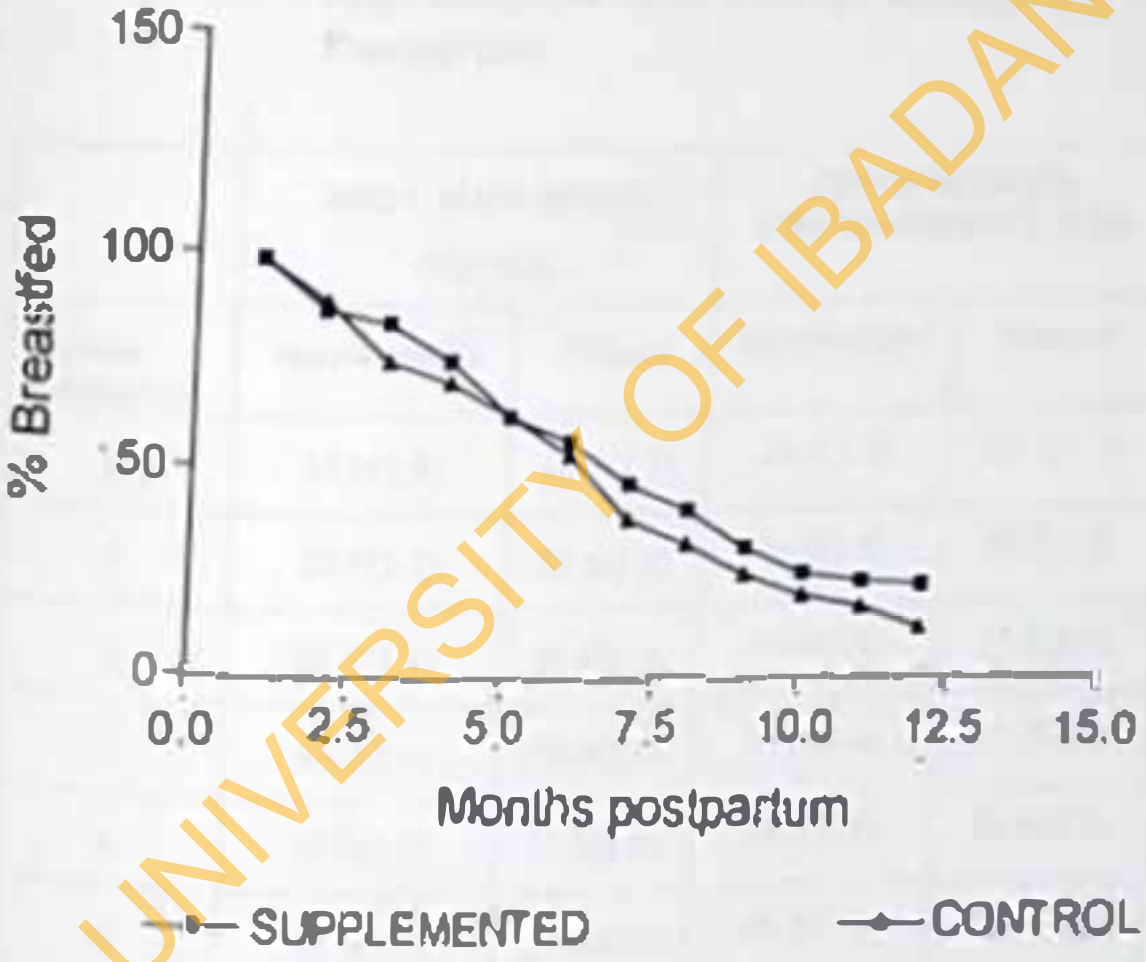
There was a progressive decline in the percentage of infants who received breastmilk with age in both groups (Table 3.28, Figure 3.10). The percentage of babies who had unlimited access to the breast was high, despite the fact that the mothers had started working outside the home by the fourth month postpartum (Table 3.28). Majority of mothers give water with glucose along side with breastmilk. This practice starts as early as the first month postpartum (Table 3.28)

TABLE 3:28: Patterns Of Breast-feeding And Feeding of Infants Of The Supplemented And Control Mothers Mothers

	% of infants being breastfed		% of infants who had unlimited access to breast		% of infants receiving food or fluid other than breastmilk(as taste)	
Months postpartum	Supplemented	Control	Supplemented	Control	Supplemented	Control
1	98	98	97	98	5	14
2	86	88	100	93	4	11
3	83	74	98	89	5	14
4	74	69	94	88	8	15
5	61	61	95	89	7	20
6	55	52	97	94	4	16
7	45	37	100	91	4	18
8	39	31	100	84	7	17
9	30	24	100	86	-	-
10	24	19	100	83	-	-
11	22	16	100	73	-	-
12	21	11	100	71	-	-

Figure 3.10

Percentage of infants being breastfed in the supplemented and control mothers and months postpartum



The body mass indices of the two groups of mothers was constant in the first few months postpartum, until 6 months postpartum when the supplemented mothers were able to maintain a higher index than the control group (Table 3.29, Figure 3.11). The upper mid arm circumference were similar in the two group of mothers (Table 3.29, Figure 3.12). The tricep ,bicep ,abdominal, subscapular, breastskinfold thickness were similar in the two group of mothers (Table 3.30 Table 3.29)

TABLE 3.29 Anthropometric Measurements Of Mothers Of The Supplemented And Control Mothers At Different Months Postpartum

	BODY MASS INDEX (Kg/m2)		UPPER MIDARM CIRCUMFERENCE (CM)		TRICEP (mm)	
Months postpartum	Supplemented	Control	Supplemented	Control	Supplemented	Control
0	20.3(1.9)	20.5(1.5)	24.3(1.7)	24.4(1.7)	7.5(2.3)	7.5(2.6)
1	20.3(2.7)	20.5(2.0)	24.4(1.8)	24.7(2.1)	8.6(2.4)	7.7(2.4)
2	20.7(2.4)	20.8(2.5)	24.4(2.0)	25.2(2.3)	8.9(2.7)	8.5(2.5)
3	20.2(2.0)	20.3(2.0)	24.3(2.2)	24.5(2.1)	8.4(3.1)	8.2(2.0)
4	20.6(2.0)	21.0(2.1)	24.5(2.2)	24.9(2.1)	8.2(2.3)	8.3(2.8)
5	20.9(2.2)	20.5(2.2)	25.9(1.9)	24.7(2.1)	7.9(2.8)	8.5(3.2)
6	21.0(2.5)	20.2(2.4)	24.9(2.3)	24.7(2.1)	7.7(2.3)	7.9(2.4)
7	21.1(2.5)	19.8(1.7)	24.8(2.2)	24.4(3.1)	7.99(2.1)	8.4(3.7)
8	20.7(2.2)	19.4(2.3)	-	-	8.0(3.3)	8.3(3.5)
9	21.0(2.9)	20.4(2.5)	-	-	8.6(3.6	8.4(2.7)

Values are expressed as mean and standard deviation

Significant p values

Figure 3.11
Body mass index of mothers of the supplemented and control mothers at different months postpartum

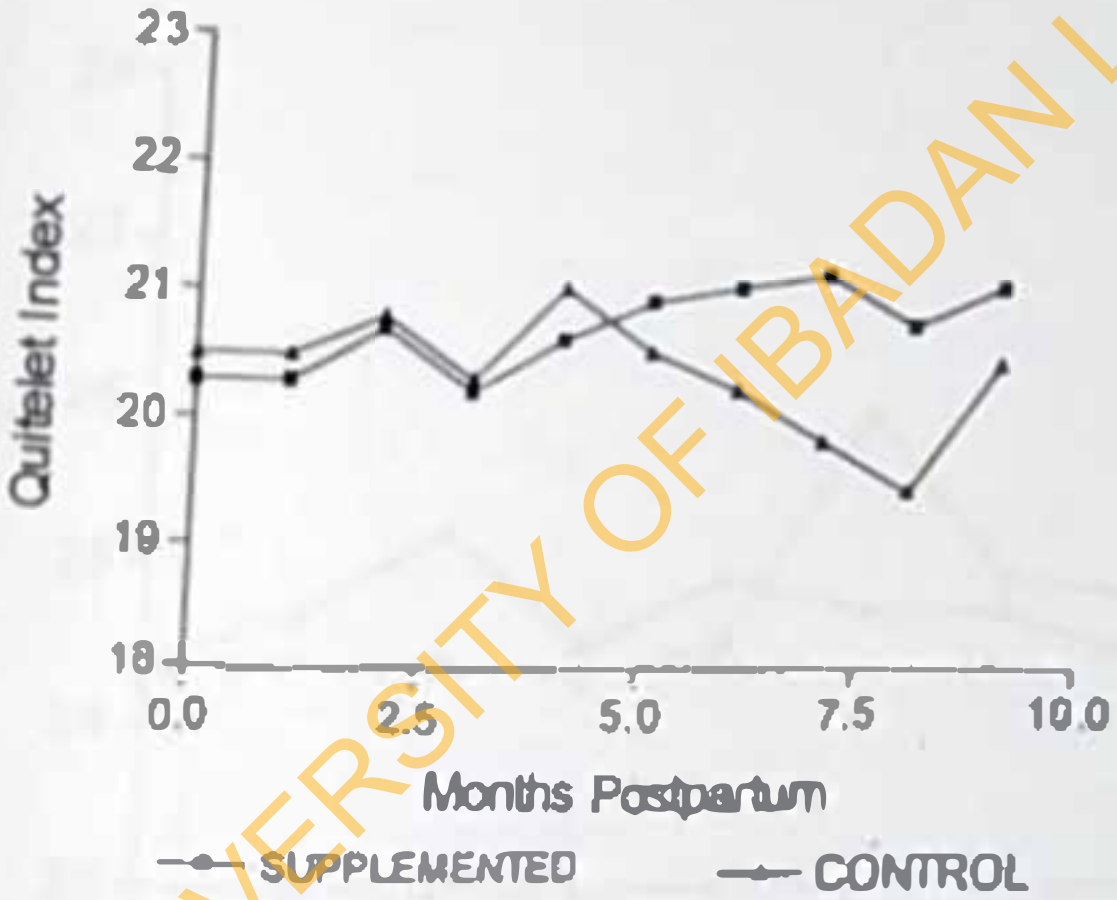


Figure 3.12
Mean uppermid-arm circumference of supplemented and control mothers and months postpartum

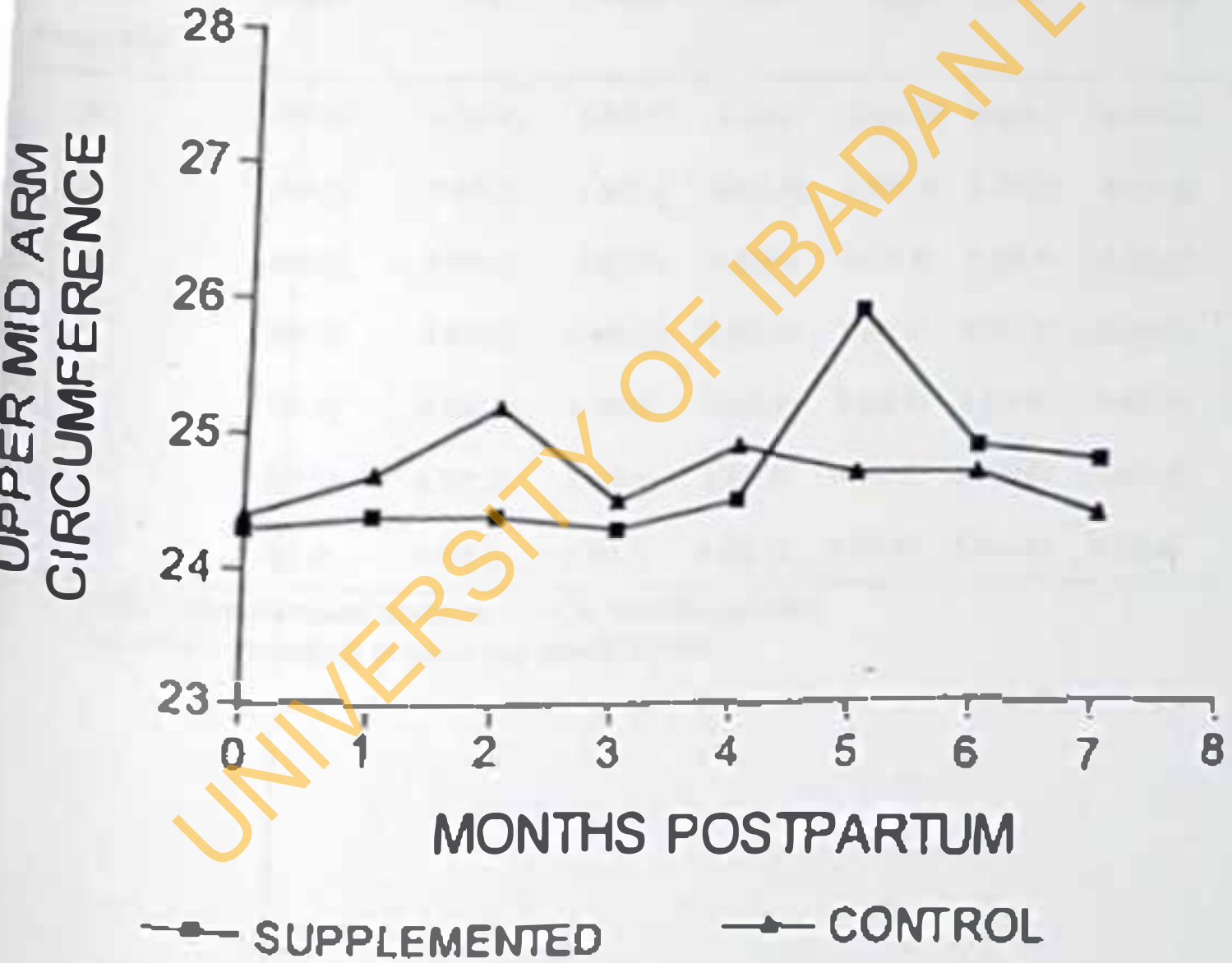


TABLE 3:30: Skinfold Measurement Of The Supplemented And Control Mothers And Months Postpartum

Months Postpartum	Bicep (Mm)		Abdominal (Mm)		Sub-Scapula (Mm)		Breast (Mm)	
	Suppl	Ctr	Suppl	Ctr	Suppl	Ctr	Suppl	Ctr
0	4.7(0.2)	4.6(0.2)	6.8(0.3)	6.1(0.2)	9.5(0.3)	9.6(0.3)	6.5(0.2)	6.9(0.3)
1	4.9(0.2)	5.0(0.2)	6.9(0.4)	6.6(0.3)	9.7(0.3)	9.7(0.3)	6.5(0.3)	6.3(0.2)
2	4.8(0.2)	4.9(0.2)	6.8(0.3)	6.4(0.4)	9.6(0.3)	9.9(0.4)	6.7(0.3)	6.4(0.3)
3	4.9(0.2)	4.8(0.2)	6.4(0.3))	6.0(0.20	9.0(0.3)	9.1(0.3)	6.1(0.3)	5.9(0.2)
4	5.1(0.3)	4.8(0.3)	6.9(0.3)	7.0(0.4)	9.3(0.4)	8.9(0.4)	6.4(0.3)	6.5(0.3)
5	4.8(0.3)	4.3(0.2)	7.0(0.4)	6.4(0.3)	9.0(0.5)	8.5(0.4)	6.1(0.3)	6.2(0.2)
6	5.0(0.3)	4.4(0.3)	6.7(0.3)	6.0(0.3)	9.5(0.5)	8.6(0.4)	6.5(0.3)	6.0(0.3)

Suppl= supplemented mothers Ctr =control mothers
Values are expressed as mean and standard error

TABLE 3. 31 Haemoglobin And Serum Albumin Concentration Of The Supplemented And Control Mothers Months postpartum

	HAEMOGLOBIN (g/100ml)			ALBUMIN (g/100ml)		
Months	Supplemented	Control	Student t test	Supplemented	Control	Student t test
0	10.2(1.9)	10.4(1.5)	P>0.05	3.3(0.9)	3.4(0.8)	P>0.05
1	10.6(3.7)	10.9(2.0)	P>0.05	3.5(0.9)	3.9(0.8)	P<0.05
2	10.9(2.4)	10.8(2.5)	P>0.05	3.7(0.9)	3.8(0.7)	P<0.05
3	10.8(2.0)	10.9(2.0)	P>0.05	4.0(0.6)	4.1(0.8)	P<0.05
4	10.3(2.0)	10.7(2.1)	P>0.05	3.9(0.6)	4.4(0.9)	P<0.05
5	10.6(2.2)	10.3(2.2)	P>0.05	4.1(0.7)	4.0(0.7)	P<0.05
6	10.8(2.5)	10.5(2.4)	P>0.05	3.8(0.8)	4.2(0.7)	P<0.05
7	10.9(1.0)	11.5(1.6)	P>0.05	4.3(0.7)	4.3(0.9)	P<0.05
8	10.9(1.4)	11.0(2.0)	P>0.05	-	-	-

Values are expressed as mean +- standard deviation * significant p-values

No significant relationship exist between the haemoglobin and albumin concentration in the two group of mothers.

The increased concentration of prolactin as a result of lactation is presented in Table 3:32 The prolactin concentration was higher in the control than in the supplemented subjects (Figure 3:13) Suckling causes an increase in prolactin concentration (Table 3:3 2, Table 3:33) There was no significant difference between the suckling induced concentration in the two groups of mothers However the effect of this suckling induced prolactin concentration decreases with time postpartum (Figure 3:14) The duration of lactational amenorhoea was similar in the two groups of mothers (Table 3:34).

TABLE 3:32 Basal serum prolactin concentration and suckling induced serum prolactin of the supplemented and control mothers

Months postpartum	Basal serum prolactin (mIU/L)		Suckling induced serum prolactin (mIU/L)	
	Supplemented	Control	Supplemented	Control
0	1967(318)	2099(360)	3070(350)	3201(441)
1	1970(255)	2275(357)	2976(356)	3952(346)
2	1905(237)	2673(451)	2933(310)	3999(504)
3	1887(330)	2419(502)	3007(353)	3366(535)
4	1583(305)	1814(313)	2385(319)	2862(400)
5	1768(306)	1925(342)	2396(418)	2997(523)
6	1205(217)	1819(606)	2095(282)	2847(749)
7	1392(263)	1243(354)	1941(328)	1886(460)
8	1841(336)	861(366)	2489(432)	1623(421)
9	*1307(285)	*550(281)	1760(413)	845(293)

Values are expressed as mean ± SD

*= significant p value

TABLE 3:33 *Differences between the basal and suckling induced Prolactin concentration of the two group of mothers.*

MONTHS POSTPARTUM	PROLACTIN INCREAMENT (MIU/L) SUPPLEMENTED	CONTROL
0	1103	1102
1	1006	1677
2	1028	1326
3	1126	947
4	802	1048
5	628	1072
6	890	1002
7	549	643
8	648	762
9	451	290

Values are differences in the mean of basal and suckling induced prolactin.

Figure 3.13
Suckling induced prolactin concentration of the supplemented and control mothers and months post partum

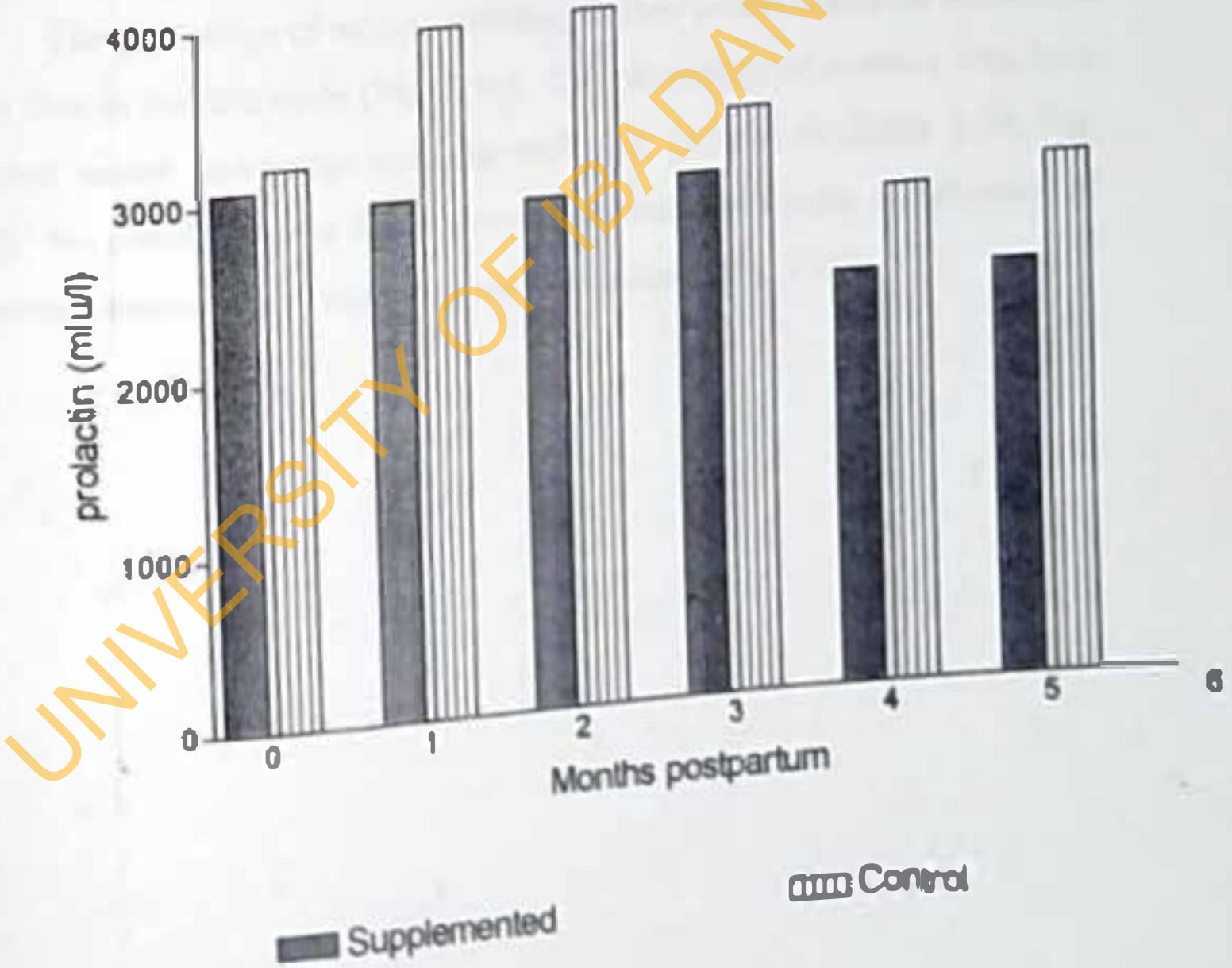


TABLE 3:34 Duration Of Lactational Amenorrhoea In Weeks Of The Supplemented And Control Mothers Mothers.

	NUMBER	MEAN (Std)	MEDIAN	MINIMUM	MAXIMUM
SUPPLEMENTED	36	28.5(19.2)	23	4.0	65
CONTROL	34	24.5(10.7)	25	6	44

• *Significant p values P> 0.05 = Not significant.*

The percentage of women ovulating in their second menstrual cycle were more than in the first cycle (Fig. 3:16). The percentage of mothers who have resumed sexual intercourse increases with time postpartum (Table 3:34, Fig. 3:17). No correlation was found between the body mass index and duration of lactational amenorrhea in the two groups of mothers (Fig 3:18).

Figure 3.14
Differences between the basal and suckling induced prolactin concentration of the supplemented and control mothers and months postpartum

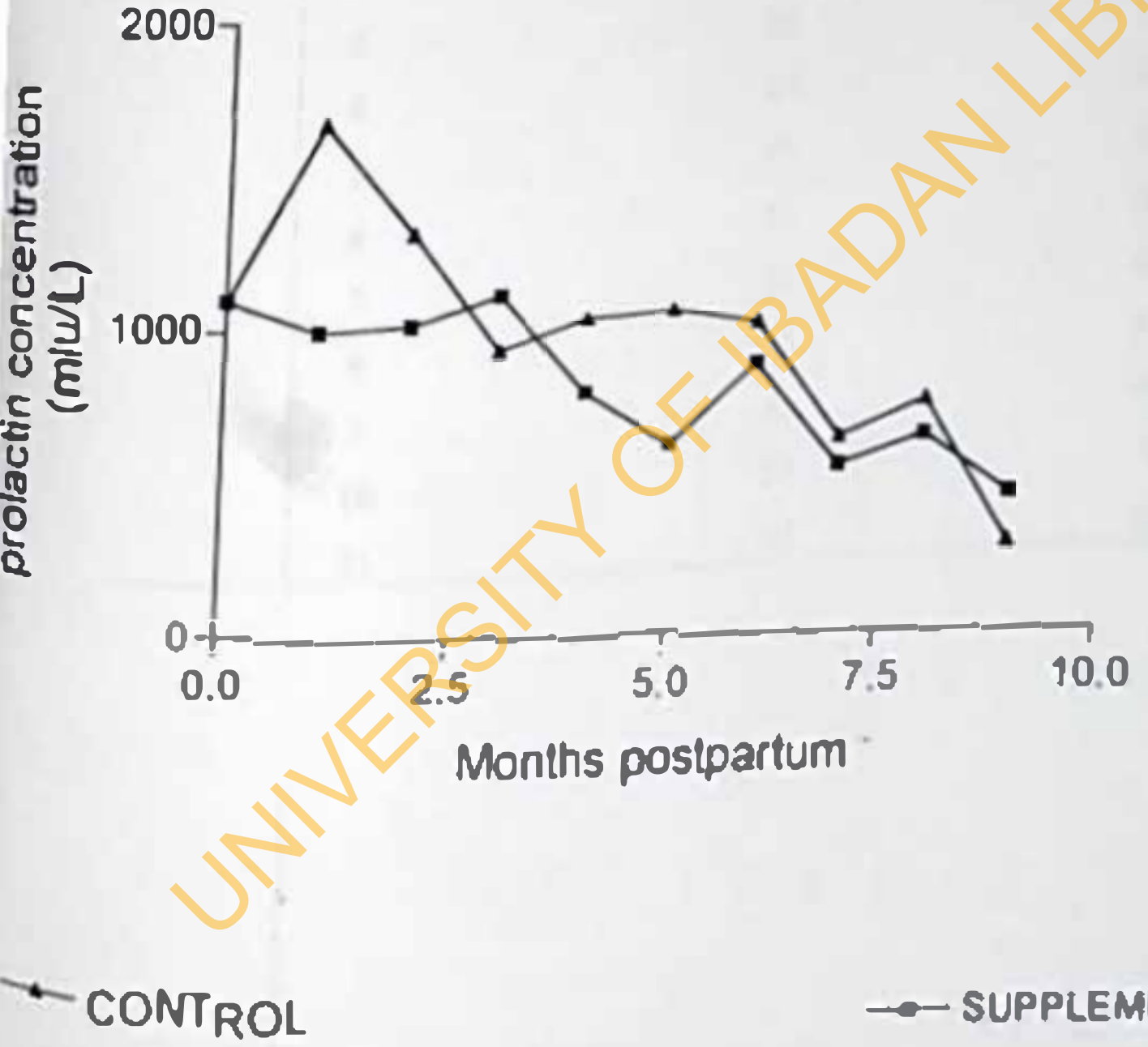


TABLE 3:35 Percentage Of The Supplemented And Control Mothers Mothers Who Have Resumed Sexual Intercourse By Months Postpartum.

Months postpartum	Supplemented	Control
1	0	0
2	14	7
3	18	19
4	20	28
5	27	33
6	31	24
7	23	43
8	35	47
9	40	53
10	31	42
11	33	50

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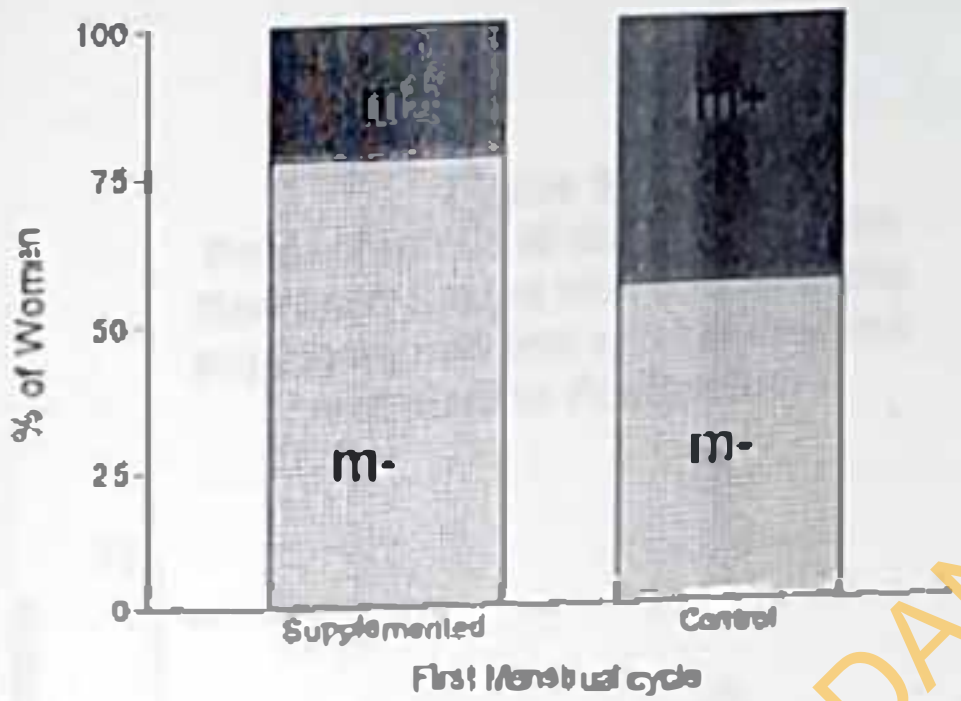
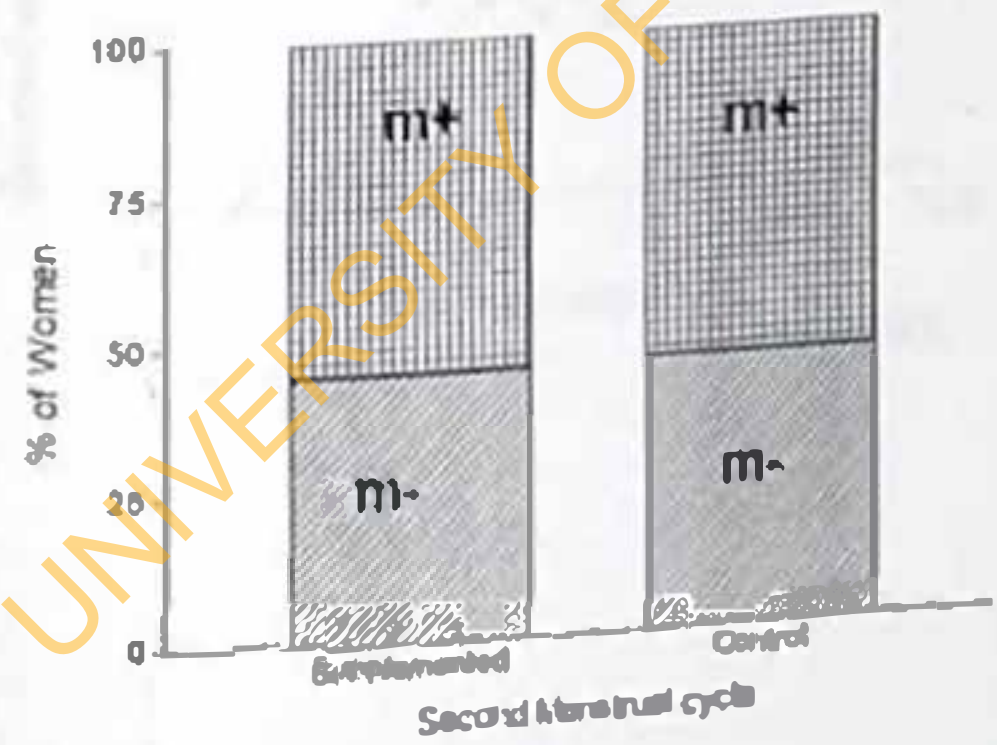
Figure 3.15

Percentage of women having regular or irregular cycles in their first and second menstruation in the supplemented and control mothers.





m = menstruation m- = menstruation but anovulatory
m+ =menstruation and ovulation (pregnanediol > 6umol/l at day m-10 to m

Figure 3.16
Percentage Of Mothers Who Have Resumed Sexual Intercourse In the supplemented and control mothers and Months Postpartum

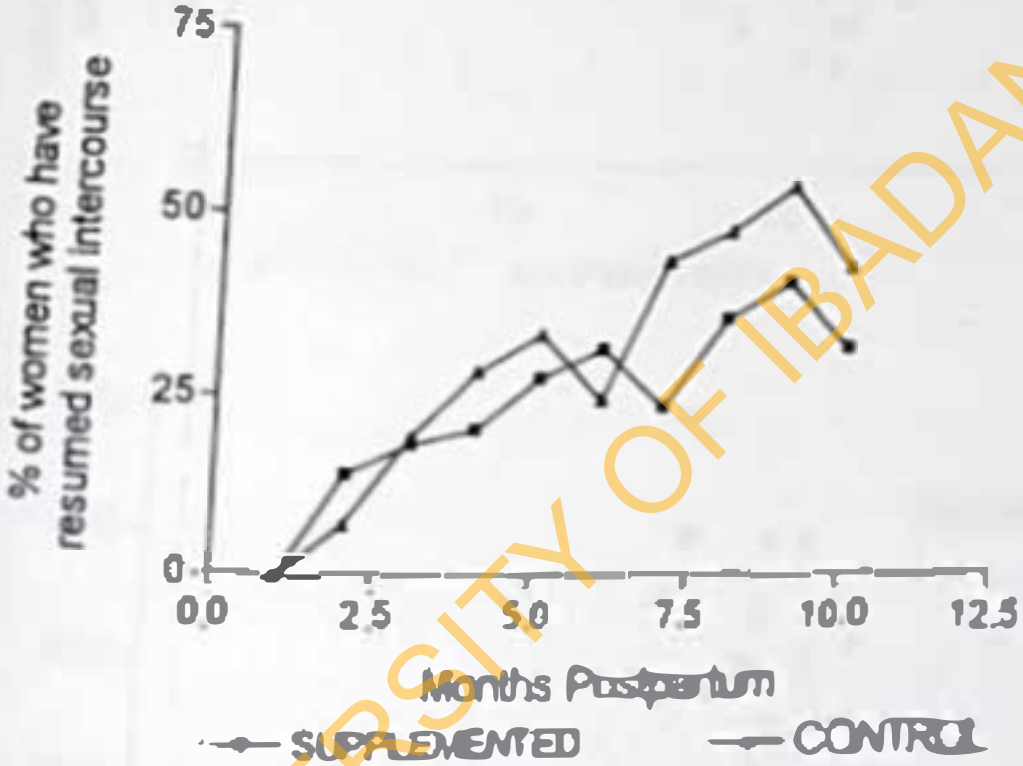
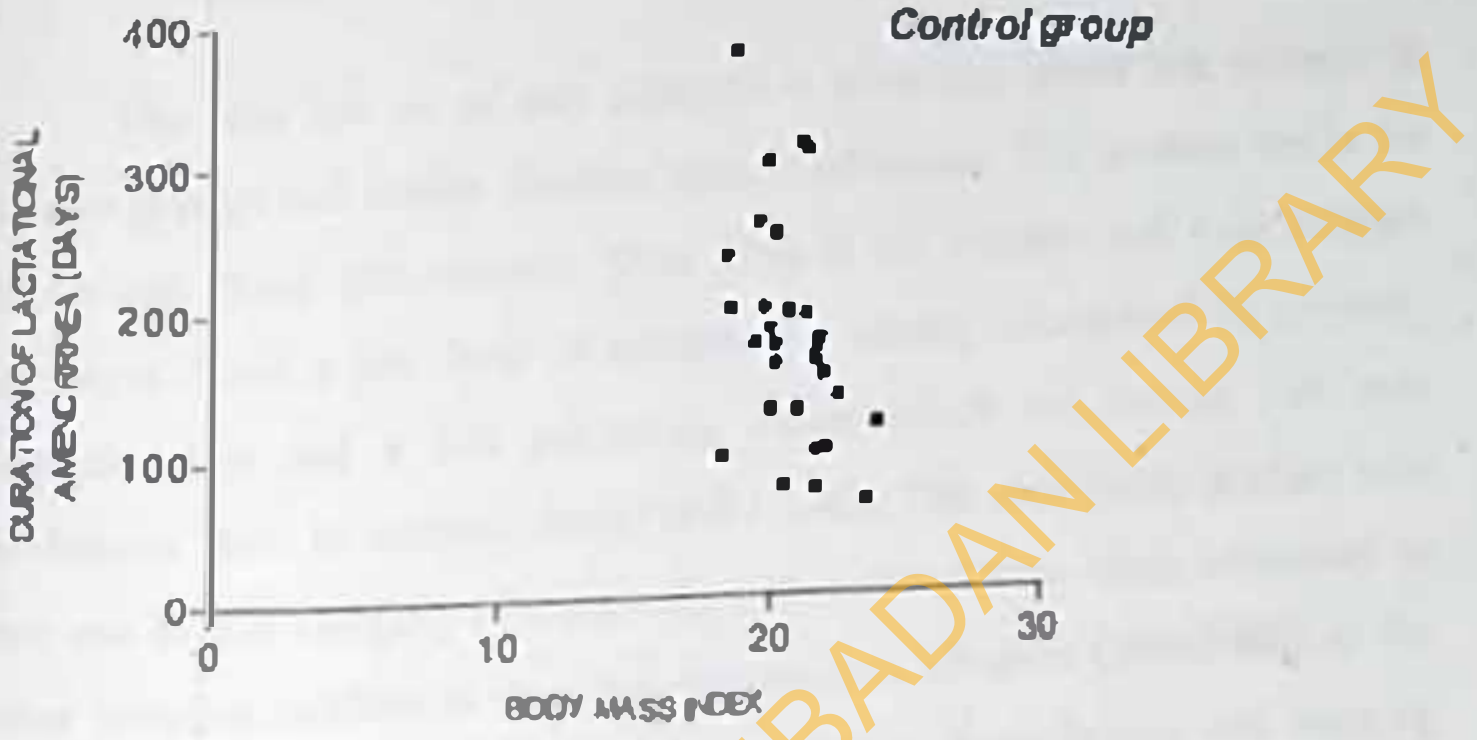
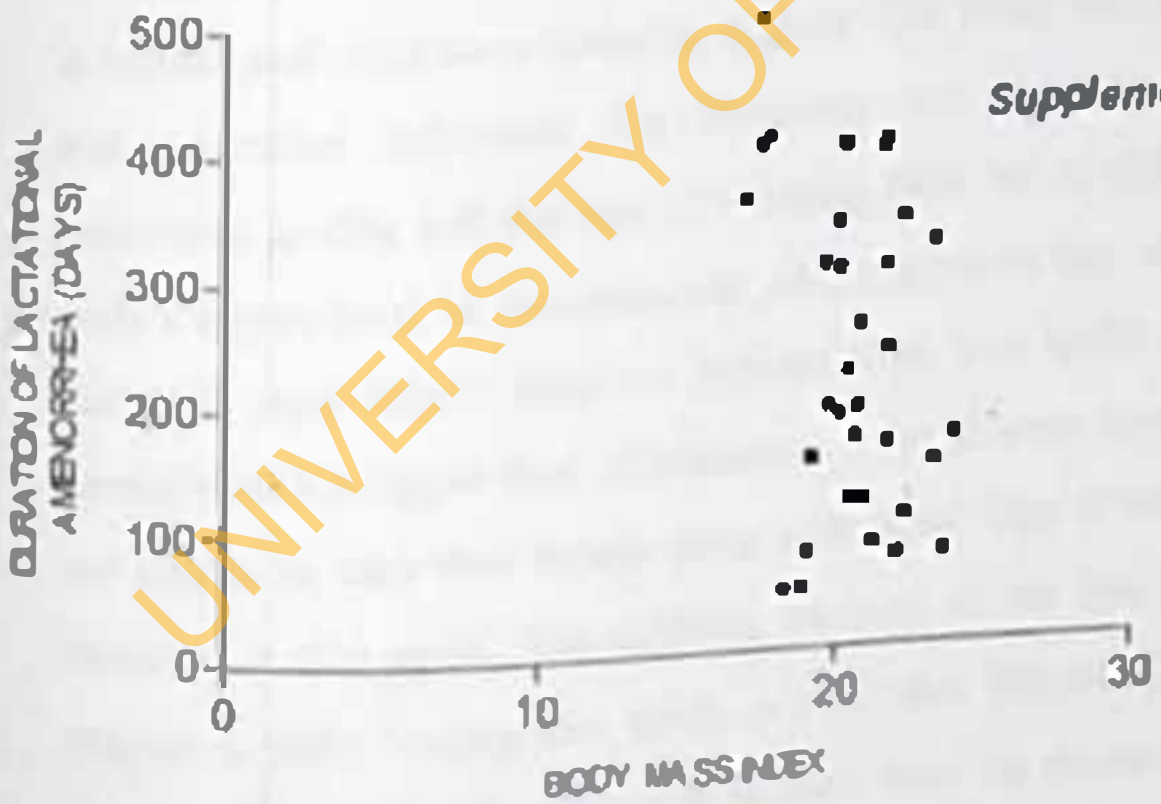


Figure 3.17

Correlates of lactational amenorrhea and body mass index in the supplemented and control mothers and months postpartum





CHAPTER 4

DISCUSSION

The base line set of data collected at admission shows that mothers in the two groups had similar characteristics at admission. The mothers are in the middle age group (20-34years). Over 80% of the mothers had only primary education. Such a low level of education is usually occasioned by poverty, unemployment and a low purchasing power which are factors that may predispose them to undernutrition (WHO 1982). This may partly explain why they are in this category of marginally malnourished state when compared to other lactating mothers in the same environment. Igbodiob (1994) studied the influence of mother's occupation and education on breastfeeding and weaning in infants and children in Makurdi, Nigeria. The result showed that occupation and education influenced the frequency and duration of breastfeeding, nutritional quality and the type of weaning food fed to their infants. Mothers with a higher level of education can afford more to buy commercial weaning diets for their infants than the mothers with low levels of education. The mothers with a higher level of education do paid work outside the homes and are unable to take their babies along with them. This is not the case with the mothers in this study. The mothers, because of the low level of education, engage in petty trading jobs inside or near their homes. The babies stay with their mothers and they were able to feed them on demand. This may be the reason why the two groups of mothers have similar breastfeeding episodes per day. Ever Hadani et al (1994) also found that long term breastfeeding (3months or more) was strongly affected ($P<0.001$) by maternal educational level,

compared with women with the fewest number of years of schooling more likely to breast feed.

The mothers in the two groups had three to four living children that were breastfed. Eighty five percent of these mothers exclusively breastfed their children for 3 months only. This figure is similar to those obtained during the global decline in breastfeeding in the last two decades. (WHO 1985). This decline was more evident when in 1921 Jundell presented evidence that infants would accept, tolerate and benefit from supplements introduced from the age of 6 months. With time it became common for the health professionals to recommend supplements from the age of three months with the purpose of adapting the child "in good time" to new taste and texture experiences (WHO 1984) In 1990 a communique was issued because of the growing concern about this practice to the effect that,

> *"Given a favourable environment, the appropriate time for the introduction of supplementary foods was 6 months postpartum and that infants should exclusively be breast fed for 6 months"* (Innocenti Declaration 1990)

At the time of this study most mothers in the environment were not aware of the Innocenti Declaration. Efforts being made by the Baby Friendly Hospital Initiative to educate mothers on the same was not in operation at the time of this study. This may partly explain why the duration of exclusive breastfeeding before the index child was 3months.

Ever Hadani et al. (1994) found parity also to be strongly associated with the duration of breast feeding. Primipara and grandmultipara (parity>4), breastfed their babies for longer periods in Jerusalem. None of the mothers in this study was a printipara and majority of the mothers had 2-4 living children. The total duration of breastfeeding of their last children was approximately 12 months for the supplemented and 10.8 months for the control subjects before they had the baby which was used for this project. The duration of lactational amenorrhea while breastfeeding the last child before the index child was 8.5 months for the supplemented mothers and 7.3 months for the control. This finding is similar to the duration of lactational amenorrhoea of 7.1 months for the supplemented mothers and 6.1 months for the control with the index children. This is also similar to the WHO findings of 1981 in which 70-80% of mothers were found menstruating at 6 months postpartum in Sweden, and the A group of Nigeria lactating mothers (Urban economically advantaged group) and India mothers. However, this values are different from the duration of lactational amenorrhoea found in the C groups(Urban poor) and Rural groups of Ethiopia, Nigeria, India, and Zaire in which the proportion of menstruating mothers at 12 months was not higher than 40%.

Maternal factors like maternal age (<20 years) maternal marital status (single) and social status of the main family provider are some of the factors that have been shown to affect the duration of breastfeeding. (Vega 1993). The likelihood that an infant would have been breastfed for less than a month was 0.84 if the infant was exposed to all the three of these risk factors. In Vega's 1993 study the mothers were not single and most of them were well over 20 years. In this study the duration of breast-feeding of their last child was 12 months for the supplemented and 10.8 months for the control. In a collaborative study in Nigeria on patterns of breastfeeding, when women were asked on what

the total duration of breastfeeding of a child should be, 50% of the economically advantaged and educated mothers said the infants should be breastfed for 9-11 months and 15% said infants should be breastfed for 3-5 months , the remaining mothers (9%) said infants should be breastfed for 12-14 months. This was not the case with the poor economically disadvantaged and rural mothers in wluch 77% of the poor mothers and 93% of the rural mothers felt that the total duration of breastfeeding should be 18 months or more. This also shows a relationship between environmental factors in controlling breastfeeding duration.

The total duration of exclusive breastfeeding (full breastfeeding) of the last child was 0-3 months in most of the mothers , with 87% of the mothers in the supplemented and 84% of mothers in the control group breastfeeding exclusively for 0-3months. These figures are similar to the finding of WHO collaborative study in Nigeria in 1981 in which 83% of the middle income women breastfed exclusively for 3 months.

Percentage of mothers with episodes of any serious illnesses was low through out the follow up period. Similarly 96% of mothers in both groups bad no history of breast or obstetrics and gynaecological surgery. The percentage of mothers with inverted nipples was very low and their breasts were prepared antenatally, so they were able to establish lactation in good time. Smoking and alcoholism were not common in the two groups of mothers. The most often consumed protein product out of fish, meat and poultry meat is fish.

At admission, the babies in the control and experimental groups had similar anthropometric measurements. The mean birth weight of the babies of the supplemented subjects at admission was 3.0Kg while that of the control was 3.2Kg. The length of the infants was 49.7cm and 49.3 for the supplemented and the control subjects respectively. None of the babies was small for

gestational age. There were no significant differences in the length, head circumference, chest circumference and the mid arm circumference values of the supplemented and control subjects at birth ($p>0.05$). Similarly, no significant differences were observed between the anthropometric measurement of the two groups of babies throughout the follow up period. Except for the height for age z score at 5 months which was significant. By the following month, this difference was not significant.

Significant differences seen in comparative weight for height measurements of infants at 3,4 and fifth months and comparative weight for age measurements at the 3rd month were not consistent after these months. This difference can not be attributed to the dietary supplement given to the mothers in the experimental group. In general, the mean birth weights of the infants in this work i.e 3.0 kg were above the weight below which infants are classified as "Low birth weight," according to the World Health Organisation definition that defines "Low birth weight" as those neonates weighing 2.5 Kilograms or less. It is also above the mean birth weight of 2.88 Kg that Osubor (1992) recorded for northern Nigerian babies in southern Zaria. The growth of babies in the study in the first 6 months is also very similar to the growth of fully breastfed infants in a poor urban Chilean population. These Similar measurements were also recorded for the length and cranial circumference of the babies in the poor chilean population and the two groups studied (Diaz 1995)

The infants can also be considered to have had mild wasting or stunting at birth with a comparative weight for height measurement of 80.3% and 85.9% in the supplemented and the control respectively. This is less than to 90.110 which have been considered to be normal by NCHS standards. This prevalence of stunting (height for age) was also recorded among Nigerian children under the age of 5years in the demographic and health survey of 1990. In the survey

43.1% of all the children had a height for age z score of –2SD.(stunting) 9.1% had weight for height z score of –2SD(wasting) and 35.7% had weight for age z-score of –2SD (underweight). However, the prevalence of this undernutrition increases with the increasing age from 12.4% among infants less than 6 months of age to 55.3% among children 36–48months of age. The high prevalence of undernutrition among 6 - 23 months of children indicate the problem during complementary feeding (NDHS 1990,UNICEF 1993).

Some improvements were observed between anthropometric measurements of the supplemented group of mothers within six months of commencement of supplementation. While the Body mass index rose from 20.3 to 21.0 at the end of the sixth month of supplementation in the supplemented subjects. It actually fell from 20.5 to 20.2 in the control subjects. The increase in the Body mass index of the experimental subjects was however, not significant ($p > 0.05$). The mid arm circumference increased from 24.3 to 24.9 cm in the experimental subjects while it rose from 24.3 to 24.7 in the control subjects (Table36)). These differences were however not found to be significant ($P>0.05$).

The serum haemoglobin value increased from 10.2g / dl to 10.8g / dl in the supplemented mothers while it dropped from 11.4g / dl to 10.5g / dl in the control subjects. This increment was not significant but the reduction in the haemoglobin concentration of the control subjects was significant ($p<0.05$). Increases in the serum albumin noted were however not significant. These findings are similar to the results of Kuiz et al (1993) who investigated the effects of maternal nutritional status and maternal energy supplementation on length of postpartum amenorrhoea among Guatemalan women.

Daily activity pattern was similar in the supplemented subjects and controls in the first three months of the study. This shows that the women were

involved in similar activities. No significant differences were observed in the quantity of breast milk produced by the two groups of mothers. This may be explained in part by the fact that the subjects were not severely malnourished and by the observation that the pattern of energy consumption in the two groups was similar. Similarly energy expenditure in the two groups of mothers is not unexpected, since they live within the same environment and belong to similar socio-economic groups. In other words, the ambient temperature to which the mothers were exposed as well as the activities they engaged in were similar in the two groups of subjects, since breast-feeding behaviour might be influenced by work demands (WHO 1981). This is not expected to happen in the two groups of mothers.

The general growth of infants of this marginally malnourished mothers was however similar when compared with the overall growth of the infants of well nourished mothers in the same environment. (Report of a multicentre longitudinal study of the duration of lactational amenorrhea in relation to breastfeeding practices 1992) and the infants of well nourished mothers in a poor Chilean population (Diaz 1995).

There was no significant difference in the weight and length of babies whose mothers had dietary supplementation and those whose mothers had no dietary supplementation in the first 8-9 months of life. This observation can be explained on the basis of an on earlier reports showing that a reduction in maternal caloric intake has minimal effect on the composition and therefore quality of breast milk (Van-Steenbergen 1983). In another study of infant feeding practices among mothers who had already been identified as marginally malnourished during pregnancy, a sub sample of 80 children was followed for four to eight months. Of those fed on breast milk alone, 76% achieved 90% of the expected weight gain for children of that age (Priyani 1981).

Although some studies have shown diminished lactation in malnourished mothers (Bassir 1958) the precise nutritional intake at which lactation is diminished is unknown. Van -Steenbergen and co-workers showed a reduction of only 8gm/24 hr in the milk production of 46 rural Kenyan women who had low weight for height during the third trimester of pregnancy when compared with their counterparts with good weight for height. Protein and lactose concentrations in milk were comparable with that of British mothers in both groups. Apart from the quantity of milk produced, it has also been shown that the growth of exclusively breast fed children through the first 4-6 months of life in developed and developing countries is adequate (Huffman 1990).

BORDERLINE NUTRITION AND BREASTFEEDING PRACTICES.

Both the babies of supplemented and control mothers had unlimited access to the breast milk of their mothers. Through out the follow up period of 10 months there was no significant difference between the way the supplemented and the control groups fed their babies on demand and how they had access to breast. Most of the babies in the two groups do not use dummy, pacifier or comforter. The mothers did not breast-feed other children (No surrogate mother). The result suggest that feeding on demand was by far the most popular practice. The results of this work also agree with the finding of WHO in 1981 on frequency of breast-feeding.

Similarly, the frequency of breast-feeding episodes during the day (6.00 am-9.59pm) and frequency of night time breast-feeding episodes were similar in the two groups of mothers. Although most of the mothers had started working outside the home by five months postpartum, majority of these women were also able to breast feed their babies on demand while working outside the home. This is possible because the main occupation of women in this type of socio-economic group is petty trading and manual work. Their babies are with them

all the time as opposed to the middle income and highly educated mothers who can not take their children to work because they work in offices. Similar observations were made in a study conducted in Makurdi, Benue State Nigeria (Igbedioh 1994). In that study, it was demonstrated that occupation and education of their mothers influenced the frequency and duration of breastfeeding. A study of Israeli women (Ever-Hadani *et al* (1994) however reported a positive correlation between number of years of schooling and duration of lactation.

Most of the mothers did not express breast milk by hand or pump, occasionally 2% of the supplemented subjects expressed milk at the fourth month and 2% of the control subjects at the first and sixth months. This is not unexpected since the subjects were marginally malnourished and most of their babies had unlimited access to the breast, and were fed on demand.

Within the first month postpartum 2% of the supplemented mothers and 5% of the control mothers had started working outside the home and by the fourth month, 53% of both groups had started working. Majority of these mothers were able to breast feed their babies on demand while working outside the home. More of the supplemented mothers were able to breast feed on demand while working. This data corresponds with what was found in Nigeria, Zaire, Chile, and Ethiopia as being the normal practice (WHO 1981).

The overall prevalence of breastfeeding was high. The percentage of infants being breast fed in the first month was 98% in both control and supplemented groups. By the second month this has fallen to 86% and 88% for supplemented and control groups respectively. But by the seventh month, over half of the mothers were not breast feeding in the two groups and by the end of one year only 21% and 11% in the supplemented and control groups respectively were still breastfeeding. This prevalence is similar to the findings of

an earlier WHO collaborative study on breastfeeding (1981). In Nigeria they found that about 100% of all the four groups of mothers studied were breastfeeding at the time of the interview. By the third month 96% of group A (economically advantaged) were breast feeding and 100% of all the other groups were still breastfeeding. However, by the sixth month 32% of group A, 91% of group B (urban middle income) and 97% of group C (Urban-poor) and 100% of group R (rural) were still breast feeding. By the twelfth month no member of group A, 22% of group B and 97% of group R were still breastfeeding. In this study breastfeeding was only well maintained in the first 4 months of lactation in the groups studied (supplemented and control). This is similar to the trends found in group A and partly in group B. This is not similar to the case in group C and R where breastfeeding was well maintained throughout the first year of the child. (WHO 1981)

The percentage of infants receiving food or fluid other than suckled breast milk as taste(a spoonful) was more prevalent in the control than experimental. The percentages in the first ten months in the supplemented subjects were less than 10% while in the control group this was between 11-20%. Most of the mothers were giving water to their babies along with the breast milk as taste. None of the infants was wet nursed or preferred the left breast. Only 2% of infants of the supplemented mothers and 3% of infants of control mothers showed preference for the right breast.

The percentage of infants with episodes of illness throughout the follow up was generally low. A slightly high figure of 25% was recorded at the 11 month in the control group. The use of dummy or pacifier or comforter was very low. The highest percentage was found in the control group at the ninth and tenth month with a percentage of 7% and 9% respectively. The presence of thumb sucking occasionally was seen in the two groups studied. The

percentages were very low in the two groups and in the cases seen, the thumb sucking was not consistent throughout the study period. The suckling intensity in the two groups was the same or more in the first 5 months of lactation. The percentage of infants suckling in the same way was constant in the two groups

The supplemented mothers on the average were eating about 51g of protein, 1940 Kcal of energy and 21.9g of fat per day. Supplementation with biscuit (40 g) increased their energy consumption by 18Kcal, protein by 8g and fat by 8g. (Appendix 2). The effect of this supplement on the growth of their suckling infants and the anthropometric measurements of their mothers has been discussed earlier in this dissertation. The most common source of protein consumed by both groups of mothers was fish. 85 - 90% of mothers in both groups do not consume poultry meat at all throughout the duration of follow up. Only 3 - 5% of the mothers in the two groups did not consume fish. Most mothers in the two groups studied consumed red meat about 3-5 times per week.

Powdered milk, liquid milk and cereals in the form of corn pap were the most common supplements taken because they were breastfeeding. The mothers were generally healthy throughout the study period. In both groups, there was less than 10% episodes of illness throughout the 10 months of lactation, with the exception of the 13% episodes of illness in the 9th month in the control group.

The average number of breastfeeding episodes per day was 6-10 in both groups and the average number of daytime feeds was 5-7. This is similar to the findings of WHO study on patterns of breast feeding that was carried out in many countries including Nigeria (WHO1981) The average number of night time breastfeeding episodes of 2-3 was however found in Nigeria as opposed to an average of 1-4 times found in this work. An average of 4 times per night was

found in the rural groups of Zaire, Guatemala and the Philippines. Night time breastfeeding was still common at the end of 12th month postpartum, but there was a gradual decline from the fourth month postpartum (WHO 1981).

The mean duration of breastfeeding in the two groups was between 9-12 minutes. Duration of breastfeeding was similar in both groups. Most mothers were giving their infants milk or milk based feeds about 3 times a day in a feeding bottle in the two groups studied. 44% of the infants of the supplemented mothers and 40% of the infants of the control mothers did not give milk or milk based cereals at the first month postpartum. At 8 months postpartum, some of the mothers had stopped milk or milk based cereals. This is not unexpected considering the purchasing power of the mothers. Most women cannot afford cereals and consequently they expose the infants quickly to adult diet normally consumed by the family. Within 8 - 9 months, most babies tolerate other foods eaten by the family, especially pap that is made from cornstarch. The mothers consequently wean the infants off the milk and milk based cereals, which are in recent times very expensive.

BORDERLINE MALNUTRITION, PROLACTIN CONCENTRATION AND RESUMPTION OF LACTATIONAL AMENORRHEA

The effect of maternal nutritional status on prolactin concentration and on the duration of lactational infertility was investigated. There were no significant differences between the basal level and suckling induced prolactin concentration in the two groups of mothers studied. This showing that supplementation of the mother's diet had no effect on the concentration of the prolactin levels. This is in agreement with the findings of Sharmgra *et al* (1982) in India. They did not observe any effect of body weight on prolactin concentration in the undernourished lactating mothers. However, Luma et al

(1980) found a reduced prolactin concentration in the Gambia with supplementation of the mother's diet

A more recent study in Srilanka on the effect of skimmed milk supplementation on lactational amenorrhoea, and maternal prolactin concentration Was undertaken by (Shatruga et al (1992) skimmed milk supplementation did not cause a reduction in prolactin secretion. The absence of a consistent significant difference in prolactin concentration between the supplemented subjects and control group can further be explained by the similar frequencies of breast feeds in both groups of mothers. Since suckling is the most potent stimulus for prolactin secretion Although, protein meals have been shown to increase prolactin secretion (Ishizuka et al (1983) Such an effect was not observed in this work, either because the amount of additional protein was an inadequate stimulus or because prolactin secretion was maximal only in response to suckling. Similarly Kurz et al (1991) found that maternal dietary intake did not affect the length of postpartum amenorrhea , but the child's lack of breast milk intake did. This is because a child's breast milk intake reduces the frequencies and duration of suckling which in turn affects the suckling stimulus which has been known to induce prolactin secretion and duration of lactational infertility

The total number of breasifeeds per 24 hours and the number of other feeds per 24hour in both groups by time postpartum were not significantly different between the two groups of mother infant pair Similarly both day time and night time breast feeds were not significantly different in the two groups

The mean duration of lactational amenorrhoea is 28.5 weeks (7 lmonths) for the supplemented mothers and 24.5 weeks (6 lmonths) for the control mothers There was no significant difference between the duration of lactational amenorrhoea of the two groups of mothers Similar duration of

lactational amenorrhoea found may be accounted for by the fact that the two groups of babies had similar suckling frequency and duration which have been shown to be directly correlated with duration of lactational amenorrhea (Mcneilly 1982)

This finding shows that maternal supplement intake was not an important variable explaining the length of postpartum amenorrhoea. The influence of nutritional status of women during lactation on the duration of lactational infertility is the same if well nourished women nurse their infants at frequencies common in populations with extended periods of lactational amenorrhea. This finding has been observed by Lewis (1985) and Short (1991) on contraceptive effects of lactational amenorrhea . They found, that the single most important controller of the duration of lactational infertility is the suckling stimulus of the baby. The duration of lactational amenorrhoea observed here is also similar to the findings of WHO (1981) in which 70-80% of the mothers in Nigeria in the economically advantaged group were found to have started menstruating by 6months postpartum.

The prolactin response to suckling were done in the evenings between 4pm and 8pm. The basal level of prolactin concentration was taken at about one hour or more after the last breastfeeding episode and the suckling induced prolactin concentration was taken 30 minutes after the commencement of a breast-feeding episode. At admission, plasma concentrations of prolactin were very high and this remained so during the first 3-4 months postpartum, declining in parallel to suckling activity post-partum. This shows that the basal concentrations of prolactin and the magnitude of the prolactin response to suckling decrease with time postpartum. This is similar to the findings of Glasier et al (1984) who found that the basal concentrations of prolactin and the magnitude of the prolactin response to suckling decrease with time

postpartum. When suckling episodes occur frequently there is insufficient time between them for prolactin levels to fall to low levels and so basal concentrations of prolactin remain elevated. When suckling episodes are less frequent due to introduction of other feeds from 4 months postpartum, concentrations of prolactin fall between feeds, and thus the prolactin concentrations in response to suckling falls. (Glassier et al 1984). Thus, basal prolactin concentrations appear to decrease more rapidly with time postpartum.

The volume of milk taken by the two groups of babies was similar throughout the month postpartum, suggesting that the intensity of the suckling stimulus was not different in the two groups of mothers. The mean duration of night time breast feeding was similar in the two groups of mothers. The average in the two groups was 5-10 minutes. The effect of night time suckling episode which has been suggested by many authors including Howie and Mcneilly (1982) to be important in maintaining lactational infertility was the same in the two groups of mothers. This has gone further to explain the absence of a significant difference in the duration of lactational infertility in the two groups of mothers. They both had similar breast-feeding practices and hence similar duration of lactational amenorrhoea.

The onset of follicular development and ovulation in the first and second menstruation was investigated in the two groups of mothers by monitoring the urinary Pregnanediol-3- alpha glucuronide and Estrone-3—glucuronide. Longitudinal studies which have used ovarian hormones as markers of the timing of ovulation have shown that continuing lactational amenorrhoea is the most important indicator of anovulation (Howie 1993). When menstruation occurs it is important to know whether the cycles are anovulatory or not.

In the first cycle 77.3% of the mothers in the supplemented group had anovular menstruation while 22.7% ovulated. In the control group 54.5 %had

anovular menstruation while 45.5% ovulated in their first menstruation In the second cycle 54.5% had anovular menstruation, 45.5% ovulated in the supplemented mothers, and 46.2% had anovular menstruation and 53.8% ovulated in the control group. The endocrine finding from this work shows that even when menstruation occurred, there is still a lot of irregularities in the follicular development and ovulation of the lactating women. This finding is similar to the observation of Howie (1993), which showed that although irregular ovarian follicular development can occur, ovulation is not observed until the cycle immediately following the return of menstruation Lowrie (1993) observed this in only one individual. However, in the present study even though more mothers had ovulated in their second menstruation, some of the mothers still had not ovulated The greatest vulnerability to unplanned pregnancies in the lactating mother is thus from the cycle preceding the first menstruation Hence, the need to educate the lactating mothers about their returning fertility by the occurrence of first menstruation. (Gray 1990, Howie 1982.) Howie (1993) showed that the cycle preceding first menstruation in the subject he studied was either anovular or just possibly, an ovular cycle associated with a grossly inadequate luteal phase incapable of sustaining a pregnancy. All these studies are pointing to the fact that there is irregular follicular development of the first few menstruation following the return of menstrual flow. Ovulation with an adequate luteal phase is also less likely to occur in nursing women who are fully or nearly fully breastfeeding (Kennedy et al. (1992) These patterns of irregular menstrual cycles during lactation after the return of menses have been reported in several studies and are consistent with the epidemiological evidence that mothers who are menstruating during breast feeding have a chance of conception which is less than that of normally cycling non-lactating women but high enough to require alternative family planning

Chen et al (1974). Hence the need to educate mother of the risk of getting pregnant. This finding is consistent with the findings of WHO (1981) in which the percentage of women with returned menstruation was remarkably uniform in all groups of mothers not breast-feeding but by contrast, the percentages among women who were breast-feeding vary widely from group to group. The data show that breast-feeding was associated with a considerable delay in the return of menstruation, but that the difference between breast-feeding and non-breast-feeding mothers gradually diminish or disappear with time.

CONCLUSION

The results of this work have shown that borderline malnutrition of the mother did not affect

- The growth of their suckling infants
- Breastfeeding patterns in terms of frequency and duration of breastfeeding of their suckling infants

Supplementing the diets of the mothers did not affect basal prolactin, suckling induced prolactin, concentrations, the return of menstruation and ovulation. Mothers in the two groups had similar duration of lactational amenorrhea.

The concentration of prolactin decreased with time postpartum along side with the suckling frequency thus suggesting a direct relationship between them. Supplementing the mother's diet had some improvement in the nutritional status of the mother. This finding suggests that breastfeeding should be encouraged where there is maternal borderline malnutrition. The young infant growth, health and well being, including the mother's health will be taken care of by supplementing the mothers' diet.

Supplementing the diet of the mother did not affect the duration of lactational amenorrhoea of the supplemented mothers. This suggests that it is not nutrition but rather suckling activities and breast feeding practices which affect the concentration of prolactin concentration. This in turn can affect duration of lactational amenorrhoea. Even when menstruation has resumed, there are a lot of irregularities in the follicular development of the two groups of mothers, the first and the second cycles may be ovulatory or anovulatory

when exclusive breast feeding is not practised. At such times mothers need to be educated on other family planning methods which can be used at this time to prevent another pregnancy, so that the health of the mother is not compromised by another pregnancy within 6 months of delivery of a child.

RECOMMENDATION

1. Breast feeding should be encouraged where there is maternal borderline malnutrition, and the diet of the mother should be supplemented. Supplementing the diet of the mother will take care of the babies nutrition as well, since maternal breast milk production is not sacrificed in the absence of good maternal nutrition and that supplementation of the mother may likely help to build maternal stores in marginally nourished lactating women.

2. If a mother is willing to use Lactational amenorrhoea as a family planning method, the mother should ensure that a high frequency and duration of breast feeding is maintained throughout the months of lactational amenorrhoea.

3. Immediately the menstruation resumes, the mother should add another family planning method to guard against unwanted pregnancies.

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ADMISSION FORM. APPENDIX I

IDENTIFICATION

(a) Form code: A D M

(b) Study number

(c) Ward number

(d) Subject number [ ][ ][ ]-[ ]

(e) Interviewer number

Date of admission: day | month | year

PERSONAL DATA OF HOUSEHOLD MEMBER

(a) Date of birth: day | month | year

(b) Age at last birthday: Years

Ethnic group, specify

Marital status

1 = Married
2 = Common-law marriage
3 = Widowed
4 = Separated
5 = Divorced
6 = Single

Usual occupation, specify

Number of completed years of education

(a) Primary: Years

(b) Secondary and/or Technical: Years

(c) University and/or Equivalent: Years

PERSONAL DATA OF HUSBAND OR CONSORT

7. Age of husband or consort at last birthday: Years

8. Ethnic group, specify

9. Usual occupation, specify

10. Number of completed years of education

(a) Primary: Years

(b) Secondary and/or Technical: Years

(c) University and/or Equivalent: Years

SOCIO-ECONOMIC CLASSIFICATION OF FAMILY

11. (a) Place of residence

1 = Urban 2 = Rural

(b) Social class

1 = Upper class
2 = Middle class
3 = No Door

MEDICAL HISTORY OF MALE

12. (a) Any history of serious illness?

1 = No 2 = Yes

(b) If YES, specify

13. (a) Any history of surgery excluding breast, gynaecological and obstetrical interventions?

1 = No 2 = Yes

(b) If YES, specify

Subject number [III]-[ ]

PREVIOUS CONTRACEPTION

14. Last method of contraception used prior to conceiving this infant [ ][ ]

01 = None
02 = Abstinence
03 = Breast-feeding
04 = Withdrawal
05 = Natural family planning method
06 = Barrier contraception
07 = Non-medicated IUD
08 = Hormone-releasing IUD
09 = Progestogen-only pill
10 = Combined oral contraceptive pill
11 = Injectable/implant contraception
12 = Hormone-releasing vaginal ring
13 = Female sterilization
14 = Male sterilization
15 = Other, specify

GYNAECOLOGICAL HISTORY

1. Average length of spontaneous menstrual cycles for the 3 months prior to conception [ ][ ] Days

4. Average duration of menstrual flow for the 3 months prior to conception [ ][ ] Days

7. (a) Any prior breast, gynaecological or obstetrical surgery?

1 = no 2 = yes

(b) If YES, specify [ ][ ][ ]

8. Has subject inverted nipple(s)?

1 = No 2 = Yes [ ]

OBSTETRIC HISTORY PRIOR TO LAST PREGNANCY

9. Total number of pregnancies (excluding the most recent that resulted in

(a) Live births
(b) Spontaneous abortions
(c) Induced abortions
(d) Stillbirths
(e) Molar pregnancies
(f) Ectopic pregnancies
(g) Other, specify

OBSTETRIC HISTORY PRIOR TO LAST PREGNANCY (CONT.)

20. Number of living children (excluding the most recent) [ ]

21. Ages of living children (excluding the most recent)

	Years	Months
a)	[ ][ ]	[ ][ ]
b)	[ ][ ]	[ ][ ]
c)	[ ][ ]	[ ][ ]

PREVIOUS BREAST-FEEDING EXPERIENCE

22. Number of infants previously breast-fed [ ]

23. (a) Was the last child breast-fed? [ ]

1 = no 2 = yes

If NO, go to 23c

(b) If YES, did she/he survive until after she/he had been weaned completely?

1 = no 2 = yes

If NO, go to 23c

If YES,

(c) Duration of breast-feeding of this child in completed months [ ][ ] Months

(d) Duration of full breast-feeding of this child in completed months [ ][ ] Months

(e) Age of infant at complete weaning [ ][ ] Months

(f) Duration of lactational amenorrhoea breast-feeding this child in completed months. [ ][ ] Months
Code 88 if conception occurred during breast-feeding.

Lactational amenorrhoea

DETAILS OF LAST PREGNANCY

24. How many months were needed to conceive this infant [ ][ ] Months

25. Duration of pregnancy [ ][ ] Weeks

Subject number

DETAILS OF LAST PREGNANCY (CONT.)

6. (a) Was the mother generally healthy during the pregnancy?

1 = No 2 = Yes

(b) If NO, specify

7. (a) Were measures taken antenatally to prepare the breast?

1 = No 2 = Yes

(b) If YES, specify

8. (a) Did the subject smoke daily?

1 = No 2 = Yes

(b) If YES, average number of cigarettes per day

9. Did the subject drink alcohol?

1 = Never
2 = Occasionally
3 = Frequently
4 = Daily

10. (a) Did subject take frequently any drug or other potentially harmful substances?

1 = No 2 = Yes

(b) If YES, specify

DETAILS OF LAST DELIVERY

11. Predicted date of delivery — day / month / year

12. Actual date of delivery — day / month / year

DETAILS OF LAST DELIVERY (CONT.)

33. Did subject deliver at or after 37 weeks of gestation?

1 = No 2 = Yes

IF NO, SUBJECT MUST BE EXCLUDED

34. (a) Place of delivery

1 = Home, unassisted
2 = Home, assisted
3 = Local health centre/hospital
4 = Other, specify

(b)

35. (a) Did subject receive any medication and/or anaesthesia (including spinal or epidural block) during labour and/or delivery?

1 = No 2 = Yes

(b) If YES, specify

36. Duration of labour — hours

37. (a) Were there any complications of delivery or immediately postpartum?

1 = No 2 = Yes

(b) If YES, specify

38. (a) Mode of delivery

1 = Vertex delivery
2 = Breech delivery
3 = Other, specify

(b)

39. Duration of stay in hospital or health centre. Code 99 if not applicable — recorded days

bject number [III]-[ ]

TAILS OF LAST DELIVERY (CONT.)

). (a) Did subject receive any medication postpartum (orally or by injection)?

1 = No 2 = Yes

(b) If YES, specify

[ ]-[ ][ ][ ]

AL CONTRACEPTION USED

. Method of contraception used since delivery [ ][ ]

01 = None
02 = Abstinence
03 = Breast-feeding
04 = Withdrawal
05 = Natural family planning method
06 = Barrier contraception
07 = Non-medicated IUD
08 = Hormone-releasing IUD
09 = Progestogen-only pill
10 = Combined oral contraceptive pill
11 = Injectable/implant contraception
12 = Hormone-releasing vaginal ring
13 = Female sterilization
14 = Male sterilization
15 = Other, specify

CODE 08-12, SUBJECT MUST BE EXCLUDED

BREAST FEEDING SINCE DELIVERY

. Interval between delivery and start of breast-feeding [ ][ ] hours

. (a) Was any fluid or food given during the interval between delivery and start of breast-feeding?

1 = No 2 = Yes

(b) If YES, specify

[ ][ ][ ]

. Is the baby using breast-feed on demand during the day (06:00-21:59)?

1 = No 2 = Yes

BREAST FEEDING SINCE DELIVERY (CONT.)

45. Number of breast-feeds during the previous day (06:00-21:59) [ ][ ]

46. (a) Does the baby sleep with the mother and have unrestricted access to the breast at night (22:00-05:59)?

1 = No
2 = Yes, but not every night
3 = Yes, every night

(b) If answered 2 or 3 above, then

1 = All night
2 = Part of night

47. Number of breast-feeds during the previous night (22:00-05:59) [ ][ ]

48. Use of a dummy/comforter/pacifier since delivery

1 = Never
2 = Occasionally
3 = Frequently

49. (a) Have there been any breast problems since delivery?

1 = None
2 = Engorgement/expected ducts/mastitis
3 = Sore or cracked nipples
4 = Abscess
5 = Other, specify

(b) [ ][ ]-[ ]

50. (a) Has the infant received any fluid or food other than breast milk since the start of breast-feeding?

1 = No 2 = Yes

(b) If YES, specify

[ ][ ][ ]

PHYSICAL DATA

51. Height of mother [ ][ ][ ] cm

52. (a) Weight of mother [ ][ ][ ] kg

(b) Where was it measured?

1 = At home
2 = At health center/hospital
3 = Other

Subject number [_I_I_I_]-[_]

PHYSICAL DATA (CONT.)

3. Sex of infant [_]

1 = Male 2 = Female

4. (a) Weight of infant at admission is [_][_].[_]

(b) Where was the infant weighed? [_]

1 = At home
2 = At health center/hospital
3 = Other

MATERNAL NUTRITION

5. How many times per week does the mother eat any of the following

(a) Red meat [_][_]

(b) Poultry [_][_]

(c) Fish [_][_]

6. (a) Is the mother taking any special dietary supplements because she is breast-feeding? [_]

1 = No 2 = Yes

If YES, which of the following

1 = No 2 = Yes

(b) Kinverale
(c) Oil palm
(d) Corn cove oil
(e) Hundred oils
(f) Liquid oils
(g) Vegetable oil
(h) Animal oil
(i) Cereals
(j) Sugar
(k) Yeast
(l) Beer
(m) Fish
(n) Meat

MATERNAL NUTRITION (CONT.)

(o) High-energy biscuits [_]

(p) Other, specify

_____ [_][_]

_____

OPTIONAL PHYSICAL DATA

57. Skin fold measurement of mother mm [_][_]

58. Upper mid arm circumference of mother cm [_][_].[_]

59. Length of infant at admission cm [_][_].[_]

60. Head circumference of infant at admission cm [_][_].[_]

61. Chest circumference of infant at admission cm [_][_].[_]

REMARKS

Interviewer's name _____

Signature _____

Date ___/___/___

Investigator's name _____

Signature _____

Date ___/___/___

APPENDIX 2

FOLLOW UP FORM

IFICATION

a) Form code: F U P

b) Study number: 8 5 9 1 7

c) Centre number: [ ][ ][ ][ ]

d) Subject number: [ ][ ][ ]-[ ]

e) Interviewer number: [ ][ ][ ]

ERAL INFORMATION

Scheduled date of visit: day [ ][ ] month [ ][ ] year [ ][ ]

quence number of follow-up visit: [ ]

ate of infant's birth: day [ ][ ] month [ ][ ] year [ ][ ]

a) Actual date of visit: day [ ][ ] month [ ][ ] year [ ][ ]

b) If visit did not take place on the scheduled date, specify reason

[ ][ ][ ] WHO code

) Was the baby still being breast-fed?
1 = No 2 = Yes [ ]

) If no, date breast-feeding stopped: day [ ][ ] month [ ][ ] year [ ][ ]

r infant

Has the infant had any episodes of illness?
1 = No 2 = Yes [ ]

If no, go to 6?

6a. Specify for each type of illness the number of days the child was ill and number of new episodes started

DETAILS OF INTAKE (CONT.)

(b) Diarrhoea
(i) Total number of days of illness [ ][ ]
(ii) No. of new episodes started [ ][ ]

(c) Cold/runny nose/cough
(i) Total number of days of illness [ ][ ]
(ii) No. of new episodes started [ ][ ]

(d) Generally unwell
(i) Total number of days of illness [ ][ ]
(ii) No. of new episodes started [ ][ ]

(e) Other illness
(i) Total number of days of illness [ ][ ]
(ii) No. of new episodes started [ ][ ]

(iii) Type of illness, specify
_____ [ ][ ][ ] WHO code
_____

(f) Other illness
(i) Total number of days of illness [ ][ ]
(ii) No. of new episodes started [ ][ ]

(iii) Type of illness, specify
_____ [ ][ ][ ] WHO code
_____

7. Did the baby show a preference for one or to either breast?
1 = None
2 = Right
3 = Left [ ]

8. Was the baby holding on vigorously as before? [ ]
1 = Less
2 = The same
3 = More

ubject number

ilt number

ETAILS OF MOTHER

. (a) Has the mother had any episodes of illness?

1 = No 2 = Yes

If YES, specify

(b) Illness

WHO CODE

(c) Treatment

WHO CODE

(b) Has the mother had any breast problems?

1 = None
2 = Engorgement/blocked ducts/mastitis
3 = Sore or cracked nipples
4 = Abscess
5 = Other, specify

b) WHO CODE

ve sexual intercourse occurred?

1 = No 2 = Yes

) Has there been a change in the family planning method used?

1 = No 2 = Yes

If NO, GO TO Q13

If YES, which change?

1 = Started any method for the first time since last visit
2 = Changed to another method
3 = Stopped using any method

Date when change took place

day	month	year

DETAILS OF MOTHER (CONT.)

(d) What method is being used now?

01 = None
02 = Abstinence
03 = Breast-feeding
04 = Withdrawal
05 = Natural family planning method
06 = Barrier contraception
07 = Non-medicated IUD
08 = Hormone-releasing IUD
09 = Progestogen-only pill
10 = Combined oral contraceptive pill
11 = Injectable/implant contraception
12 = Hormone-releasing vaginal ring
13 = Female sterilization
14 = Male sterilization
15 = Other, specify

IF CODES 08-12, SUBJECT MUST BE DISCONTINUED

13. Does the mother think that she is pregnant?

1 = No 2 = Yes

14. (a) Did the subject smoke daily?

1 = No 2 = Yes

(b) If YES, average number of cigarettes per day

15. Did the subject drink alcohol?

1 = Never
2 = Occasionally
3 = Frequently
4 = Daily

16. (a) Did subject take frequently any drugs or other potentially harmful substances?

1 = No 2 = Yes

If YES, specify

(b) WHO CODE

17. (a) Has the mother been working outside her home?

1 = No 2 = Yes

If NO, GO TO Q18

If YES,

bject number [ ][ ][ ]-[ ]

it number [ ][ ]

TAILS OF MOTHER (cont.)

(b) Could she breast-feed the baby on demand while working?

1 = No 2 = Yes

(c) Did the mother do paid work?

1 = No 2 = Yes

(d) Did mother work night shifts?

1 = No 2 = Yes

(e) How many hours did she work each week outside the home? Hours [ ][ ]

ART OF DAILY RECORD CARD

e the chart kept during the period nder review?

1 = No 2 = Yes

If NO, GO TO Q20

mber of days included in the daily ord card [ ][ ]

s the baby breast-fed on demand ring the day (06:00-21:59)?

1 = No 2 = Yes

erage number of breast-feeding ds during the day(06:00-21:59)? [ ][ ].[ ]

Did the baby sleep with the mother and have unrestricted access to the breast at night (22:00-05:59)?

1 = Never
2 = Occasionally
3 = Frequently
4 = Daily

If answered 3 or 4 above, then

1 = All night
2 = Part of night

erage number of breast-feeding ds at night (22:00-05:59)? [ ][ ].[ ]

Did the mother express breast milk by hand or pump?

1 = No 2 = Yes

If YES, and DAILY,

Average number per day [ ][ ].[ ]

SUMMARY OF DAILY RECORD CARD (cont.)

(c) If YES, and NOT DAILY,

Number of days [ ][ ]

25. (a) Did the infant receive any food or fluid other than suckled breast milk?

1 = No 2 = Yes

If NO, GO TO Q30 [ ]

(b) Were the amounts given very small ("tastes")?

1 = No 2 = Yes [ ]

If YES, GO TO Q30

26. MILK OR MILK-BASED FEEDS

(a) Number of times given during this period [ ][ ]

If 00, GO TO Q27

(b) Was this type of food started or restarted during the period? [ ]

1 = No 2 = Yes

If YES,

(c) Give date

day	month	year
[ ][ ]	[ ][ ]	[ ][ ]

(d) Specify reason _____ [ ][ ][ ]

USE CODE

(e) Was this type of food stopped during the period? [ ]

1 = No 2 = Yes

If YES,

(f) Give date

day	month	year
[ ][ ]	[ ][ ]	[ ][ ]

(g) Specify reason _____ [ ][ ][ ]

USE CODE

27. WATER AND OTHER NON-CALORIC FLUID FEEDS

(a) Number of times given during this period [ ][ ]

If 00, GO TO Q28

ct number

number

RY OF DAILY RECORD CARD (cont.)

) Was this type of food started or restarted during the period?

1 = No 2 = Yes

If YES,

day | month | year

) Give date

) Specify reason _____ WHO CODE

Was this type of food stopped during the period?

1 = No 2 = Yes

If YES,

day | month | year

Give date

Specify reason _____ WHO CODE

LORIC FLUID FEEDS

Number of times given during this period

If 00, GO TO Q20

Was this type of food started or restarted during the period?

1 = No 2 = Yes

If YES,

day | month | year

Give date

Specify reason _____ WHO CODE

SUMMARY OF DAILY RECORD CARD (cont.)

(e) Was this type of food stopped during the period?

1 = No 2 = Yes

If YES,

day | month | year

(f) Give date

(g) Specify reason _____ WHO CODE

29. SEMI-SOLID OR SOLID FEEDS

(a) Number of times given during this period

If 00, GO TO Q30

(b) Was this type of food started or restarted during the period?

1 = No 2 = Yes

If YES,

day | month | year

(c) Give date

(d) Specify reason _____ WHO CODE

(e) Was this type of food stopped during the period?

1 = No 2 = Yes

If YES,

day | month | year

(f) Give date

(g) Specify reason _____ WHO CODE

30. (a) How many times has the infant been breast-fed by [illegible]?

(b) How many times has it together breast-fed any other child?

[I_I_I]-

number

number

F DAILY RECORD CARD (cont.)

of dummy/pacifier/comforter

1 = Never
2 = Occasionally
3 = Frequently

ence of thumbsucking (finger, toe)

1 = Never
2 = Occasionally
3 = Frequently

date lochia ended

day	month	year

(999997 if lochia has not yet ended or if has been given in previous follow-up form)

there been one or more vaginal bleeding ides during the period under review?

1 = No 2 = Yes

F NO, GO TO 040

complete bleeding episode or incomplete ding episode carried over from previous orm

as this episode related to a necological procedure including nsertion of IUD?

= No 2 = Yes

F YES, specify procedure and GO TO 036

WHO CODE

F NO,

hen did it start?

day	month	year

w many days did it last?
code 99 if still bleeding, and
plete information on this
eeding episode on next FUP form)

w did the episode compare to
rmal menstruation?

= Less
= The same
= More

as this the second normal
nstruation after delivery?

= No 2 = Yes

SUMMARY OF DAILY RECORD CARD (cont.)

NOTE: IF IT IS THE SECOND NORMAL MENSTRUATION, SUBJECT SHOULD BE DISCONTINUED

36. Was there a second bleeding episode?

1 = No 2 = Yes

If NO, GO TO 040

37. Second bleeding episode

(a) Was this episode related to a gynaecological procedure including insertion of IUD?

1 = No 2 = Yes

(b) If YES, specify procedure and GO TO 038

WHO CODE

If NO,

(c) When did it start?

day	month	year

(d) How many days did it last? (code 99 if still bleeding, and complete information on this bleeding episode on next FUP form)

(e) How did the episode compare to normal menstruation?

1 = Less
2 = The same
3 = More

(f) Was this the second normal menstruation after delivery?

1 = No 2 = Yes

NOTE: IF IT IS THE SECOND NORMAL MENSTRUATION, SUBJECT SHOULD BE DISCONTINUED

38. Was there a third bleeding episode?

1 = No 2 = Yes

If NO, GO TO 040

39. Third bleeding episode

(a) Was this episode related to a gynaecological procedure including insertion of IUD?

1 = No 2 = Yes

number

uber

OF DAILY RECORD CARD (cont.)

If YES, specify procedure and GO TO Q60

WHO CODE

If so,

day	month	year

When did it start?

How many days did it last?
(code 99 if still bleeding, and complete information on this bleeding episode on next FUP form)

How did this episode compare to normal menstruation?

1 = less
2 = the same
3 = More

Was this the second normal menstruation after delivery?

1 = No 2 = Yes

IF IT IS THE SECOND NORMAL MENSTRUATION, SUBJECT SHOULD BE DISCONTINUED

Have mother and baby been separated for more than 8 hours on any occasion?

1 = No 2 = Yes

If YES, how often?

RY OF DETAILED RECORD DAY

Was the detailed record day chart kept?

1 = No 2 = Yes

If NO, GO TO Q57

If YES, on which date was the record have been kept)

day	month	year

Actual date kept?

day	month	year

SUMMARY OF DETAILED RECORD DAY (CONT.)

42. Total number of breast-feeding episodes

43. (a) Longest interval between two successive episodes of breast-feeding / expression — Mins

(b) Was this interval during the day or night?

1 = Day 2 = Night

44. Was the baby breast-fed on demand during the day (06:00-21:59)?

1 = No 2 = Yes

45. Number of daytime breast-feeding episodes

46. Mean duration of daytime breast-feeding episodes — Mins

47. (a) Did the baby sleep with the mother and have unrestricted access to the breast at night (22:00-05:59)?

1 = Never
2 = Occasionally
3 = Frequently
4 = Daily

(b) If answered 3 or 4 above, then

1 = All night
2 = Part of night

48. Number of night-time breast-feeding episodes

49. Mean duration of night-time breast-feeding episodes — Mins

50. (a) Did the mother express breast milk by hand or pump?

1 = No 2 = Yes

(b) If YES, how many times?

51 (a) Did the infant receive any food or fluid other than sucked breast milk?

1 = No 2 = Yes

If NO, GO TO Q54

(b) Were the amounts given very small (teaspoons)?

1 = No 2 = Yes

If YES, GO TO Q54

number

umber

T OF DAILY RECORD CARD (cont.)

If YES, specify procedure and GO TO 060

VAG CODE

If NO,

day	month	year

When did it start?

How many days did it last?
(code 99 if still bleeding, and complete information on this bleeding episode on next FIP form)

How did the episode compare to normal menstruation?

1 = Less
2 = The same
3 = More

Was this the second normal menstruation after delivery?

1 = No 2 = Yes

IF IT IS THE SECOND NORMAL MENSTRUATION, SUBJECT SHOULD BE DISCONTINUED

Have mother and baby been separated for more than 8 hours on any occasion?

1 = No 2 = Yes

If YES, how often?

RY OF DETAILED RECORD DAY

Was the detailed record day chart kept?

1 = No 2 = Yes

If NO, GO TO 057

If YES, on which date should the record have been kept?

day	month	year

Actual date kept?

day	month	year

SUMMARY OF DETAILED RECORD DAY (CONT.)

62. Total number of breast-feeding episodes

63. (a) Longest interval between two successive episodes of breast-feeding / expression — Mins

(b) Was this interval during the day or night?

1 = Day 2 = Night

64. Was the baby breast-fed on demand during the day (08:00-21:59)?

1 = No 2 = Yes

65. Number of daytime breast-feeding episodes

66. Mean duration of daytime breast-feeding episodes — Mins

67. (a) Did the baby sleep with the mother and have unrestricted access to the breast at night (22:00-05:59)?

1 = Never
2 = Occasionally
3 = Frequently
4 = Daily

(b) If answered 3 or 4 above, then

1 = All night
2 = Part of night

68. Number of night-time breast-feeding episodes

69. Mean duration of night-time breast-feeding episodes — Mins

70. (a) Did the mother express breast milk by hand or pump?

1 = No 2 = Yes

(b) If YES, how many times?

71 (a) Did the infant receive any food or fluid other than matched breast milk?

1 = No 2 = Yes

If NO, GO TO 076

(b) Were the amounts given very small (=teaspoon)?

1 = No 2 = Yes

If YES, GO TO 076

ect number [I I I]-[ ]

t number [ ][ ]

MARY OF DETAILED RECORD DAY (Cont.)

MILK OR MILK-BASED FEEDS

a) Number of times given during this 24-hour period

If 00, GO TO Q53

b) When were these normally given?

1 = Before a breast-feed
2 = After a breast-feed
3 = During a breast-feed
4 = Unrelated to a breast-feed
5 = No consistent pattern

c) How were they normally given?

1 = Bottle
2 = Spoon/fingers
3 = Cup
4 = Combination or other

WATER AND OTHER NON-CALORIC LUID FEEDS

a) Number of times given during this 24-hour period

If 00, GO TO Q54

b) When were these normally given?

1 = Before a breast-feed
2 = After a breast-feed
3 = During a breast-feed
4 = Unrelated to a breast-feed
5 = No consistent pattern

c) How were they normally given?

1 = Bottle
2 = Spoon/fingers
3 = Cup
4 = Combination or other

SUMMARY OF DETAILED RECORD DAY (Cont.)

54. CALORIC FLUID FEEDS

(a) Number of times given during this 24-hour period

If 00, GO TO Q55

(b) When were these normally given?

1 = Before a breast-feed
2 = After a breast-feed
3 = During a breast-feed
4 = Unrelated to a breast-feed
5 = No consistent pattern

(c) How were they normally given?

1 = Bottle
2 = Spoon/fingers
3 = Cup
4 = Combination or other

55. SEMI-SOLID OR SOLID FEEDS

(a) Number of times given during this 24-hour period

If 00, GO TO Q56

(b) When were these normally given?

1 = Before a breast-feed
2 = After a breast-feed
3 = During a breast-feed
4 = Unrelated to a breast-feed
5 = No consistent pattern

(c) How were they normally given?

1 = Bottle
2 = Spoon/fingers
3 = Cup
4 = Combination or other

56. (a) Does the detailed record day represent a typical day?

1 = No 2 = Yes

(b) If NO, why not _____ [ ][ ] WHO CODE

_____

(c) Is the recorded information likely to be accurate?

1 = No 2 = Yes

(d) If NO, why not _____ [ ][ ] WHO CODE

_____

number

mber

KENTS

Weight of mother at visit kg

Where was the mother weighed?

1 = At home
2 = At health center/hospital
3 = Other

Weight of infant at visit kg

Here was the infant weighed?

1 = At home
2 = At health center/hospital
3 = Other

NUTRITION

any times per week did the mother eat
f the following

ed meat

oultry

ish

es the mother taking any special dietary
upplements because she is breast-feeding?

1 = No 2 = Yes

f YES, which of the following

1 = No 2 = Yes

Minerals

Vitamins

Iron-rich syrup

Fortified milk

Liquid milk

Vegetable oil

Animal oil

Cereals

Sugar

Yeast

Eggs

MATERNAL NUTRITION (CONT.)

(m) Fish

(n) Meat

(o) High-energy biscuits

(p) Other, specify

WHO CODE

OPTIONAL PHYSICAL DATA

61. Skin fold measurement of mother mm

62. Upper mid arm circumference of mother cm

63. Length of infant cm

64. Head circumference of infant cm

65. Chest circumference of infant cm

66. Is the subject continuing with the study?

1 = No 2 = Yes

IF NO, COMPLETE DISCONTINUATION FORM

REMARKS

Interviewer's name

Signature

Date

Investigator's name

Signature

Date

APPENDIX J

DAILY RECORD CHART

Infant feeding chart – Daily Record

APPENDIX 3

Name	Volunteer number	Dates covered from to

date and day of the week	day or night ☼ 0600 to 2200 ☾ 2200 to 0600	Write in how many times any of these foods were given							Tick the box	
	☼									
	☾									
	☼									
	☾									
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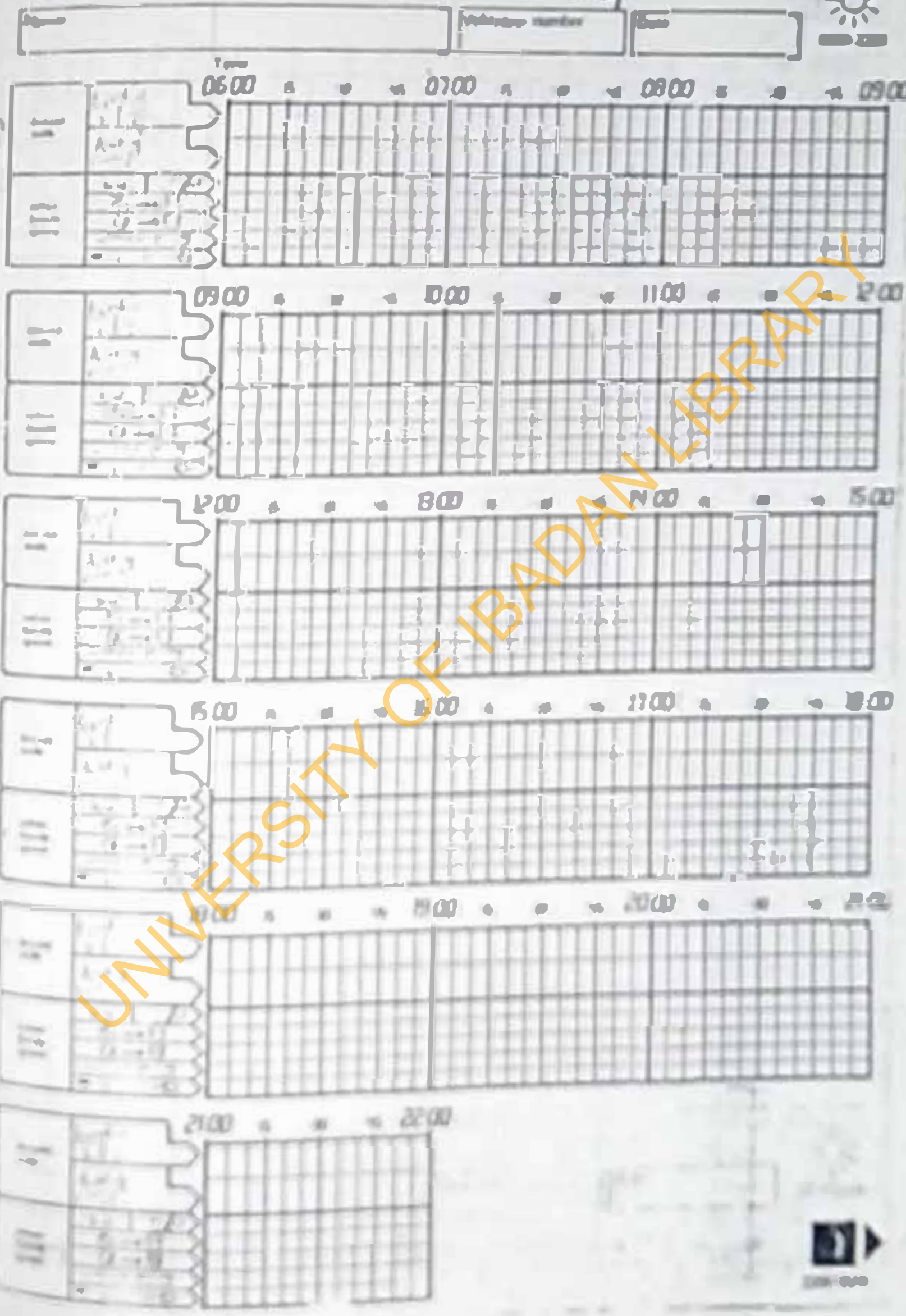
APPENDIX 4

DETAILED RECORD CHART

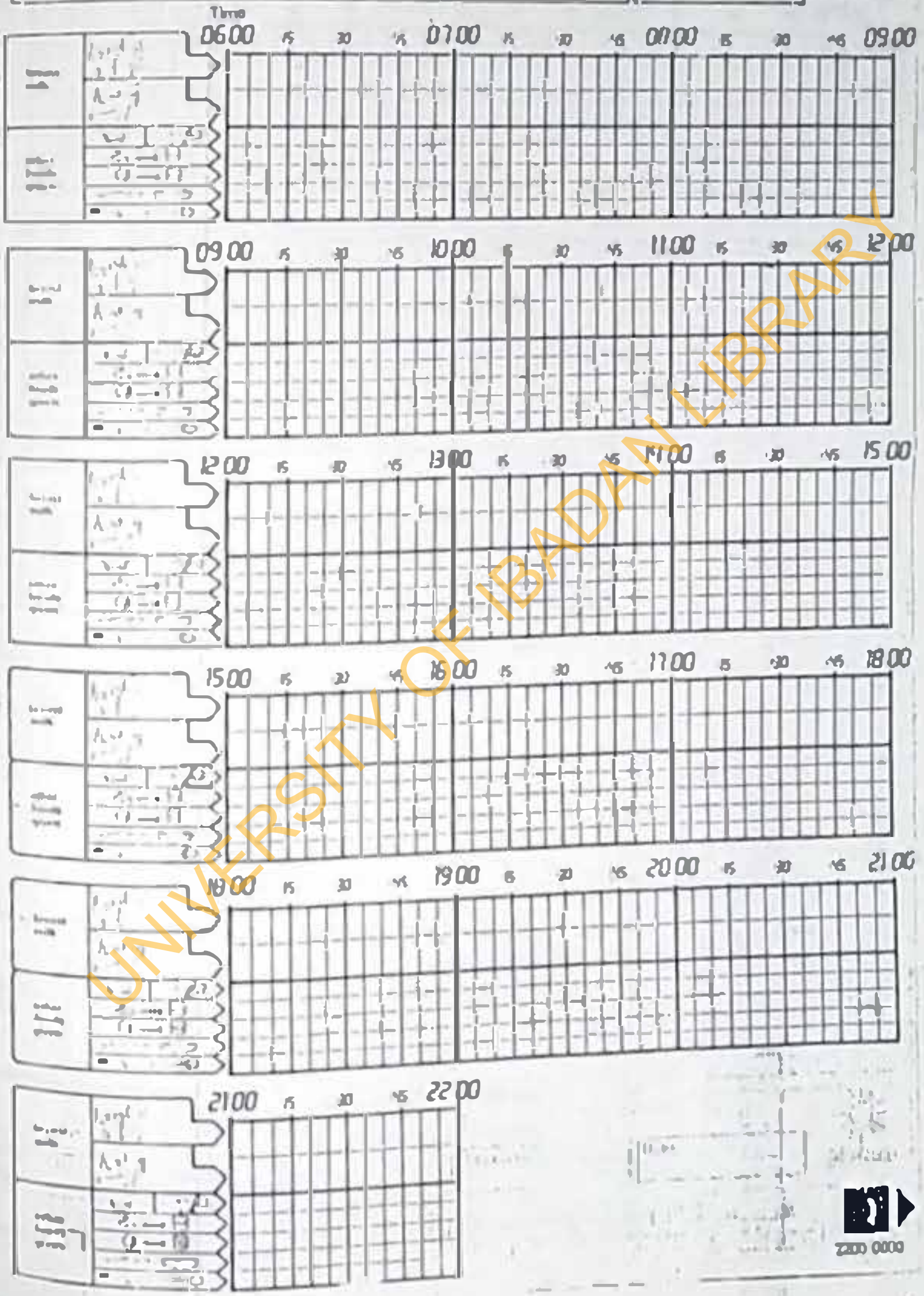
Infant feeding chart – Detailed Record Day

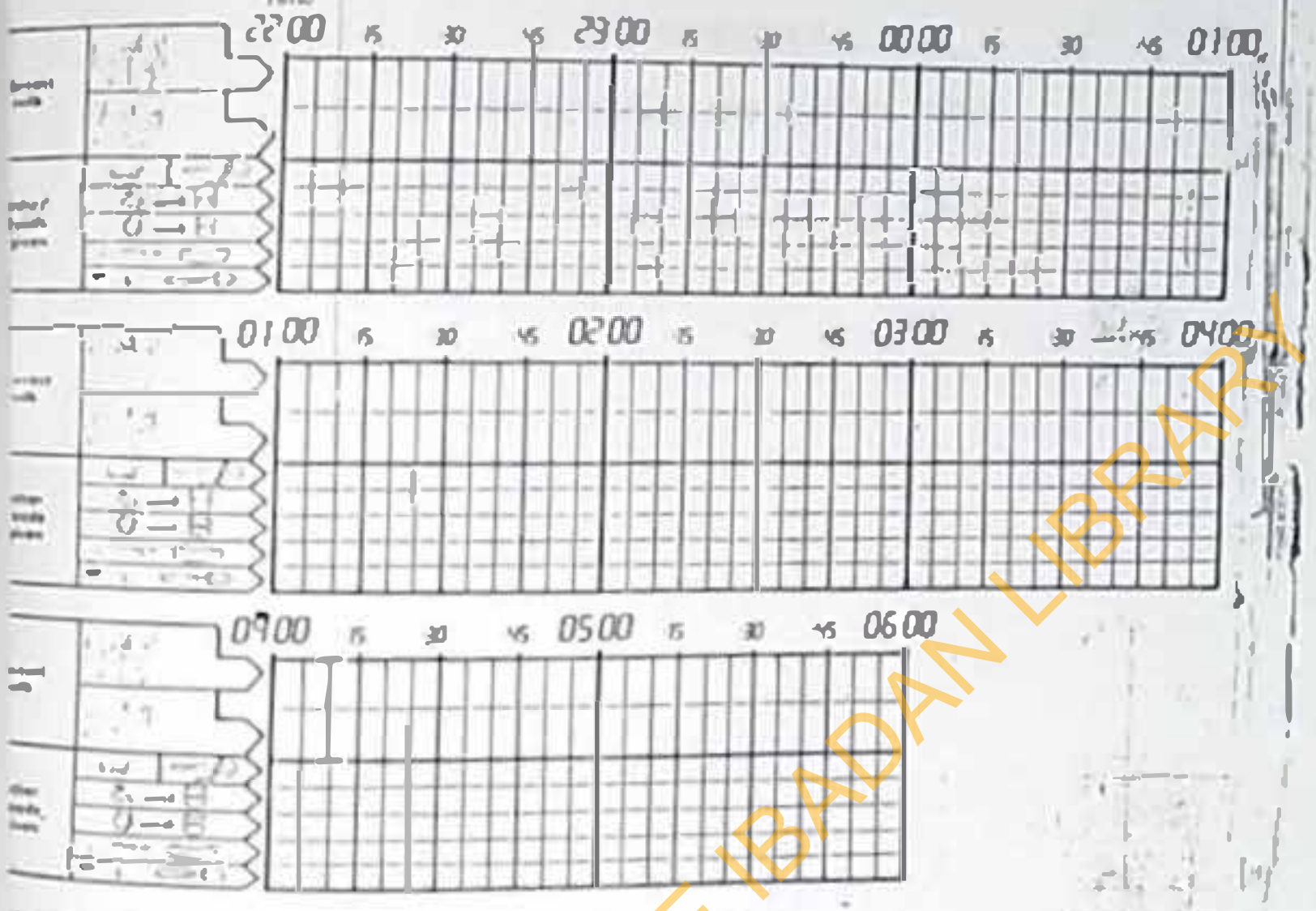


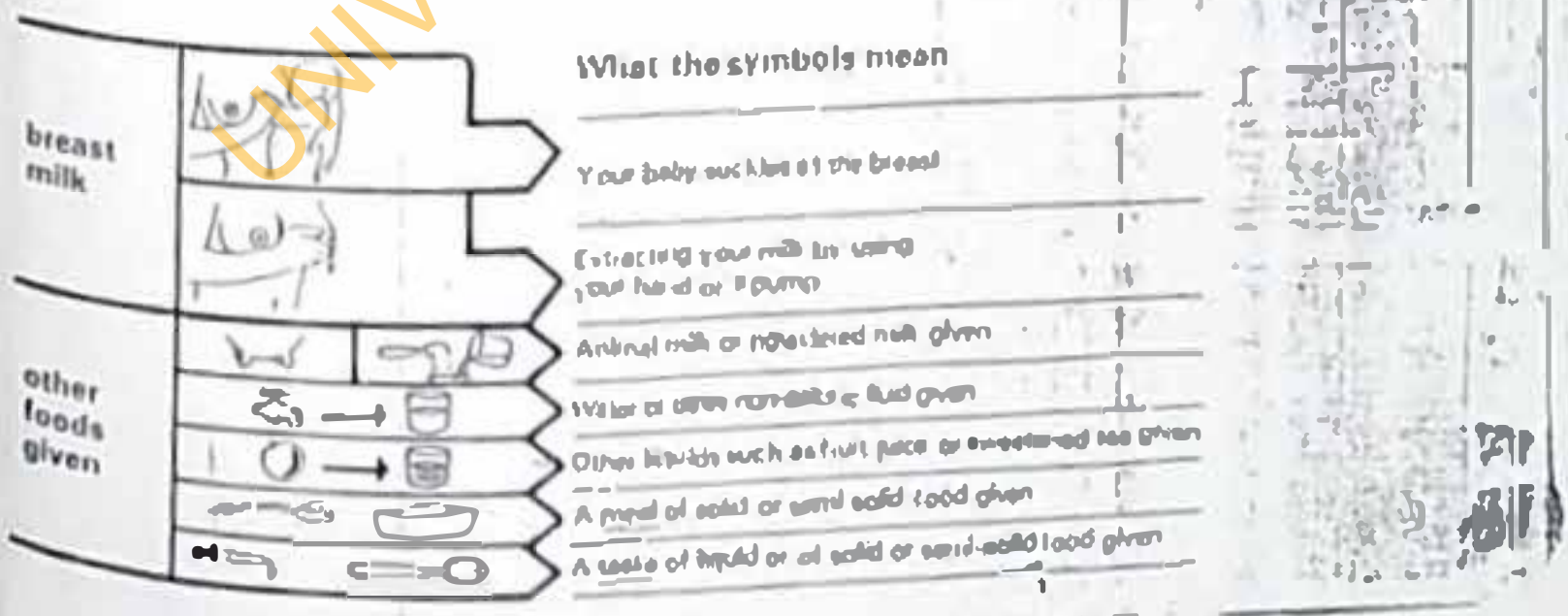
Infant feeding chart – Detailed Record Day

Name	Volunteer number	Date

0600-2200







APPENDIX 9

NUTRITIONAL ASSESSMENT FORM

Appendix 5

NUTRITIONAL ASSESSMENT FORM

MOTHER'S NAME .... CHILD'S NAME ....

APPOINTMENT NO .... ADDRESS ....

STUDY GROUP [ ]

DAY	MONTH	YEAR

SERIAL NO: .... AGE OR DATE OF BIRTH OF MOTHER ....

SEX .... WEIGHT AT BIRTH ....

DATE OF BIRTH OF INDEX CHILD ....

ELIGIBILITY FOR TEST WEIGHING AND NUTRITIONAL ASSESSMENT

1. MOTHER PREGNANT [ ] YES [ ] NO

2. HORMONAL CONTRACEPTIVES [ ] USING [ ] NOT USING

3. CHILD HEALTH CONDITION

   CHILD WELL   NOT WELL (REQUIRING DOCTOR PRESCRIBING FEVER, GASTRO INTESTINAL PROBLEM, RESPIRATORY PROBLEM COLD COUGH, DIARRHOEA)

4. MOTHER'S HEALTH CONDITION

   MOTHER WELL [ ]   NOT WELL [ ]

5. STATUS

   A. ELIGIBILITY FOR TEST WEIGHING [ ]

   B. TEST WEIGHING PERFORMED [ ]

   C. TEST WEIGHING CANCELLED [ ]

IF TEST WEIGHING POSTPONED, SPECIFY REASON ....

NEW APPOINTMENT (IF APPLICABLE)

DATE .... TIME .... PLACE ....

WEIGHT OF BABY BEFORE BREASTFEEDING .... KG

" " AFTER " FORTY-FIVE MINUTES ....

" MOTHER .... KG WEIGHT OF MOTHER .... KG

[illegible] MEASUREMENTS .... MID. ARM CIRCUMFERENCE OF MOTHER ....

TRICEP .... BICEP .... SUB SCAPULAR ....

TRICEPS .... BICEP .... SUB [illegible] ....

[illegible] MEASUREMENTS .... MID ARM CIRCUMFERENCE OF MOTHER .... KG

" MOTHER .... KG WEIGHT OF MOTHER ....

[illegible]

ABNORMAL ..... BREAST .....

HAEMOGLOBIN TONE ..... GM/100ml.

PLASMAALBUMEN .....

PROLACTIN RESPIRATION 30 MINS AFTER COMMENCEMENT OF A BREASTFEEDING

EPISODE ..... PROLACTIN CONCENTRATION 60 - 90 MINS

AFTER A SUCKLING EPISODE ..... TIME TAKEN .....

URINE SAMPLES

1ST WEEK ☐ 2ND WEEK ☐ 3RD WEEK ☐ 4TH WEEK ☐

SICK IF URINE SAMPLE WAS COLLECTED

WEIGHT OF BABY ..... NO LENGTH OF BABY .....

MID UPPER ..... CM CHEST CIRCUM OF BABY .....

MID ARM CIRCUMFERENCE OF BABY ..... CM .....CM

☐

PROJECT 89112

Appendix 6

TYPICAL DAILY WORK LOAD ASSESSMENT FORM

NAME..... OCCUPATION..... VILLAGE..... DATE.....

	05. 00	06. 30	07. 00	07. 30	08. 00	08. 30	09. 00	09. 30	10. 00	10. 30	11. 00	11. 30	12. 00	12. 30	13. 00	13. 30	14. 00	14. 30
Lying at rest																		
Walking																		
Sleeping																		
Standing																		
Cooking																		
House hold chores																		
Dressing																		
Washing																		
Office work																		
Operating foot machine																		
Operating electric machine																		
Driving																		
Teaching																		

	15. 00	15. 30	16. 00	16. 30	17. 00	17. 30	18. 00	18. 30	19. 00	19. 30	20. 00	20. 30	21. 00	21. 30	22. 00	22. 30	23. 00	23. 30	24. 00	24. 30
Lying at rest																				
Walking																				
Sleeping																				
Standing																				
Cooking																				
House hold chores																				
Dressing																				
Washing																				
Office work																				
Operating foot machine																				
Operating electric machine																				
Driving																				
Teaching																				