PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 1(3): 106-118, 2002

Why Breast Milk Has Health Benefits for Infants and Children: A Review

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Abstract: Breastfeeding is superior to formula feeding because it has specific and non-specific factors that have long term consequences for early metabolism and disease later in life. Human milk enhances the immature immunologic system of the neonate and strengthens host defense mechanisms against infective and other foreign agents. Mechanisms that explain active stimulation of the infant's immune system by breastfeeding are through bioactive factors in human milk such as hormones, growth factors, colony stimulating factors and specific nutrients. Human milk may show a reduced occurrence of disease because: 1) Mammalian evolution promotes survival advantage. 2) Factors that promote gastrointestinal mucosal maturation. 3) Factors that decrease the incidence of infection and alter the gut microflora. 4) Functional immunomodulatory and anti-inflammatory factors. 5) Hormones, growth factors and cytokines that may modulate the development of disease. 6) Reduced exposure to foreign dietary antigen. Following the termination of breastfeeding there is evidence of ongoing protection against illness due to influences on the immune system mediated via human milk. Industry continues to attempt to improve formula with the addition of compounds such as fatty acids, oligosaccharides, nucleotides and lactoferrin. However, human milk has such far reaching effects on the infant's immune response that normal development depends heavily on its provision. All mothers should be encouraged and supported to continue breastfeeding for six months and beyond in order to promote the good health of their infants.

Key words: Breastfeeding, infections, long-term effects, child health

Introduction

Breastfeeding is superior to formula feeding because it has specific and non-specific factors that have long term consequences for early metabolism and disease later in life. In this paper the scientific evidence in support of why breastmilk is beneficial for infants and children is summarised and the mechanisms whereby breastfeeding impacts on disease are explored.

The Epidemiological Evidence: The health hazards of bottle feeding were first reported in the early 20th century (Grulee C.G. *et al.*, 1934; Grulee C.G *et al.*, 1935). Mortality charts at that time showed a clear difference in risk of death between breastfed and bottle-fed babies. Since these earlier studies, evidence has emerged that breastfeeding may be directly responsible for reducing the incidence of many illnesses in infancy and childhood including acute diarrhea (Feachem and Koblinsky, 1984), lower respiratory tract infections (Wright *et al.*, 1989; César *et al.*, 1999), ear infections (Saarinen, 1982; Duncan, *et al.*, 1993) and asthma (Saarinen and Kajosaari, 1995; Oddy *et al.*, 1999). In addition, there is evidence that breastfeeding protects against less common illnesses such as necrotising enterocolitis (Lucas and Cole., 1990), botulism (Wigginton and Thill, 1993), urinary tract infections (Pisacane *et al.*,1992; Mårild *et al.*, 1990; Mårild *et al.*, 1989) sudden infant death syndrome (Ford *et al.*, 1993) insulin dependent diabetes mellitus (Gerstein, 1994) and childhood lymphoma (Davis, 1998).

There is also evidence that breastfeeding reduces the risk of immunologic disease including coeliac disease (Falth-Magnusson *et al.*, 1996), Crohn's disease [Peters *et al.*, 2001) and ulcerative colitis (Corrao *et al.*, 1998) although further confirmation of protection against these illnesses is required. In the longer term, breastfeeding appears to have beneficial consequences for metabolism, cognitive development and diseases later in life including cardiovascular disease, rheumatoid arthritis, multiple sclerosis and some cancers (Davis, 1998; Barker, 1997; Bergstrom *et al.*, 1995; Greene *et al.*, 1995; Drane and Logemann, 2000; Anderson *et al.*, 1999; Mason *et al.*, 1998; Pisacane *et al.*, 1994; Davis *et al.*, 1988).

Because breastfeeding stimulates the neonatal immune system, it may protect against autoimmune disease. Of interest is that breastfeeding enhances tolerance of maternal renal grafts (Campbell *et al.*, 1984), and that diabetes mellitus (an expression of autoimmunity) is reduced in breastfed children (Ellis and Atkinson, 1996; Borch-Johnsen *et al.*, 1984; Virtanen *et al.*, 1993). The possibility that the early introduction of 'other milk' (Vaarala *et al.*, 1998; Willems *et al.*, 1993) may increase the risk of type I and type II (Pettitt *et al.*, 1997) diabetes has been debated (Ellis and Atkinson, 1996) but the possible biological role of these observations is unknown (Mason *et al.*, 1998; Pisacane *et al.*, 1994; Fort *et al.*, 1990).

Compelling evidence for a relationship between breastfeeding and cognitive development now exists from longitudinal (Rogan and Gladen, 1993; Fergusson *et al.*, 1982; Rodgers, 1978; Lucas *et al.*, 1992; Horwood and Fergusson, 1998) experimental (Koletzko *et al.*, 1998) and neurodevelopmental studies (Wharton, 1992; Clandinin *et al.*, 1980; Farquharson *et al.*, 1992; Makrides *et al.*, 1994; Hamosh and Salem, 1998; Jenson *et al.*, 1992; Gibson and Makrides, 1998; Uauy and De Andraca, 1995).

It is biologically plausible that increased fatty acid exposure through breast milk may enhance brain development and learning ability. Epidemiological data do not allow certainty of knowledge that breast milk exposure does or does not enhance neurodevelopmental outcome (Feldman and Feldman, 1996). However, the effect of full breastfeeding to at least six months on the mean IQ of a population can be conceptualised as shifting the mean upward by 11 points for term infants and 8 points for preterm infants. Rogan and Gladden (Rogan and Gladen, 1993) noted that even minor changes in the mean IQ could significantly alter the number of children falling below a cut-off level.

Some of the levels of evidence in support of risk associated with lack of breastfeeding are summarised in Table 1.

Why Breastfeed? : Most women when asked why they want to breastfeed emphasise the bonding aspect. In fact, breast milk contains many substances that act as mediators between mother and child and establish a physiological and biochemical communication network, as an evolutionary extension of the intrauterine to extrauterine environment (Bernt and Walker, 1999). The extrauterine development of the human immune system is delayed (Goldman *et al.*, 1998) partly explaining why susceptibility of young infants to infections increases with prematurity. Immunologic agents are transmitted through amniotic fluid or the placenta during fetal life but the risk of infection to the newborn is lessened by breast milk feeding.

Table 1: Illness, disease and development feeding measure and risk ratio range.

Common illnesses	Reference	Feeding measure	Risk Ratio range*
Acute diarrhea	Victora and Barros, 2000	Breastfed < 3 months	6.10 (4.1-9.0)
Lower respiratory tract infections	(Wright *et al.*, 1989)	Breastfed < 4 mo/ sharing bedroom	3.29 (1.8-6.0)
Pneumonia	César *et al.*, 1999	No breastfeeding	16.7 (7.7, 36.0)
Ear infections (Recurring vs acute)	Duncan *et al.*, 1993	Breastfed < 6 months	1.61 (1.27, 1.79)*
Asthma	Oddy *et al.*, 1999	Breastfed < 4 months	1.25 (1.02, 1.52)
Atopy	Oddy *et al.*, 1999	Breastfed < 4 months	1.30 (1.04, 1.61)
Less common illnesses			
Necrotising enterocolitis	Lucas and Cole 1990	39% formula fed/ 7% breastfed	4.50 (3.00, 6.00)*
Urinary tract infections	Pisacane *et al.*, 1992	Never breastfed	1.62 (1.35, 1.78)*
Insulin dependent diabetes mellitus	Gerstein, 1994	Breastfed < 4 months	1.63 (1.22, 2.17)
Acute lymphobastic leukemia	Shu *et al.*, 1999	Never breastfed	1.21 (1.09, 1.30)*
Sudden infant death syndrome	Ford *et al.*, 1993	Current formula feeding	1.35 (1.09. 1.54)*
Cholera	Clemens *et al.*, 1990)	Not breastfeeding	1.70 p<.0001
Immunologic disease			
Coeliac disease	Falth-Magnusson *et al.*, 1996; Peters *et al.*, 2001	Breastfed < 3 months	1.63 (1.36, 1.79)*
Crohn's disease	Corrao *et al.*, 1998; Koletzko *et al.*, 1989a	Lack of breastfeeding	1.90 (1.50, 3.60)
Ulcerative colitis	Corrao *et al.*, 1998; Koletzko *et al.*, 1991b	Lack of breastfeeding	1.50 (1.10, 2.10)
Juvenile rheumatoid arthritis	Mason *et al.*, 1998	Lack of breastfeeding	1.60 (1.19, 1.80)
Multiple sclerosis	Pisacane *et al.*, 1994	Breastfed < 7 months	1.62 (1.26, 1.81)
Development			
Cognitive development in preterm	Lucas *et al.*, 1992	Lack of breastfeeding	↑ mean IQ of 8.3 pts
Cardiovascular disease	Bergstrom *et al.*, 1995	Lack of breastfeeding	↑ mean Tot Cholesterol
Metabolic development	Bergstrom *et al.*, 1995)	Lack of breastfeeding	↑ ApoB values
Obesity	von Kries *et al.*, 1999	Breastfed < 6 months	1.25 (1.02, 1.43)

* The risk ratios have been adjusted to reflect a level of risk of formula rather than protection of breast milk. This was done to ensure consistency of results.

Furthermore breastfeeding protects against infection well beyond the neonatal period (Clemens *et al.*, 1990).

The biochemistry of human milk encompasses a massive body of scientific literature most of which has been generated since the 1970's (Goldman and Goldblum, 1995; Wold and Hanson, 1994; Xanthou *et al.*, 1995; Hanson, 1998). Human milk changes composition from colostrum, to transitional, to mature milk and over time of day and as time goes by. Concentrations of protein, fat, carbohydrates, minerals and cells differ and physical properties such as osmolarity and pH change impacting on the physiology of the infant gut (Lawrence, 1999). More than 200 constituents have been discovered in human milk and the bioactivity and immunologic significance are yet to be explored in many. We now know that human milk contains an array of antimicrobial, anti-inflammatory, immunomodulating and bioactive molecules and compounds that are often multifunctional are adapted to mucosal sites and are not well represented in formula milks.

When human milk feeding is not practiced, reliable data on human milk constituents and their significance to infant health must be available for the preparation of formula by industry. However, there are myriads of factors that cannot be incorporated into formula. The adequacy of formula cannot be predicted from compositional analysis due to possible differences in compartmentalisation and the molecular form of nutrients (Picciano, 2001).

Industry continues to improve formula with the addition of compounds such as fatty acids, oligosaccharides, nucleotides and lactoferrin. It is clear however, that human milk has such far-reaching effects on the infant's immune response that normal immunologic development depends heavily on its provision.

Mechanisms Whereby Breastfeeding Could Impact On Disease: There are a number of reasons to expect that breastfed children may show a reduced occurrence of disease and illness (Table 2) (adapted from Chandra, 1991). The full sophistication of human milk has only recently become evident with many components exhibiting pleiotropic functionality (Garofalo and Goldman, 1999).

Mammalian Evolution Promotes Survival Advantage: Paleontologic evidence suggests that vertebrates evolved gradually from deuterostome ancestors about 500-600 million years ago, where many innovations were retained in their evolutionary descendants (Smith and Davidson, 1994). The evolutionary innovation defining mammals was the mammary gland developed in evolution about 190 million years ago (Blackburn, 1993), as a consequence of newborn receiving nutrients from secretions of the maternal ventral thorax/abdomen favouring maternal infant interaction. Evolutionary success is determined by an ability to not only reproduce but to cope with the environment, reach the reproductive period and assure the long term survival of offspring (Goldman *et al.*, 1998). In an evolutionary context, milk allows the infant to mature to a sexually active adult better able to cope with the environment.

The mammary gland evolved prior to the placenta, evidenced by monotremes, mammals with no placenta (Griffiths, 1988). Milk from humans displays certain similarities of the immune system to these. Lysozyme, abundant in milk and produced by mammalian apocrine glands (Ansai *et al.*, 1995) is phylogenetically very ancient and found in both monotreme and human milks (Tehan *et al.*, 1991). So too are transferrins and difucosyl lactose, an oligosaccharide that interferes with attachment of common toxins to epithelial cells (C. jejuni, e coli) (Crane *et al.*, 1994). These primordial immunologic adaptations may have evolved to protect the recipient infant from maternal bacterial flora by secreting antimicrobial agents.

Homosapiens emerged at least 100 000 – 200 000 years ago (Ayala, 1995) with evolutionary relationships in the milk composition from various species evident. Part of the switch from immune factors provided in eggs to the immune system produced by the mammary gland, was suggested by the discovery of lysozyme in tortoise eggs (Aschaffenburg *et al.*, 1980). A further clue was the discovery that the human immune system including IgA type is most similar to that of the chimpanzee (Cole *et al.*, 1992). The immune activities of the mammary gland allowed newborns of primitive mammals who had evolved a slow rate of

Table 2 : Mechanisms whereby breastfeeding has long-term health benefits

Mammalian evolution promotes survival advantage
Factors that promote gastrointestinal mucosal maturation
Factors that decrease the incidence of infection and alter the gut microflora/ breast milk microbiology
Functional immunomodulatory and anti-inflammatory factors.
Hormones, growth factors and cytokines that may modulate the development of disease
Reduced exposure to foreign dietary antigen

immune development, to survive (Goldman *et al.*, 1998).

Defense factors in the milk of species that experience developmental delays exist reciprocally with the production of immune factors by the lactating mammary gland in all mammalian species investigated to date. Thus the evolution of infant protection interacts with the mother. Certain immunological components are highly conserved, others vary according to the species with variations evolving by genetic mutation and natural selection.

Evolutionary adaptations enhance the survival of specific defense factors in milk in the recipient infant. Defense factors such as lactoferrin, lysozyme and secretory IgA are inherently resistant to digestion (Brines and Brock, 1983; Lindh, 1985) whereas others are compartmentalised and are shielded from digestive enzymes or denaturing conditions (Rudloff *et al.*, 1992; Rudloff *et al.*, 1993; Garofalo *et al.*, 1995) Anti-proteases in human milk such as α_1-antichymotrypsin and α_1-antitrypsin protect immune agents composed of protein in milk from digestion (Lindberg *et al.*, 1982). Moreover, factors in milk are protected at least during the first month of life because gastric production of hydrochloric acid (Euler *et al.*, 1979; Agunod *et al.*, 1969) and pancreatic secretion of chymotrypsin are very low in the neonatal period (Lebenthal and Lee, 1980; Boehm *et al.*, 1995).

Factors that promote gastrointestinal mucosal maturation: Biologically active factors in the infant gut have profound effects beyond nutrition (Thorell *et al.*, 1996). During life in utero, the infant swallowed amniotic fluid and epidermal growth factor and gastric inhibitory peptide in the fluid influenced intestinal growth and function (Perin *et al.*, 1997). Following the first feed of mother's milk in many mammalian species the most striking interaction between diet and intestinal development immediately occurs. This interaction may be due to an expression of genes triggered by the constituents in the milk.

Unlike formula milk, human milk contains hormones and growth factors that promote gastrointestinal maturation (Sheard and Walker, 1988) and host defense (Xanthou *et al.*, 1995; Xanthou, 1998). The growth modulating effect of human milk has been ascribed to a range of growth factors, hormones and other similar biologically active substances, which act synergistically. Systemic absorption and subsequent biologic activity at distant sites of these factors have been reported in new-born animals (Grosvenor *et al.*, 1992), and are currently under investigation in human neonates (Garofalo and Goldman, 1999). By direct uptake into the infant's circulation, the bioactive components in human milk may significantly influence general long-term tissue development (Hasselbalch *et al.*, 1996) as demonstrated in animal studies (Jain *et al.*, 1989).

A large number of enzymes are present in human milk (Hamosh, 1995). Some of these enzymes provide potential protection against disease. Anti-proteases and proteases modulate the proteolytic breakdown of milk proteins, many which have specific functions in the neonate (Lindberg *et al.*, 1982; Lindberg, 1979). Milk bile-salt-dependent-lipase digests milk triglycerides (99% of milk fat) in the intestine to free fatty acids and monoglycerides, contributing indirectly to lytic activity through resistance to bacterial and viral growth in milk (Sbarra *et al.*, 1996). The addition of lipases to human milk or formula increases anti-viral and anti-gram-positive microbial activity. Platelet-activating factor acetylhydrolase, an enzyme in human milk, protects the intestine of the neonate (Furukawa *et al.*, 1993) from inflammatory diseases (Caplan and Hsueh, 1990).

Factors that decrease the incidence of infection and alter gut microflora:

Human milk cells: Human milk cells found in colostrum and mature milk are functional and active (Goldman, 1993) and include macrophages, polymorphoneutrophils and lymphocytes (Carlsson and Hanson, 1994) that have the ability to phagocytose and kill bacteria and fungi (Robinson *et al.*, 1978; Keeney *et al.*, 1993). As well as phagocytes, numerous other epithelial cells and cell fragments are present in human milk (Michie *et al.*, 1998).

The lymphocytes in human milk are mainly T-cell in origin (about 80%) and contain both CD4 (helper-inducer) and CD8 (suppressor-cytotoxic) cells with a ratio similar or less than in peripheral blood (Keller *et al.*, 1986; Slade and Schwartz, 1989). Selective colonisation of the mammary gland during lactation by memory T-cells could be one of the mechanisms through which a breastfeeding infant gains from the mother's own immunologic experience. Human milk lymphocytes like macrophages elaborate a wide number of lymphokines (chemical factors produced and released by T lymphocytes that attract macrophages to the site of infection or inflammation and prepare them for attack).

Milk immunocompetent cells have the ability to survive in the gastrointestinal tract, to secrete bioactive factors including hormones, growth factors and cytokines, and to migrate across the neonatal intestinal mucosa into the systemic circulation. Milk cells are able to traverse intact human fetal intestinal implants in mice and migrate into mice tissues (Xanthou, 1998; Xanthou, 1997). This bioactivity allows milk cells to potentiate not only the local response of the gastrointestinal tract but also systemic immune responses (Xanthou, 1998).

Recently, innovative studies have demonstrated that the primary immunological organ in infancy and early childhood, the thymus gland, is significantly larger in four-month-old babies exclusively or partially breastfed compared to those not breastfed (Hasselbalch *et al.*, 1996; Hasselbalch, 1999). By direct uptake into the infant's circulation, the bioactive components of human milk appear to significantly influence general long-term tissue development. Imprinting in the neonatal period may determine these apparent long-term health outcomes (Michie *et al.*, 1998) particularly in relation to endocrinological development (Phillips *et al.*, 1993) by traversing gut epithelium in the presence of milk (Michie *et al.*, 1998) and stimulating the growth and development of other organ systems (Hasselbalch *et al.*, 1996).

Protein: Because the gut mucosa is immature, the newborn infant has a high macromolecule absorption that leads to small amounts of foreign protein activating the immunological system (Delire *et al.*, 1978). Exposure to cow's milk in early infancy has long-lasting effects on the humoral antigen-specific responses, indicating less effective tolerance-inducing mechanisms in the intestinal mucosa during the first months of life (Jenmalm and Bjorksten, 1998). The immunomodulatory function of milk proteins (Xanthou, 1998) has been well documented elsewhere (Garofalo and Goldman, 1999). In summary, protein is an important determinant of immune responses (Chandra, 1997).

Immunoglobulins: Immunoglobulins (Ig) are a group of glycoproteins present in the serum and tissue fluids of all mammals and are often called antibodies because they have the ability to fight foreign proteins (Roitt and Brostoff, 1996). The main immunoglobulin in human milk is secretory IgA (sIgA) (Hanson *et al.*, 1978). The specificity's of sIgA antibodies in breast milk reflect the maternal enteric and respiratory antigens,

providing the immunologically immature infant with protection against environmental pathogens that the mother has come into contact with. The antibodies in human milk have specific functions and protect against numerous illnesses (Clemens *et al.*, 1990; Tsutsumi *et al.*, 1989; Bell *et al.*, 1988; Cravioto *et al.*, 1991; Cruz *et al.*, 1985; Kim *et al.*, 1984).

Enterobronchomammary pathway: Because the immune system of the human baby is not fully developed and functional at birth (Hanson, 1998) a unique system called the enterobronchomammary pathway has developed whereby maternal protection is passed to the infant through breast milk. During gestation, tissue defense is transferred across the placenta. After birth, sIgA antibodies in the mothers' milk provide defense to the newborn's gastrointestinal mucosal surfaces (Roux *et al.*, 1977).

Gut microflora and breast milk microbiology : The mucosal immune system's most important task is control of colonisation of mucosal surfaces by organisms. The commensal (normal, healthy) microbial flora of the gastrointestinal tract as well as respiratory microbial agents stimulate the functional maturation of the immune system (Holt *et al.*, 1997). This is accomplished through humoral and cellular mechanisms which control the growth of bacterial, viral and parasitic organisms and non-cellular elements. Microbial products of the gastrointestinal flora may activate the antigen presenting mechanism of dendritic cells, polarising towards a Th_1 memory (Heufler *et al.*, 1996; Ridge *et al.*, 1996). In early life Th_1 and Th_2 cell populations possess the potential of reversibility towards the alternate cytokine type but the reversibility is lost after long term stimulation by microbes (Murphey *et al.*, 1996). The microbiology of breast milk has a large impact on bowel development and gut microflora with breastfed babies having a more healthy microflora than formula-fed babies' (Orrhage and Nord, 1999). Formula-fed infants have higher numbers and isolation frequencies of enterococci and clostridia in their faecal biliary than breastfed infants.
Other defense agents are created after partial digestion in the gastrointestinal tract of the infant. For example, antiviral lipids and monoglycerides once freed from milk fat by in vivo lipolysis disrupt enveloped viruses (May, 1994), and lactoferricin created by partial hydolysis of lactoferrin kills Candida albicans (Bellamy *et al.*, 1993), some enteric bacteria (Yamauchi *et al.*, 1993) and Giardia (Turchany *et al.*, 1995) by damaging their outer cell membranes (Bellamy *et al.*, 1993; Yamauchi *et al.*, 1993; Turchany *et al.*, 1995).

Functional immunomodulatory and antiinflammatory factors: Specific cell-mediated immunity and humoral response tests to antigen suggest that breastfeeding may have an immunoregulatory purpose (Garofalo and Goldman, 1999). Breastfed infants appear to have more effective immune function, reflected by an ability to mount a targeted response to a potential pathogen (Pabst *et al.*, 1997; Pabst, 1997).
Immunomodulating factors in human milk include α-tocopherol, β-casomorphins, prolactin and anti-inflammatory components. These direct-acting agents protect by non-inflammatory mechanisms, including enzymatic activity that degrades inflammatory mediators.

Anti-inflammatory components: Generally the anti-inflammatory components in human milk include vitamins A, C and E, enzymes, E prostaglandins, enzyme inhibitors, protease inhibitors, growth factors (eg. Epidermal growth factor and transforming growth factor alpha (TGFα) that promote gut maturation); anti-inflammatory cytokines and specific receptors for inflammatory cytokines (Hamosh, 2001).

Antioxidant defense system: Mammalian cells have developed an elaborate antioxidant defense system that includes both non-enzymatic antioxidants (e.g. glutathione, vitamins C and E {α-tocopherol} and β-carotene) and lactoferrin as well as enzymatic activities (e.g. glutathione peroxidase, catalase, and other hemoprotein peroxidases) both of which play a significant part in the anti-inflammatory system of human milk. As by-products of reactive oxygen species, oxidative stress (which is an excess production of reactive oxygen species) can damage cells by lipid peroxidation and alteration of protein and nucleic acid structures. The antioxidants, α-tocopherol and β-carotene are readily absorbed into the systemic circulation (Ostrea *et al.*, 1986) and are potent scavengers of oxygen radicals that may be produced at the mucosal sites of the infant. Lactoferrin in human milk may also act as an important antioxidant (Nuijens *et al.*, 1996).

Vaccine responsiveness: An infant's active immune response to specific antigens during the first year of life may be different in breastfed and formula-fed babies (Hahn-Zoric *et al.*, 1990). Evidence for this is that breastfed infants had enhanced vaccine responses (Hahn-Zoric *et al.*, 1990; Pabst *et al.*, 1989; Pickering *et al.*, 1998) when tested between nine and 20 months of age. Breastfed babies given BCG vaccination (Pabst *et al.*, 1989) either at birth or later had a significantly higher lymphocyte blast transformation response to purified protein derivative than those who were never breastfed. Following these findings a trial was conducted with Haemophilus influenzae B conjugate vaccine in breastfed versus formula fed infants (Pabst and Spady, 1990). Antibody response to foreign protein at seven and 12 months was significantly higher in breastfed compared to formula fed infants. Similarly at 21 to 40 months breastfed children had higher serum neutralising antibody titers and higher concentrations of salivary secretory IgA antibodies to polio, tetanus and diptheria toxoid (Hahn-Zoric *et al.*, 1990). Furthermore by in vivo measures, breastfed infants had a T-helper 1 type response to measles, mumps and rubella (MMR) vaccination, a response that enables division, differentiation and production of antibodies. Breastfed infants may have a clinical advantage over formula fed infants, due to their enhanced immune response (Pabst *et al.*, 1997).

Hormones, growth factors and cytokines that may modulate the development of disease: Milk provides essential nutrients for infant growth and development but also serves as transport from mother to infant of molecules that regulate development. Recent clinical and experimental observations suggest that, unlike formula milk, human milk not only provides passive protection but can also directly modulate the recipient infants developing immune system. The study of human milk is difficult due to the number of biochemically active substances and their biochemical complexity, the small concentration of some bioactive components, the compartmentalisation of some agents, the dynamic quantitative and qualitative changes of milk during lactation, and the lack of specific reagents to quantify the components. In spite of these difficulties hormones, growth factors and cytokines have been identified in human milk (Table 3) (Garofalo and Goldman, 1999; Goldman and Rudloff, 1991; Garofalo and Goldman, 1998; Srivastava *et al.*, 1996). It is likely that some of these substances are important for control and maturation of the neonatal immune system and gastrointestinal mucosa (Sheard and Walker, 1988).

Hormones: Milk hormones may be the product of local mammary synthesis, maternal transfer or mammary modification of blood borne- hormones. Many of these hormones are transferred from maternal blood to milk. They affect various aspects of growth, differentiation and functional maturation of specific organs in the infant. Resistance to digestion in the infant's digestive tract is increased by post transcriptional modification of peptide hormones in the mammary gland before secretion into milk (Hamosh, 2001). During critical periods of development the infant may be conditioned by the transfer of milk borne hormones (Ellis *et al.*, 1997). The effects of hormones may be immediate in the newborn, or delayed.

Table 3: Some of the biochemically active substances in human milk

Hormones		Cytokines	
Hypothalamic	GRH	Interleukins	IL-1αβ
	Somatostatin		IL-2
	TSH		IL-4
	Dopamine		IL-6
Pituitary	Growth hormone		IL-8
	ACTH		IL-10
	TSH		
	FSH/LH	Other cytokines	TNF-α
	Prolactin		IFN-γ
Thyroid	Triiodothyronin		TGF-β
	Thyroxin		RANTES
	Calcitonin		GROα
Parathyroid	PTH and PTH related peptides		MCP-1
Adrenal	Cortisol		MIP-1α
	Progesterone		
	Estradiol/Estriol	Colonystimulating factors	
	Testosterone		GM-CSF
Gastrointestinal	Gastrin		G-CSF
	Cholecystokinin		M-CSF
	GIP		
	VIP	Nutrients	
	Peptide YY		Protein
	Erythropoietin (EPO)		Nucleotides
			Glutamine
Growth factors	IGF		Lactoferrin
	EGF		Lipids
	NGF		Oligosaccharides
	MGF		
	bFGF		

Adapted with permission of the authors (Bernt and Walker, 1999)

Adrenocorticotropic hormone (ACTH) and cortisol have both been isolated in human milk. While ACTH modulates cortisol level, cortisol exhibits multiple functions including gene regulation. An example of this is the glycosylation pattern of the intestinal microvillus membrane favouring colonisation of the gut by non-pathogenic bacteria thereby providing protection against infection (Mahmood and Torres-Pinedo, 1985). Because the primary cause of necrotising enterocolitis is intestinal barrier immaturity cortisol's influence on intestinal barrier maturation is of particular interest. Cortisol also has immunomodulatory effects, increasing leukocyte counts while selectively suppressing B and T cell activation and lymphocyte counts, and inhibiting prostaglandin and leukotriene generation depending on conditions. Finally cortisol is involved in the regulation of intermediate metabolism and activation of energy in response to stress, a model for the transmission of environmental information through a biochemical message.

Prolactin (PRL) in milk is biologically active in the neonate and may regulate differentiation and maturation of neonatal neuroendocrine, reproductive and immune systems (Ellis *et al.*, 1997). This early conditioning may regulate neuroendocrine function later in life (Grosvenor *et al.*, 1992; Kacsoh *et al.*, 1991).

A wide array of gastrointestinal hormones has been isolated from milk. Gastrointestinal hormones constitute important components of epithelial host defense helping to prevent or delay gastrointestinal allergy and playing a role in gastrointestinal tract function as well as growth and maturation (Berseth *et al.*, 1990.

Growth hormone (GH) and growth hormone releasing hormone (GRH) have been isolated from human milk. There is good evidence that maternal GRH is involved in neonatal stimulation of pituitary GH synthesis in suckling rats (Kuhn *et al.*, 1978).

Erythropoietin (EPO) another hormone found in milk stimulates erythropoiesis in suckling rats (Carmichael *et al.*, 1992). Physiologically active EPO may be transferred to the infant from the mother or increased if a supplement is added to formula.

Other hormones have been found in milk including thyroid-releasing hormone, thyroid stimulating hormone, triiodothyronin and thyroxin, somatostatin and the estrogens, progesterone, androgens, calcitonin and parathyroid hormone. Interactions of these hormones with the infant endocrine system can be demonstrated but their clinical relevance for the infant's development remains unclear (Grosvenor *et al.*, 1992).

Growth factors: Human milk contains several known growth factors including Insulin like Growth Factor (IGF), Epidermal Growth Factor (EGF) and Transforming Growth Factor (TGF) (Koldovsky and Goldman, 1998). These factors promote the maturation of gastrointestinal mucosa restricting the penetration of harmful antigenic material indirectly contributing to the anti-inflammatory effect of human milk. In general IGF is a comprehensive mediator of growth and development and some animal studies have detected a positive effect of IGF supplementation to formula fed infants (Houle *et al.*, 1997)

Transforming Growth Factor Beta (TGF-β) in human milk has important immunomodulatory properties (Cummins and Thompson, 1997) and directly affects immunity and inflammation by suppressing the proliferation and modulating the activity of leukocytes. TGF-β inhibits the production of cytokines such as IL-1, IL-6, and TNF; reduces the expression of the human leukocyte antigen (HLA)-DR on antigen-presenting cells; and inhibits the synthesis of nitric oxide by IFN-γ-activated macrophages (Ding *et al.*, 1990).

Nerve growth factor (NGF) found in milk triggered speculation about its role in neuronal development and cognitive function. It's uptake in a newborn rat model ileum has been shown (Siminoski *et al.*, 1986) and although there is no evidence yet that NGF stimulates cerebral growth or function some studies have reported a statistically improved outcome for cerebral dysfunction in premature infants fed breast milk (Morley and Lucas, 1994) and better school results in breastfed 'normal' children (Rogan and Gladen, 1993).

The presence of growth factors in milk provides a fascinating model for the developmental benefit of breastfeeding. However, even though numerous factors including mammary-derived growth factor and basic fibroblast factor have been isolated from milk many of their roles are unresolved.

Cytokines: Cytokines are pluripotent polypeptides that act in autocrine/paracrine fashions by binding to specific cellular receptors. They operate in networks and orchestrate the development and functions of the immune system. Cytokines influence the development and immunologic function of the mammary gland and the neonate. The cytokines IL1, IL6, IL10, G-CSF, MCSF, TNFα, interferon gamma, have all been found in human milk (Goldman *et al.*, 1996).
Mononuclear cells of human milk have a potential for production of many different cytokines and when nitrogen stimulated in vitro (Skansen-Saphir *et al.*, 1993) mononuclear cells are potent producers of cytokines. However in vivo implications of these findings remain to be investigated.

Colony-stimulating factors: Unlike hormones that act as systemic messengers, Colony-stimulating factors (CSF) stimulate cell growth and are synthesised in the bone marrow. Granulocyte (G)-CSF stimulates the proliferation of granulocyte progenitor cells and leads to their increase in circulation.

Nutrients: Besides mediators such as protein, hormones, growth factors and cytokines some other simple nutrients are able to transmit biochemical messages to the developing infant.

Nucleotides: Nucleotides in milk have multiple functions (Thorell *et al.*, 1996; Moya, 1991; Ebrahim, 1998), which include effects on gut microflora (Gil *et al.*, 1986), effects on intestinal growth and development (Uauy *et al.*, 1990) and effects on the response to immunisation (Pickering *et al.*, 1998). Nucleotides are involved in a wide variety of biological processes that include serving as precursors of RNA and DNA and comprising bases for the high energy source ATP, regulatory signals (cyclic AMP and cyclic GMP), coenzyme components and methyl donors.
The effects of nucleotides support the hypothesis that human milk is a potentially potent biological food, which serves to fine-tune the growth and maturity of function in a number of physiological systems as well as providing nutrition. Infant formulae supplemented with nucleotides have been developed by industry and are on the market although the full implications of such additions are not properly understood or biologically tested.

Glutamine: The amino acid glutamine, rich in human milk, has important influences on the metabolism and function of enterocytes and cells of the lymphatic system (Newsholme and Calder, 1997). In an experimental animal model of gut-derived sepsis, oral glutamine demonstrated an ability to decrease bacterial translocation through the intestinal wall and to enhance the destruction of bacteria following infection with E coli, an effect that improved considerably when the animals were substituted with glutamine (Gianotti *et al.*, 1995).

Lactoferrin: Lactoferrin, the major milk protein is lymphostimulatory, anti-inflammatory, bactericidal, viricidal and fungicidal. The protective function of lactoferrin was initially ascribed to its iron binding capacity, but its low iron saturation (6% - 9%) and high iron affinity suggests that it could act as a bacteriostatic agent in human milk (Peterson *et al.*, 1998). Indeed, a broad spectrum bactericidal peptide has been isolated following gastric cleavage of lactoferrin (Bellamy *et al.*, 1992; Tomita *et al.*, 1994).
The immunomodulating activity of lactoferrin is due to specific binding of lactoferrin receptors on monocytes or macrophages (Miyazawa *et al.*, 1991) that appear to inhibit cytokine production (Misra *et al.*, 1994). Lactoferrin inhibits the discharge of interleukins 1,2 & 6 and TNF-α from monocytes and of prostaglandin E_2 from macrophages protecting against inflammation (Mattsby-Baltzer *et al.*, 1996; Bartal *et al.*, 1987; Mechnicki *et al.*, 1993).

Lipids: Afte proteins milk fat globules are the second most abundant component of human milk. The fat in human milk is contained within these globules, the core of which is made up of fatty acids and triglycerides. Unsaturated fatty acid chains have been shown to inhibit parasite adherence (Crouch *et al.*, 1991) and disrupt enveloped viruses (Thormar *et al.*, 1987; Isaacs and Thormar, 1991). Although lipolysis generates similar products for formula-fed and breastfed infants, breastfed infants have higher rates of lypolysis associated with the fat structure and the presence of the enzyme, milk-salt-dependent-lipase.
Lipids in human milk are a source of the "nutritionally essential" fatty acids, linoleic (18:2 n-6) and linolenic (18:3 n-3). Breast milk also contains other very long chain polyunsaturated fatty acids such as docosahexanoic acid (DHA) and arachidonic acid (AA) that have been linked to visual and cognitive function in human infants through randomised controlled trials. As a result of these trials some formulas for preterm and term infants are now supplemented with DHA and AA (Gibson *et al.*, 2001).
Human milk contains a wide range of long chain polyunsaturated fatty acids (LCFUFA) most importantly DHA (22:6ω3), whereas conventional formulas contain only a small amount although there is no convincing evidence concerning the effects of long-chain PUFA supplementation on long-term cognitive development (Jenson *et al.*, 1992). Lipids also act as carriers of fat-soluble vitamins and hormones (Gibson and Makrides, 1998; Makrides *et al.*, 1996), as precursors of biologically potent mediators (eg prostaglandins, thromboxanes, leukotrienes) and as vital structural components of membrane systems in all tissues (Innis, 1994; Calder, 1997). In particular the long chain fatty acids (Calder, 1997) in breast milk have been implicated as potential modulators of the immune system (Hasselbalch *et al.*, 1996; Goldman, 1993; Pabst *et al.*, 1997; Miles and Calder, 1998; Wold and Adlerberth, 1998; Howie *et al.*, 1990). For the possible influence of DHA and AA on the developing immune system a lower proportion of CD45RO+ cells and deficient Interleukin-10 production by formula-fed infants, compared with human-fed was corrected with supplementation of long-chain polyunsaturated fatty acids (Field *et al.*, 2000).

Oligosaccharides and glycoconjugates: Human milk is unique because of its high concentration of complex oligosaccharides compared with milk from other species (Pabst, 1997). The oligosaccharides are quantitatively one of the three main components of human milk in addition to protein and fat with more than 100 structures described (Peterson *et al.*, 1998; Newburg, 1996). The structure of oligosaccharide mimics that of bacterial receptors on intestinal cells, blocking bacterial attachment to intestinal cell membranes (Goldman *et al.*, 1986). They can function as receptor analogs for bacteria and viruses and inhibit the adhesion of microbes (including pneumococci) (Andersson *et al.*, 1986). Oligosaccharides also influence intestinal flora growth by the provision of substrate for lactobacillus bifidus, the healthy bacteria, while limiting the growth of potentially pathogenic bacteria (Carlson, 1985).

Reduced exposure to foreign dietary antigen: Although a breastfed infant is less exposed to foreign dietary antigen in cow's milk (Höst, 1994), there are also antigens in maternal milk. More than 70 years ago it was hypothesised that infants might react to foods in the mother's diet, such as egg or cow's milk protein, transmitted through her milk (Shannon, 1921; Vandenplas, 1997). Some exclusively breastfed infants develop allergic reactions to cow's milk protein (β-lactoglobulin) (Lifschitz *et al.*, 1988) but the incidence of this is very low (0.5-1.7%) in comparison to the incidence in unselected populations of infants (2%-3%) (Businco *et al.*, 1993).
In most lactating women (50-95%) the cow's milk protein, β-lactoglobulin can be detected in small concentrations about eight hours after ingestion with continuous testing of breast milk (Sorva *et al.*, 1994). Given the low frequency of cow's milk allergy in breastfed infants, the small measure of β-lactoglobulin found in

Table 4: Ten steps to successful breastfeeding (World Health Organisation, 1989)

Have a written breastfeeding policy that is routinely communicated to all health care staff.
Train all health care staff in skills necessary to implement this policy.
Inform all pregnant women about the benefits and management of breastfeeding.
Help mothers initiate breastfeeding within a half-hour of birth.
Show mothers how to breastfeed, and how to maintain lactation even if they should be separated from their infants.
Give newborn infants no food or drink other than breastmilk, unless medically indicated.
Practice rooming-in - allow mothers and infants to remain together 24 hours a day.
Encourage breastfeeding on demand.
Give no artificial teats or pacifiers (also called dummies or soothers) to breastfeeding infants.
Foster the establishment of breastfeeding support groups and refer mothers to them on discharge from the hospital or clinic.

breast milk may induce tolerance rather than allergic sensitisation (Vandenplas, 1997) and may help to explain the almost constant presence of β-lactoglobulin in mother's milk (Host *et al.*, 1995; Stuart *et al.*, 1984). Furthermore, dietary proteins other than cow's milk antigens (such as egg proteins) are transferred to breast milk and can induce adverse reactions in hypersensitive infants (De Boissieu *et al.*, 1994; De Boissieu *et al.*, 1997).

On the other hand, a maternal diet low in allergens in a lactating mother until the infant is six-months-of-age may be the most relevant factor in protecting the infant from the development of atopic disease (Zeiger and Heller, 1995; Zeiger, 1994; Hide and Guyer, 1981; Bruno *et al.*, 1993) . Family history however, may be one of the confounding factors for allergy independent of breastfeeding (Lucas *et al.*, 1990). Although evidence is conflicting, current advice from the European Society of Paediatrics is that if a family history of asthma is present it is best to breastfeed, and cow's milk and eggs should be avoided by the mother (Host *et al.*, 1999). Many children *do* receive cow's milk proteins in the first days of life (Höst, 1994; Saarinen, 1997) that may initiate sensitisation in susceptible individuals, and subsequent exposure even to minute quantities of β-lactoglobulin in breast milk may elicit an allergic manifestation that may be associated with IgE mediated adverse reactions.

The young infant's gut is immature, and may poorly exclude multiple allergens or large quantities of allergens that can react with the system of sensitisation. The benefits of exclusive breastfeeding derive not only from elimination of cow's milk protein but from local protection of human milk in the bowel. For example secretory IgA coats the mucosa and blocks entrance of antigens (Taylor, 1973). Because of this, recommendations to exclusively breastfeed and to withhold infants from solid food until after six months of age may be well founded.

Contraindications to breastfeeding : A balanced discussion of the contraindications to breastfeeding is credible only when the enormous benefits of breastfeeding to infants and to mothers are considered together with the risks for any possible contraindication (Lawrence and Lawrence, 2001).

Poor maternal diet is not a contraindication to breastfeeding and all mothers should be counseled to eat appropriately. Even in apparently well nourished women milk vitamins D and K may not always provide adequate amounts for infants. Therefore, it is recommended that all infants receive vitamin K at birth to prevent hemorrhagic disease of the newborn caused by vitamin K deficiency in the first few days of life (Lawrence, 1999).

If and when a medical situation arises in the mother that poses a threat to the breastfeeding infant, the theoretic risk needs to be measured against the projected benefits of breastfeeding (Lawrence, 1997). Maternal infectious disease is not a contraindication to breastfeeding in most cases (Beaudry *et al.*, 1995). The most infectious disease that is a contraindication to breastfeeding however, is Human Immunodeficiency Virus (HIV). HIV type 1 can be transmitted through human milk, and detailed suggestions for implementation of the WHO current policy are available (World Health Organisation, 1998). The policy states that in most countries policy must cover a range of socioeconomic conditions, and the aim should be to promote and protect breastfeeding for the majority of women while offering as many choices as possible to women who are HIV positive, enabling them to decide what is most appropriate for their circumstance and supporting them in their choice. Human T-cell Lymphotropic Virus (HTLV-1) is another distinct retrovirus, epidemic in parts of the world and the only other infectious disease that is an absolute contraindication to breastfeeding (Lawrence and Howard, 1999).

Certain maternal diseases may be a contraindication to breastfeeding because of the treatments that may cross into the mother's milk. An example is penacillamine because it binds minerals and the effect on the infant's microminerals may present a significant risk.

In rare cases an infant has unique nutritional needs for example if the infant has a metabolic enzyme deficiency disease such as galactosemia, phenylketonuria or maple syrup urine disease. These infants may be partially breastfed, supplemented with special formula and monitored closely (Lawrence, 1999).

Chemicals in the environment may pose a risk to breast milk where unusual exposure does occur. A massive exposure of polybrominated biphenyls entered the food chain in the 1970's exposing women in the immediate geographic area (Poland and Cohen, 1980). The exposure of the general public to herbicides and dioxins is generally minimal and the WHO does not consider DDT a major cause for concern. Breastfeeding is not contraindicated in association with environmental hazards under ordinary circumstances with excessive exposure assessed on an individual basis.

In face of any potential contraindication to breastfeeding the benefits to the infant of being breastfed must be compared with the theoretic risk for the determined hazard and a decision made on an individual basis.

Effects of breast milk in premature infants: The American Academy of Paediatrics in 1997 acknowledged that human milk is beneficial in the care and management of premature infants. The special needs of premature infants that arise due to metabolic and gastrointestinal immaturity, immunologic compromise and associated medical conditions must be considered in order for adequate nutrition to be provided to meet the needs for intrauterine rates of growth and nutrient accretion (Ziegler *et al.*, 1976).

Although the optimum nutrition of premature infants is not known, recent data suggest that human milk fortified with additional nutrients is more appropriate for tube fed infants than unfortified human milk (Schanler, 2001). Such manipulation of milk may affect milk's intrinsic host defense properties but fortified human milk may provide significant protection from infection and necrotising enterocolitis (NEC). Furthermore skin to skin contact provides species specific anti-microbial protection. Additional investigation is required but neonatal centers should encourage the feeding of fortified human milk in addition to skin to skin contact as methods to enhance maternal milk production and the development of the enteromammary response.

Premature infants fed their own mothers milk have fewer episodes of NEC, diarrhea and urinary tract infection than premature infants fed formula suggesting that human milk may enhance premature infants host defenses. Because di*et al*so affects fecal flora, the flora of human milk-fed babies is less pathogenic than those fed formula.

International support for breastfeeding: Universal breastfeeding has been the goal of the World Health Organisation; American Academy of Pediatrics and many other organisations for nearly a quarter of a century. The World Health Organisation International Code Of Marketing Of Breastmilk Substitutes was developed in 1981 (World Health Organisation, 1981). The aim of the Code was to contribute to the provision of safe and adequate nutrition for infants, by the protection and promotion of breastfeeding and by the proper use of breast-milk substitutes, when these are necessary, on the basis of adequate information and through appropriate marketing and distribution. The Code also forbid inducements to health workers (Taylor, 1998) to promote specific breast-milk substitutes (Chren and Landefeld, 1994).

There is enough evidence to support maternity facilities and the ten steps to successful breastfeeding (Table 4). This will ensure that hospitals become 'baby friendly', that mothers are encouraged and supported to commence breastfeeding and that there is adequate community support for the continuation of full breastfeeding for at least the first six months of an infants life and beyond.

In conclusion, breastfeeding has numerous factors such as hormones, cytokines and other bioactive compounds that protect against disease in infancy and promote optimum development. The current level of knowledge about breast milk and the mechanisms whereby breastfeeding impacts on infant health and development provides evidence that breastfeeding should be promoted to the public. The aim must be to increase the duration of full breastfeeding for all infants to at least six months and beyond up to two years.

Acknowledgement

This article has been adapted from Oddy WH. Long term health benefits and mechanisms associated with breast feeding. Expert Rev. Pharmacoeconomics Outcomes Res. 2002; 2(2): 89-105.

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Pakistan Journal of Nutrition 1 (3): 119-120, 2002

Adjuvant Nutritional Therapy in the Management of Malnourished Cancer Patients

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Abstract: A new Nutritional Adjunct was tested as an adjuvant with radiotherapy for cervical cancer in Dhaka Medical College Hospital, Bangladesh. In addition to the cancer induced malnutrition, most Bangladeshi people are basically malnourished. The main objective of this study was, therefore, to examine if this newly patented Nutritional Adjunct could at least be useful in preventing further deterioration of the nutritional status of cancer patients receiving chemo- and/or radiotherapy. Twenty female subjects with stage III cervical cancer participated in this preliminary case-control clinical trial. Ten subjects receiving the Nutritional Adjunct showed a significant improvement in their nutritional status, at least in terms body weight changes and a general feeling of wellness, compared to the patients in the control group. Furthermore, the bone marrow status, as measured by the platelet counts (PC), was found to be enhanced significantly ($p < 0.001$), i.e. PC was increased from $2.6 \pm 0.10 \times 10^4$ Cu mm to $3.9 \pm 0.3 \times 10^4$ Cu mm, in the supplemented group. These preliminary results, therefore, strongly suggest prospective full clinical trial to ascertain any therapeutic efficacy of this new Nutritional Adjunct.

Key Words: Nutrition, cervical cancer, radiotherapy

Introduction

Malnutrition plays a major role in the morbidity of cancer patients receiving chemo-and/or radiotherapies. Such health condition may be attributed to the nutritional status of the patients prior to the development of cancer, to the carcinogenesis and/or to the therapeutic regimens. Aggressive therapeutic modalities along with progressive malnutrition may result in the death of a patient. Cachexia and anorexia are the common features observed clinically in cancer patients. Even well-nourished patients undergoing radiation therapy for the cervical, prostate and bladder cancers develop diarrhoea and nausea resulting in anorexia and cachexia(Bosch and Frias, 1977; Weisbrot *et al.*, 1975; Harding *et al.*, 1990). Parenteral and enteral nutritional supports are usually given as adjunct to the cancer therapies (Robuck and Fleetwood, 1992; Sax and Souba, 1993), although with little or no significant success in alleviating these conditions. Nutritional pharmacology, however, is an emerging field where one should manipulate nutritional requirement of cancer patients to achieve desired physiological results. Most Bangladeshi patients are basically malnourished. We always, therefore, search for any nutritional adjuvant for the managements of our cancer patients receiving either chemo- and/or radiotherapies. In this respect, a new Nutritional Adjunct has recently been patented (Khaled, 1999) and claimed to combat antineoplastic therapy-induced toxicities. This product is a composite of several nutrients chelated together in order to enhance their bio-availability. We procured this product and conducted a preliminary a case-control clinical trial on cervical cancer patients receiving radiotherapy.

Materials and Methods

Female patients, between the age of 25 to 50 years, with cervical cancer, stage III, were recruited for this study under the approval of the Dhaka Medical College Hospital, Dhaka, Bangladesh. Stage III cervical cancer indicates an advance state of carcinoma that involves the lower third of the vagina extending up to the lateral pelvic wall. Patients normally at this stage report to the hospital with poor health conditions, particularly with progressive weight loss and anemia. Twenty such patients participated in this preliminary study, half belonging to control groups (10 patients) who received placebo (similar capsules filled with cooked and dried rice powder) and other half (10 patients) received the Nutritional Adjunct (2 caps, three times/day with meals) four days prior to the initiation of radiation therapy and continued until the end of the therapy. The Nutritional Adjunct was supplied by the Life Sciences' Technologies, Inc. (LSTI), Birmingham, Alabama (USA). A cobalt-60 teletherapy machine was used in this study. Irradiation dose was 45 to 50 Gray given over a period of 4.5 to 5.0 weeks.

Blood samples were collected before giving either placebo or the Nutritional Adjunct and after completing the radiation therapy. These samples were analyzed particularly for hemoglobin (Hb), white blood cell (WBC) and platelet count (PC). Clinically patients conditions were monitored for weight loss, vomiting, nausea, diarrhea, loss of appetite (leading to anorexia), lethargy (loss of physical energy) and most of all, their feeling of wellness. The blood biochemical parameters, as obtained in this study, are listed in Table 1. Table 2 gives the subjective clinical observations that were made during the period of radiation therapy. Energy and/or a feeling of wellness in each patient were assessed from their day to day's activity. For example, the control patient who received placebo could walk only a few steps compared to the patients receiving this Nutritional Adjunct. The control patients always felt tired and some of them even had to be carried with a stretcher from their bed to the radiation clinic, whereas the supplemented patients walked the same distance (about 250 ft) easily and comfortably.

Results and Discussion

Loss of body weight (cachexia) and appetite (anorexia) is a serious problem usually encountered during the treatment of cancer patients with chemo- and/or radiotherapies. Such condition was obviously observed in the control patients in this study whose mean body weight decreased to 36.7 ± 5.5 kg from a pretreatment body weight of 41.1 ± 4.4 kg. Statistically this is a significant ($p = 0.06$) weight loss over a period of 4 to 5 weeks. Nutritionally supplemented patients, on the other hand, did not show any reduction in their body weight (Table 1), rather, some of them gained some weight, albeit not significantly. Stability of body weight, particularly during the therapy, is highly desirable.

Bone marrow toxicity (myelosuppression) due to chemo-and/or radiotherapies many times poses life threatening problem to the cancer patients by making them more prone to many opportunistic diseases (Fyles *et al.*, 1995; Makino *et al.*, 1995). In fact, antineoplastic therapy-associated toxicities may be the secondary factors of patients mortality (Rudolph *et al.*, 1994; Hahn *et al.*, 1994). Moreover, success of any anticancer therapy could be dependent on immuno-compatibility, which may be

Table 1: Objective observations on the biochemical parameters of cervical patients before and after the treatment

Parameters		Control (n = 10)	p^a	Supplemented (n = 10)	p^a	p^b
Age (year)		39.6 ± 11.8	---	40.1 ± 11.2	-----	0.92
Height (cm)		151.1 ± 5.9	---	152.8 ± 6.4	-----	0.54
Weight (kg)						
	Pre[c]	41.1 ± 4.4	0.06	38.1 ± 4.1	0.96	0.13
	Post[c]	36.7 ± 5.5		38.2 ± 3.8		0.49
Hemoglobin (%)						
	Pre	61.7 ± 3.3	< 0.001	61.3 ± 3.6	< 0.01	0.80
	Post	48.7 ± 3.3		53.2 ± 4.3		0.02
WBC (Cu mm x 10^3)						
	Pre	10.3 ± 0.9	< 0.001	9.4 ± 1.0	0.59	0.049
	Post	8.2 ± 0.5		9.6 ± 0.6		< 0.001
Platelet (Cu mm x 10^4)						
	Pre	2.5 ± 0.2	0.017	2.6 ± 0.10	< 0.001	0.17
	Post	2.2 ± 0.3		3.9 ± 0.30		< 0.001

p^a = Statistical significance within the group, p^b = Statistical significance between the groups, i.e. control and supplemented
c = Before (pre) and after (post) supplementation

Table 2: Subjective observations on the health status of cervical patients at the end of the treatment

Parameters	Control	Supplemented
Cachexia	Yes	No
Anorexia	Yes	No
Nausea	Yes	No
Anemia	Yes	No
Feeling of wellness	No	Yes
Feeling of energy	No	Yes
Consent to further participation	No	Yes
Attitude towards life	Negative	Positive

severely compromised in malnourished patients and/or may be altered due to the therapeutic modalities. The measured mean value of platelet count (PC), i.e. the bone marrow status, in the nutritionally supplemented patients in this study was found to be enhanced from 2.6 ± 0.10 x 10^4 Cu mm to 3.9 ± 0.3 x 10^4 Cu mm which is significantly higher within the group and between the groups, i.e. control versus supplemented. This therefore indicated that the Nutritional Adjunct not only prevented the bone marrow depletion, rather increased it significantly (Table 1).

From the subjective clinical examinations, as listed in Table 2, it could be noted that the most important observation was the feeling of wellness and energy by the patients receiving Nutritional Adjunct. This is extremely helpful to cancer patients undergoing any aggressive therapeutic modalities. It was surprising to see that the nutritionally supplemented patients left hospital after completion of radiation therapy with a feeling of wellness and energy while the control patients appeared more malnourished with some kind of discomfort prevailing in them. While a follow-up examination of these patients will determine any therapeutic efficacy, these preliminary results, however, warrant prospective full clinical trial of this new Nutritional Adjunct on a larger cohort of cancer patients populations using perhaps more cytotoxic therapeutic modalities.

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Pakistan Journal of Nutrition 1(3): 121-123, 2002

The Nutritional Status of School-Aged Children in an Urban Squatter Settlement in Pakistan

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Abstract: A study was carried out to determine the nutritional status of school-aged children living in an urban squatter settlement in Islamabad, Pakistan. 200 children were selected through systematic random sampling from 1479 children aged 5-10 years living in 1147 households. Measurement of height and body weight revealed a high prevalence of malnutrition among these children. The prevalence of underweight (< 2 standard deviations below the NCHS standard for weight-for-age) was 29.5%, wasting (< 2 SD below standard weight-for-height) 13% and stunting (< 2 SD below standard weight-for-height) 35%. Overall 44% of the children had one or more of underweight, wasting or stunting. Severe malnutrition (< 3 SD below the standard value) was present in 15.4% of the children. The prevalence of malnutrition was significantly higher among older children and those from larger, poorer households. The study recommends the inclusion of school-aged children in the Pakistan National Nutrition Survey.

Key words: School-aged, children, Pakistan, malnutrition

Introduction

Malnutrition is a major public health problem in Pakistan, a South Asian nation with over 130 million people. Half of its children aged five years or less are stunted, over a third (38%) are underweight, and a quarter of all births are low birth weight (Bhutta, 2000). These high levels of malnutrition contribute to about half of the 740,000 child deaths that occur every year in Pakistan (UNICEF, 1996).

In view of the scale of the problem in children under five, nutritional programmes in Pakistan during the last few decades have been targeted at this age group. However, malnutrition is a significant problem in older children as well, a fact that is often overlooked by policy makers and programme managers. Though little is known about the state of nutrition in this older group, studies conducted in the 1980s indicate that malnutrition is a significant problem in this population, with prevalences ranging from 47-70% in male school children in rural Pakistan (Wahab *et al.*, 1993; Ahmad and Gilani, 1998).

The situation among school-aged children in urban squatter settlements in Pakistan is even less well known. These settlements contain a large proportion of the rapidly growing urban population, with high levels of malnutrition already documented in the under-five child population (Fikree *et al.*, 2000).

Given the potential for both short and long term health and behavioural consequences of malnutrition in school-aged children (Brown and Sherman, 1995; Haas *et al.*, 1996; Martorell, 1995), a nutritional study was conducted in 1999 in a peri-urban squatter settlement near Islamabad, the capital city to assess the magnitude of the problem. This paper describes the nutritional status, and associated socio-demographic characteristics, of children aged 5-10 years in the squatter settlement.

Materials and Methods

A descriptive cross-sectional design was used for the study, employing a combination of quantitative and qualitative research methods. These included anthropometric measurements of the children, structured and semi-structured interviews with their mothers about socio-economic status and feeding practices, and focus group discussions with mothers, fathers and mother-in-laws.

An electronic weighing scale, which was standardised daily with a standardised weight, was used to measure the weight of children. A standard height scale was used for measuring height. Time and resource constraints dictated that only 200 households containing children aged 5-10 years could be studied. As there were just over a thousand households in the area, systematic random sampling of every fifth household was carried out. The household for the starting point was randomly selected. If there were no children aged 5-10 years in a household, the next fifth household was selected. If there were more than one child in a household, the children were allotted numbers, and one child randomly selected by drawing lots.

The squatter settlement chosen was Muslim Colony at Noorpur Shahan, 7 kms north of Islamabad. A mazaar or shrine in memory of a renowned 16th century Sufi mystic, Syed Abdul Latif Shah Mashadi, is located nearby. Most of the residents have been displaced by economic hardships, natural calamities or war in their home villages in Kashmir, the North-West Frontier Province or Punjab. Besides being broadly representative of other squatter settlements in Islamabad, the area was also chosen because it was accessible and familiar to the principal researcher (RMAM) who had previously worked in the Federal Government Services Hospital which provides preventive and curative services to the residents of Islamabad and its surrounding areas. The population of the community was 5914, living in 1147 households. The number of children aged 5-10 years was 1479.

Quantitative data were analysed using the SPSS statistical software. Qualitative data analysis involved content analysis, identification of themes, summarising, interpretation and presentation as outlined by Bernard, 1995.

Results

Socio-economic context: Most of the houses in the area are built of mud brick with tin roofs. There is no piped water supply, the only source being a few communal standpipes. Cooking is with wood-fired stoves outside the house during summer and inside during winter. There is no power supply to the community, although a few of the better-off households share the use of a generator.

The average household size was 5 members. The majority of households (67%) surveyed reported a monthly income of less than Rs 3000 (approximately US$70). The great majority (95%) of mothers worked as housewives, with the rest working as domestic helps, cooks, or seamstresses. The occupations of fathers included daily labour (40%), salaried work (26%), and small business or trade (18%). The mean maternal age was 33 years (median 34 years), with about half between the ages of 25 and 34 years. The great majority of mothers (84%) had no schooling at

Table 1: Nutritional status of children aged 5-10 years

Z score	Weight-for-age		Height-for-age		Weight-for-height	
> 0	17	(8.5)	22	(11)	58	(29.5)
0 to –1	46	(23)	42	(21)	55	(27)
-1 to –2	78	(39)	66	(33)	61	(30.5)
-2 to –3	47	(23)	47	(23.5)	17	(8.5)
-3 to <-4	12	(6.5)	20	(11.5)	9	(4.5)
Total	200	(100)	200	(100)	200	(100)

Note:Figures in parentheses are row percents.

Table 2: Age and malnutrition

Age group (years)	No.of children malnourished		Total	
5-6	30	(44%)	69	(100%)
6-7	19	(35%)	55	(100%)
7-8	20	(47%)	43	(100%)
8-9	10	(59%)	17	(100%)
9-10	9	(56%)	16	(100%)
Total	88	(44%)	200	(100%)

Malnutrition defined as <-2SD for any indices, Percentages are row percents, Chi square for linear trend: 9.7, p value < 0.01

all, compared to 53% of fathers.

Nutritional status: Overall, 44% of the children in the study could be classified as having one or more of underweight, wasting, or stunting. Just over 15% of the study children were severely malnourished, having a z score less than – 3 standard deviations (SD) for any index. Table 1 shows the distribution of nutritional indices in the study children.

Weight-for-age: Almost one third of children (30%) were underweight, as judged by a z score for weight-for-age less than –2 SD. Twelve children (6%) were severely underweight (z score < – 3 SD).

Height-for-age: Over a third of the children (35%) had a z score for height-for-age less than – 2 SD. Twenty children (10%) were severely stunted (z score < – 3 SD).

Weight-for-height: Wasting, as defined by a z score less than – 2 SD for weight-for-height, was present in 13% of the study children. Nine children (5%) were severely wasted (z score <– 3 SD).

Socio-demographic correlates of malnutrition:

Age: Interestingly, the prevalence of malnutrition in any form (underweight, stunting or wasting) was higher among older children than younger ones, as shown in Table 2.

Family income: As expected, there was a linear trend between nutritional status and monthly family income, with malnutrition being present in 49% of families in the lowest bracket (< Rs 3000) compared to 33% in the Rs 3000-6000 bracket and 29% in the > Rs 6000 bracket (chi square for linear trend: 4.9, p value < 0.03).

Family size: While malnutrition was more commonly observed in large families, this relationship was not statistically significant.

Gender: There was no significant or consistent association between nutritional status and gender. Although proportionately more girls than boys were stunted (37% vs 33%), more boys than girls were underweight (31% vs 28%) and wasted (14% vs 12%). However, none of these differences were statistically significant.

Discussion

The study found a high prevalence (44%) of malnutrition, including severe malnutrition (15%), among the surveyed school-aged children in the squatter community near Islamabad. Although there has not been any research in Pakistan directly comparable to the present study, a few studies conducted in school-aged children allow a broad comparison with respect to the prevalence of malnutrition.

The study prevalence is comparable to the 47% prevalence of malnutrition found in a 1993 study among children aged 5-10 years in rural Peshawar, Pakistan, although the latter used the Gomez classification based on weight-for-age (Wahab *et al.*, 1993). Similarly high prevalences of malnutrition have been observed among school-aged children in low-income developing countries such as India and Indonesia (Chhabra *et al.*, 1996; Hadju *et al.*, 1995).

The Pakistan National Nutritional Survey 1990-94 provides data only for children under five years of age. The survey found that 38% of these children were malnourished, between 30 to 40% were underweight, and equal proportions (14%) stunted or wasted (Pakistan Medical Research Council, 1998). Our figures, in broad terms, indicate a continuation of these levels of malnutrition in older children as well and would suggest that malnutrition in under-fives does not disappear magically when children cross the 'critical' threshold of 5 years.

Indeed, older children in our study were more likely to be malnourished than younger ones. Within the 7-10 year age bracket, 36% were underweight, 39% stunted and 20% wasted, compared to 26% who were underweight, 32% stunted and 8% wasted in the 5-7 year age bracket. Overall, 51% of children in the older group were malnourished compared to 40% in the younger group. Thus, while generally children below the age of five are considered to be a nutritionally vulnerable group, older, school-age children may be as nutritionally vulnerable.

Due to time and resource constraints, the present study did not attempt to explore causative factors responsible for the high prevalence of malnutrition. However, similarly high rates of stunting and wasting that have been observed in younger children in a Karachi study were strongly associated with intrauterine growth retardation (IUGR), and thus raises the role of maternal nutritional status as an aetiological factor (Fikree *et al.*, 2000). At the same time, the heavy burden of infectious diseases in these children also needs to be considered in the aetiology of malnutrition in Pakistani children. This is indicated by the finding from a low-income urban community in Mahmoodabad that the average child suffered from two episodes each of diarrhoea and acute respiratory infections every year (Qureshi *et al.*, 1989). Socio-cultural reasons may also be important. In one study, mothers of marasmic children in squatter settlements in Karachi generally attributed their children's condition to a supernatural causation, and did not readily make the connection between food intake and nutritional status (Mull, 1991) and thus may not be aware of the need for nutritional rehabilitation in these children.

As mentioned earlier, malnutrition in the older age group has serious short- and long-term implications. One, the cognitive and behavioural development of these malnourished children are likely to be impaired, leading to educational failure (Brown and Sherman, 1995). Two, there is evidence that malnutrition in childhood will impair the capacity for physical work later in adulthood (Haas *et al.*, 1996; Martorell, 1995). Finally, the health consequences of malnutrition in terms of impaired immune function and vulnerability to development of chronic, degenerative diseases in adult life are well known.

Fortunately, nutritional supplementation even at this relatively late stage in childhood may ameliorate some of these effects. Thus, children participating in school breakfast programmes in the United States showed marked improvements in academic performance which was associated with reduced absenteeism (Kennedy and Davis, 1998).

While the determinants of malnutrition were not explored to any great extent in this study, the strikingly high rate of illiteracy observed among mothers is of concern. There is now strong evidence and consensus that maternal education can have a very

large effect on reducing child mortality and malnutrition (Cleland and Ginneken, 1982; Caldwell and McDonald, 1982).

A number of recommendations follow from this study. One, the National Nutrition Survey in Pakistan should include older children aged 5 years and above. Two, the nutritional component of school health programmes needs to be strengthened, with particular consideration being given to nutritional supplements. Three, ongoing efforts to develop and popularise low-cost nutritional home supplements should be encouraged and expanded. Finally, there needs to be much more effort and priority given to address Pakistan's high levels of female illiteracy.

Acknowledgement

The assistance of the Government of the Islamic Republic of Pakistan and the Australian Agency for International Development in supporting the Master of Medical Science studies of Raheela Mohammed Ali Mian at the University of Western Australia, of which this research was a part, is gratefully acknowledged. We gratefully acknowledge the role of the Department of General Practice at the University of Western Australia in Raheela's Masters studies. We also thank Sue Gillieatt for her editorial assistance.

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Pakistan Journal of Nutrition 1 (3): 124-126, 2002

Nutritional intake during a 244 km multisport ultraendurance race

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Abstract: Data about the nutrition during ultraendurance competitions are scarce, with the exceptions of few case reports. Because very long lasting sports events become more and more popular, we aimed to describe the nutritional intake during an extreme ultraendurance race carried out in Switzerland in 1998. The ultraendurance multisport race was 244 km long (48 km mountain biking, 122 km road cycling, 28 km roller blading, 3.5 km swimming, 42.5 km running; total altitude difference ± 4000 m). The 12 male finishers participating in the study completed the race in a median (and range) time of 18.6 (17.0–19.8) hours. Their energy intake during the race was 22.6 (12.4–33.6) MJ and corresponded to 44 % of their estimated energy expenditure. Carbohydrate, protein, net fluid, and net sodium intake amounted to 60 (36–90) $g \cdot h^{-1}$, 0.8 (0.1–2.4) $g \cdot kg^{-1}$ body mass, 560 (310–790) $mL \cdot h^{-1}$, and 13 (7–19) $mmol \cdot L^{-1}$ net fluid intake, respectively. In conclusion, the nutrition during the ultraendurance race was similar to the one recommended for shorter events like a marathon run and the focus was set upon a high carbohydrate intake.

Key words: Exercise, marathon, triathlon

Introduction

Ultraendurance events lasting ten or more hours have become very popular. A careful race preparation is in any case mandatory for all competitors and the successful accomplishment of such a race depends on many factors with nutrition certainly being one of them. It is now widely accepted that the nutrition during endurance events lasting up to a few hours like a marathon should mainly focus upon an adequate fluid and carbohydrate supply. But, with the exception of sodium, it is largely unknown if additional nutrients would be necessary for an optimal nutritional supply during ultraendurance races. Sodium has been suggested to be a critical nutrient during very prolonged races (Rehrer, 2001) and some cases of hyponatremia were reported after an ultraendurance race (Speedy *et al.*, 1999). It could be argued that some further nutrients might become critical (e.g. some amino acids), but this would be pure speculation according to current knowledge.

Because even descriptive studies about the nutrition during ultraendurance competitions are scarce and most of the published reports were case studies (Clark *et al.*, 1992; Gabel *et al.*, 1995; Eden and Abernethy, 1994; Rontoyannis *et al.*, 1989; Lindeman, 1991; Hill and Davies, 2001), we aimed to describe the nutrition during an extreme ultraendurance race on a large scale. The ultraendurance race which took place in Switzerland in 1998 was the Gigathlon. It consisted to cross the country from south to north and to cover 244 km with an altitude difference of ± 4000 m by mountain biking, road cycling, roller blading, swimming, and running. The goal of the study was therefore to track the nutritional intake on the race day of the greatest possible number of competitors.

Materials and Methods

Subjects: Fifty-three of the 216 registrants answered the advertisement of the study, which was published with the application form. They were informed about the purpose and the procedure of the study and, in order to familiarize with the study design, they received a copy of the nutrition log books to be used during the study a few weeks before the race. Any question related to the study was answered by phone and a final and compulsory information meeting was held just after the check-in to the race on the day before the race. Twenty-seven competitors either did not start the race, did not appear at the information meeting, or preferred to withdraw from the study. The study was therefore carried out with the 26 remaining competitors. Fourteen competitors did not finish the race, so that only results of the 12 finisher (=subjects) are presented. The study drop out rate corresponded to the overall drop out rate of the race (114 finisher out of 216 starter). The median (range) age of the 12 male subjects was 34 (20–48) y and they weighed 74 (63–84) kg.

The exercise volume of the subjects was assessed for a common week (CW) and for an "intensive" week (IW), such as during the preparation for a competition. The mean and range exercise volume in kilometers per week was for mountain biking CW=25 (0-60), IW=45 (25–130); for road cycling CW=113 (40–300), IW=313 (150–750); for roller blading CW=10 (0–40), IW=30 (20–60); for swimming CW=2 (0–8), IW=5 (3–20); and for running CW=25 (15–55), IW=60 (35–120).

Ultraendurance race: The 244 km long race "Swiss Gigathlon 1998" crossed Switzerland from south to north and consisted of 48 km mountain biking, 122 km road cycling, 28 km roller blading, 3.5 km swimming, and 42.5 km running with a total altitude difference of ± 4000 m. The start of the race was set at 6 am and the race had to be completed within 20 hours. The weather conditions varied along the route, but they were in general cool and humid. Each athlete had one or more personal assistant(s) and coaching was allowed in the transition areas after each stage of the race.

Nutritional intake: Because planning the race nutrition was part of the preparation of a subject, the food items to be consumed during the race were known in advance. All food items that a subject planned to consume were therefore listed in an individualized log book used for the nutrition recall. The recall was logged immediately after completing each stage in the transition area by the personal assistants of the subject (the assistants were previously instructed in keeping note of the log book). The nutrition of the last stage was logged either by the assistants of a subject or by a member of the research staff.

The nutrition recall of the just completed stage was usually made by subtraction of the amount of a given food item carried by the subject at the end of the stage with the amount of items the subject had taken with him at the beginning of the stage. During the race it was moreover allowed to pick up food from the official aid stations of the organizing committee. Because the number of the stations and the food items provided were known to the subject, picking up food was part of the planned nutrition schedule of a subject. The recall of the food picked up during a

Table 1: Median and range race time and energy balance (n = 12) for each stage of the 244 km multisport ultraendurance race Gigathlon. The altitude difference of the race as ± 4000 m and it had to be completed within 20 hours

	Mountain biking 48 km	Road cycling 122 km	Roller blading 28 km	Swimming 3.5 km	Running 42.5 km	Total 244 km
Race time h	4.0 (3.5-4.3)	5.2 (4.9-6.5)	1.8 (1.4-2.5)	1.7 (1.4-2.0)	5.7 (4.4-6.9)	18.6(17.0-19.8)
Energy expenditure[1]MJ	11.6 (9.0-13.3)	19.5 (16.1-22.0)	4.3 (3.6-4.6)	3.7 (3.3-4.3)	13.3 (11.2-15.1)	51.7(43.7-58.9)
Energy intake MJ	4.4 (1.8-10.6)	8.0 (3.0-11.6)	0.3 (0.0-6.7)	2.6 (0.5-5.5)	6.2 (1.9-12.1)	22.6(12.8-33.6)
Energy balance	-7.2	-11.5	-4.0	-1.1	-7.1	-28.1

[1] The energy expenditure during the race was estimated using energy expenditure tables (Williams, 1997; Anonymous, 1999) and race times for each stage and athlete individually. To consider the altitude difference of the race, the energy amount to lift a defined mass to a defined height (E = body mass x gravity force x altitude difference) was added to the energy expenditure estimation. The mass of the mountain bike and the racing bike were included in the energy expenditure calculations.

Table 2: Median and range relative nutrient intake (n = 12) for each stage of the 244 km multisport ultraendurance race Gigathlon. The altitude difference of the race as ± 4000 m and it had to be completed within 20 hours

	Mountain biking 48 km	Road cycling 122 km	Roller blading 28 km	Swimming 3.5 km	Running 42.5 km	Total 244 km
Energy kJ·min^{-1}	17.6 (7.5-41)	24.0 (9.5-37.0)	2.8 (0.0-60.0)	25.0 (6.1-53.0)	18.5 (5.6-40.6)	19.3 (11.5-29.4)
Carbohydrate g·min^{-1}	0.9 (0.4-2.2)	1.1 (0.5-0.8)	0.1 (0.0-3.0)	1.2 (0.3-2.7)	0.9 (0.3-2.2)	1.0 (0.6-1.5)
Water L·h^{-1}	0.57 (0.30-1)	0.61 (0.09-0.81)	0.12 (0.0-0.49)	0.64 (0.19-1.62)	0.50 (0.16-1.35)	0.56 (0.31-0.79)
Sodium from drinks[1] mmol·L^{-1}	5 (1-14)	5 (2-12)	60 (0-90)	5 (0-30)	4 (0-10)	9 (5-13)
Total sodium [1,2] mmol·L^{-1}	6 (3-17)	12 (5-41)	79 (0-90)	7 (3-40)	7 (4-34)	13 (7-19)

[1] Data of only six subjects were included in the sodium results, because the sodium content of part of the food items ingested by the remainder six athletes could not be uncovered.
[2] Total sodium intake was set in relation to total fluid intake for better comparability with sodium intake from drinks.

stage was usually made by recalling how many deviations from the scheduled pick ups were done.
Food items were evaluated for water, energy, carbohydrate, protein, fat, and sodium content using in descending order nutritional information supplied on the food labels, nutritional information provided on request by the manufacturer of the product, or by use of the nutrition analysis software EBIS (version 1.1, University Hohenheim, Stuttgart, Germany), which is based on the official Federal German Food Key. Because the sodium content of some food items could not be uncovered for six subjects, the sodium results were presented for the remainder six athletes.

Body mass and race energy expenditure: Body mass was recorded after the breakfast before the start and after completing the race on a scale with a 50 g precision. The energy expenditure during the race was estimated using energy expenditure tables (Williams, 1997; Anonymous, 1999) and individual race times for each stage. To consider the altitude difference of the race, the energy amount to lift a defined mass to a defined height (E = body mass x gravity force x altitude difference) was added to the energy expenditure estimation. The mass of the mountain bike and the racing bike were included in the calculation of the energy expenditure of the two bike stages.

Statistics: Data were presented as median and range in text and tables.

Results

The race was completed in a median time of about 18 ½ hours (Table 1) and the body mass of the subjects was reduced by 5.5 % at the end of the race (73.6 (63.1-83.8) kg before the race vs. 70.3 (59.3-79.8) kg after the race).
The energy balance was negative for each individual stage resulting in an overall negative balance of 28.1 MJ (Table 1). The energy intake corresponded to about 45 % of the energy expenditure. The median relative nutrient intake was 15.0 (9.9-24.0) g·kg^{-1} body mass for carbohydrates, 0.8 (0.1-2.4) g·kg^{-1} body mass for protein, and 0.4 (0.1-1.5) g·kg^{-1} body mass for fat resulting in a median energy provision of 90 (65-99) % from carbohydrates, 5 (1-15) % from protein, and 7 (1-20) % from fat. The median fluid intake was 560 mL·h^{-1} and the overall sodium intake (n = 6) of 13 mmol per liter net water intake corresponded to an intake of 0.17 (0.06-0.31) g·h^{-1} (Table 2).

Discussion

Energy balance: The energy intake during the 18 ½ hours of the Gigathlon was 23 MJ and resulted in a negative energy balance of 28 MJ. A large part of the energy provision on the race day derived therefore from endogenous stores. Interestingly, well-adjusted energy balances were more often reported during ultraendurance events lasting several days compared to one-day events, irrespective of the magnitude of the energy expenditure (Gabel *et al.*, 1995; Saris *et al.*, 1989; Rontoyannis *et al.*, 1989).

Carbohydrate intake: The carbohydrate intake of 60 g·h^{-1} during the race was probably sufficient to sustain a high carbohydrate oxidation rate, because maximal oxidation rates occur at exogenous intakes of 1.0 to 1.5 g·min^{-1} (Jeukendrup and Jentjens, 2000). This is supported by a recent finding, that providing 60 g of carbohydrates per hour during a six hours ride at 55 % VO_2max indeed sustained a high oxidation rate of nearly 50 g·h^{-1} in the later stages of the exercise bout (Rauch *et al.*, 1998).
A main benefit of an adequately high carbohydrate intake during an exercise bout is probably the prevention of a rapid hepatic glycogen depletion and the maintenance of a stable blood glucose concentration, which would reduce the need to increase gluconeogenesis. Support for this notion comes from a study in which already a moderate carbohydrate ingestion of 35 g·h^{-1} during an exercise bout at 50 % VO_2max reduced the hepatic glucose production by about 60 % (Jeukendrup *et al.*, 1999). An ingestion of 175 g·h^{-1} even completely blocked the hepatic glucose production.
The carbohydrate intake of about 60 g·h^{-1} corresponded to an intake of 15 g·kg^{-1} body mass, which is more than the amount recommended for extremely prolonged and intense exercise (10-12 g·kg^{-1} body mass (Hawley and Burke, 1998)).

Protein intake: The relative protein intake was low during the race and contributed to about five energy percent to the energy intake. Quantitative amino acid oxidation is only marginal during endurance exercise (Hargreaves and Snow, 2001) but it might become qualitatively important (Gibala, 2001) in particular with depleted glycogen stores. A major fate of protein degraded in condition of liver glycogen depletion is to provide gluconeogenetic precursors. This raises the question if the endogenous protein degradation during exercise would be attenuated by exogenously

increasing the amount of circulating amino acids (similar to a liver glycogen sparing by carbohydrate intake), but according to our knowledge, for prolonged exercise situations this issue was not addressed up to now.

Fluid and sodium intake: Ninety-six percent of the total fluid intake of 560 mL·h^{-1} derived from drinks and only 4 % from solid food. The fluid intake was within the range reported in literature (550 mL·h^{-1} during a 100 km run (Fallon *et al.*, 1998) and 800-900 mL·h^{-1} during a 960 km run (Rontoyannis *et al.*, 1989)) and within the range of general recommendations for fluid replacement during exercise (450 and 1200 mL·h^{-1} (Horswill, 1998)).
Fluid replacement prescriptions should by now probably be the best known nutritional recommendation for athletes, because dehydration is a major performance deteriorating aspect. However, the fluid intake of four athletes in the present study was lower than 450 mL·h^{-1}, and an insufficient fluid intake of only 250 mL·h^{-1} was even reported for competing professional cyclists (GarciaRoves *et al.*, 1998). It seems therefore that not all athletes are aware of the importance of an adequate fluid intake.
Excessive water intake, on the other hand, might also be deleterious and lead to hyponatremia. For example, 18 % of the finisher of the 1997 New Zealand ironman triathlon were reported to be hyponatremic (Speedy *et al.*, 1999). Although the reasons for its occurrence are not conclusively uncovered, hyponatremia is discussed to develop as a consequence of salt depletion from massive sweat loss associated with net dehydration, excessive water intake, and large fluid shifts (Vrijens and Rehrer, 1999). To prevent hyponatremia during ultraendurance races, it was even recommended to ingest 1 g sodium per hour, which corresponds to 32 to 46 mmol·L^{-1} with a fluid intake of 500 to 700 mL·h^{-1} (Douglas and Hiller, 1989). For comparison, sports drinks commonly have a sodium content of only 10 to 25 mmol·L^{-1} (Maughan, 1998). The median sodium content of the ingested drinks in the present study was 9 mmol·L^{-1} with a fluid intake of 560 mL·h^{-1}. And even if the sodium intake derived from solid food was taken into account, a hypothetical content of only 13 mmol per liter net fluid intake would result. It would have been of interest to assess the blood sodium content to verify if the low sodium intake had caused hyponatremia, but because of logistical problems it was not possible to draw blood samples immediately before and after the race.

Concluding remarks: Assessing the nutrition during a competition is a difficult task because several factors could affect its accuracy. However, because of the following reasons, we believe that the accuracy in the present study was high. First of all, the race was an extraordinary event and carefully planned by the subjects. Second, all food items available during the race were known in advance which allowed for an individual preparation of the log book for the nutrition recall. Third, the recall was simplified because the subjects and their assistants had scheduled the nutrition so that any deviation from the planned schedule could be remembered more easily.
To summarize, the nutrition during the extreme ultraendurance race Gigathlon was similar to the one recommended for exercise events lasting only a few hours, and the focus was set primarily upon a high carbohydrate intake, which reached an amount allowing for a high carbohydrate oxidation rate. The sodium intake was low, reflecting on the one hand the low sodium content of the ingested drinks and on the other hand the rather low fluid intake.

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Pakistan Journal of Nutrition 1(3): 127-131, 2002

Responses of Laying Chickens to Diets Formulated by Following Different Feeding Standards

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Abstract: Responses of laying chickens to diets based on formulations following different feeding standards were investigated during March to July months under Bangladesh conditions. One hundred twenty Shaver 579 layers of 35-week old were distributed randomly into 5 diet groups with 3 replicates, each of 8 birds. The birds were reared in laying batteries with 2 birds in each cage and 8 birds in four adjacent cages constituted a replicate. The feeding standards that constituted different dietary treatments were: Shaver 579 (1997), recommended by the breeders of the birds considered in this study; NRC (National Research Council, 1994); INSP (International Nutrition Standards for Poultry, 1983) recommended for Asiatic regions; ISI (Indian Standard Institute, 1992) and BSTI (Bangladesh Standard Testing Institute, 1988). Other cares and management were identical for birds of all diet groups. The feeding trial was conducted for 15 weeks and the data on laying performance and egg quality were evaluated. Evaluation of results revealed that responses of laying birds fed diet based on formulation following the recommendation for Shaver 579 (control) were at least equal to or better than those of other standards during March to July months under Bangladesh conditions.

Key words: Responses, laying chickens, feeding standards, performance, egg quality

Introduction

Feed constitutes the major cost of poultry meat and egg production, usually 65-70%, all over the world. The poultry producers are always interested for high production but with minimum expenditure of nutrients to economize their feeding practices. In addition, there is a recent trend to reduce unnecessary wastage of nutrients that are excreted through excreta and therefore become potential pollutants for the environment. Use of a feeding standard well suited to birds raised within a particular agro-ecological climatic zone or a local situation may be helpful to achieve production target in an economic way with minimum detrimental effect on the environment. Although feeding standards have been established and the nutrient requirements reported there in are based on research results, the commercial producers mostly follow the suggestions of the breeding companies which sometimes considers the climatic zone where the birds go for farming. On the other hand, paucity of information with regard to suitability of feeding standards in different climatic zones has made these difficult to apply for commercial use although standards are always considered as guides in feeding practices but not as inflexible rules (McDonald *et al.*, 1995). Experimentations to compare different nutrient specifications at different locations may show variable responses because of variations in climatic or agro-ecological zone. Thus, the suitability of feeding standards including those recommended by breeders for specific strains of chicken must be examined under local condition. Despite the fact that feeding experiments and nutritional studies are mostly based on diet formulations in accordance with a well known standard, responses of birds to diets based on nutrient specifications of different standards has probably not been compared in the same trial. The present study was, therefore, an attempt to investigate responses of laying chickens (performance and egg quality) to diets formulated by following feeding standards recommended by five different sources. The trial was conducted in Bangladesh during summer when the temperature ranged from 26 to 34. 5 °C.

Materials and Methods

Feed ingredients and their nutrients: The local feed ingredients commonly used for poultry feed formulations were considered. Samples were subjected to chemical analyses for proximate components (AOAC, 1990), Ca and total P (Page *et al.*, 1982), starch (Rangana, 1977) and free sugar (Hodge and Hofreiter, 1962). The ME was calculated in accordance with the formula suggested by Wiseman (1987).

Birds and diets: One hundred twenty, 35-week old Shaver 579 commercial laying chickens almost similar in weight were randomly distributed to 5 diet groups with 3 replicates each of 8 birds. Birds of each replicate were housed in adjacent 4 cages on tiers of laying batteries with 2 birds in each cage. Five experimental diets were prepared on the basis of nutrient specifications suggested by five sources. Since Shaver 579 layers were used as the experimental birds, nutrient specifications suggested by the breeder of this strain were considered as control (Shaver 579, 1997). Other 4 test diets were formulated according to nutrient specifications of the standards published by National Research Council (NRC, 1994), International Nutrition Standards for Poultry (INSP, Blair, *et al.*, 1983) for Asiatic regions, Indian Standard Institute (ISI, 1992) and Bangladesh Standard Testing Institute (BSTI, 1988). All diets were formulated keeping nutrient concentrations as closely as possible to the recommended allowances of the respective standards. A computer package "User-Friendly Feed Formulation, Done Again" (UFFDA, 1992) developed at the University of Georgia, USA was used for least-cost and accuracy in diet formulations. The ingredient composition and nutrient concentrations of the diets are shown in Table 1 and Table 2 respectively.

Feeding and watering: All experimental birds had an adjustment on the control diet for a week to eliminate residual effects of the previous diet fed to them. At 35 weeks and onwards, the feeding of the control diet was continued for a treatment group while the other 4 groups received their respective test diets. All diets were randomly allocated to different treatment groups. Birds of all treatments were fed in two phases: phase I (35 to 41 weeks) and phase II (41 to 50 weeks). Feed was offered *ad. libitum*. Availability of fresh, clean and cool drinking water was ensured throughout the experimental period.

Routine management: The experimental birds were reared on a 16 hours lighting schedule. Feeders and waterers were kept clean. Droppings were cleaned twice in a week. Eggs were collected and weighed everyday in the morning and afternoon. Birds of all treatment groups received identical care and management.

Egg quality measurement: Both internal and external quality characteristics were measured from eggs laid by layers received different treatments. These measurements were carried out at the end of phase I (41 weeks of age) and phase II (50 weeks of age).

Table 1: Ingredient composition of experimental diets (g/kg)

Feed ingredients	Control		NRC		INSP		ISI		BSTI	
	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks
Maize	487.0	0.0	553.0	0.0	507.0	0.0	556.0	0.0	458.0	0.0
Wheat	0.0	607.0	0.0	659.0	0.0	616.0	0.0	577.0	0.0	440.0
Full- fat soybean	267.0	210.0	184.0	111.0	203.0	130.0	180.0	157.0	236.0	201.0
Rice polish	71.0	26.0	58.0	60.0	26.0	84.0	89.0	85.0	0.0	115.0
Soybean meal	0.0	6.0	35.5	49.0	0.0	24.5	0.0	7.5	0.0	64.0
Sesame oil cake	20.0	0.0	12.0	0.0	89.0	0.0	38.0	0.0	125.0	0.0
Fish meal	0.0	0.0	20.0	0.0	20.0	36.0	20.0	48.0	20.0	25.5
Soybean oil	0.0	0.0	0.0	14.0	0.0	0.0	0.0	16.0	0.0	0.0
Oyster shell	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	115.0	100.0
Bone meal	45.0	44.5	27.0	0.0	45.0	0.0	5.5	0.0	36.0	45.0
Common salt	5.0	2.3	5.0	2.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral-amino acid premix*	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-Lysine	0.5	0.0	1.0	0.5	1.5	0.0	2.0	0.0	1.5	0.0
DL-Methionine	2.0	1.7	2.0	2.0	1.0	2.0	2.0	2.0	1.0	2.0

Control, Shaver 579, 1997; NRC, National Research Council, 1994; INSP, International Nutrition Standards for Poultry, 1983; ISI, Indian Standard Institute, 1992 and BSTI, Bangladesh Standard Institute, 1988.

*Jayson Agrovet Ltd., Dhaka, Bangladesh. Added Supramix Layer; Usage rate 2.5 g/kg feed which contained per kg: Vitamin A, 4800000 I.U.; Vitamin D3, 960000 I.U.; Vitamin E acetate (Alfa-tocoferol 91%), 8000 mg; Menadion Sodium Bisulfat (Vitamin K), 800 mg; Vitamin B1, 800 mg; Vitamin B2, 2000 mg; Nicotinic Acic, 12000 mg; D-Pantothenic acid, 3200 mg; Vitamin B6, 1600 mg; Vitamin B12, 4 mg; Folic acid, 320 mg; biotin, 20 mg; Choline chloride, 100000 mg; Zinc, 16000 mg; Copper, 2400 mg; Cobalt, 100 mg; Iodine, 200 mg; Iron, 9600 mg; Selenium, 48 mg; Manganese, 20000 mg; Methionine, 20000 mg

Table 2: Nutrient concentrations of formulated diets

Nutrients per kg	Control		NRC		INSP		ISI		BSTI	
	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks	35-41 weeks	41-50 weeks
ME (kcal)	2863.0	2750.0	2850.0	2850.0	2738.0	2788.0	2900.0	2900.0	2700.0	2701.0
Protein (g)	184.8	170.0	183.0	165.0	189.0	176.6	180.0	180.0	203.0	193.4
Linoleic acid (g)	36.3	24.1	29.8	16.2	29.3	19.0	30.0	21.3	30.4	25.6
Calcium (g)	43.3	41.0	42.9	38.6	44.3	41.4	42.2	42.2	47.6	45.3
Total phosphorus (g)	9.6	7.6	9.4	5.6	10.7	6.2	8.3	6.3	11.5	8.8
Sodium (g)	2.1	1.7	2.1	1.7	2.1	2.7	2.1	2.7	2.0	2.6
Arginine (g)	12.2	14.7	9.5	11.3	10.0	11.8	9.3	12.4	10.9	12.4
Lysine (g)	3.0	3.2	4.9	4.9	5.0	5.0	4.9	4.9	5.0	5.7
Methionine (g)	2.3	2.3	2.3	2.3	2.8	2.8	2.5	2.9	3.2	3.2
Methionine plus Cystine (g)	7.0	6.4	6.9	6.4	7.1	6.6	6.8	6.8	7.6	7.3
Threonine (g)	7.0	7.0	5.6	5.2	5.8	5.4	5.5	5.8	6.2	5.9
Tryptophan (g)	2.0	2.0	1.7	1.9	1.8	1.9	1.7	1.9	2.0	2.4

Control, Shaver 579 commercial layers, 1997; NRC, National Research Council, 1994; INSP, International Nutrition Standards for Poultry, 1983; ISI, Indian Standard Institute, 1992 and BSTI, Bangladesh Standard Institute, 1988.

Each time, 4 fresh eggs from each replicate group were considered during 4 consecutive days of the respective weeks. Following the measurements of weight, length and breadth of each egg, they were broken on a glass plate and different components were separated and weighed (Chowdhury, 1988). Albumen and yolk indices were determined following the established procedures. Haugh units were measured by adjusting egg weight and height of the albumen in an Egg Quality Scale (Ogawa Seiki Co., Tokyo, Japan). Yolk colour scores were recorded by a Roche Yolk Colour Fan (F. Hoffman-La Roche Ltd., Switzerland). Shell thickness was measured by a Shell Thickness Meter (Ogawa Seiki Co., Tokyo, Japan) from the pointed, blunt end and waist regions of each egg to obtain average values. Shell surface area and breaking strength were determined by following the formulae suggested by Carter (1975) and Arad and Marder (1982) respectively. Shape indices were calculated from the values of length and breadth of the eggs.

Statistical analyses: Statgraphic (1993), a computer packaged programme was used for ANOVA in accordance with a completely randomized design. Treatment means were separated when the test on a variable was found to be significant. Real differences in mean values were based on $P < 0.05$ or better.

Results

Laying performance: The results of laying performance are shown in Table 3. No significant differences in body weight were found among different treatments although the control birds had the highest weight gain. A significant increase in feed intake was observed with BSTI treatment compared to ISI ($P < 0.05$) but the values including those of NRC and INSP were comparable to the control. Egg production was significantly increased in BSTI treatment compared to both NRC and ISI ($P < 0.05$) but again, all data including that of INSP were comparable to the control. The most interesting result was obtained for egg weight. The data on egg weight were significantly decreased in NRC, INSP, ISI and BSTI compared to the control ($P < 0.01$). Fig. 1 shows the trend in weekly egg weight. The birds that received the control diet had always an upward trend following two weeks of feeding and this increasing trend in egg weight continued till the termination of the experiment except during 43 to 45 weeks of age when, birds from

Table 3: Performance of layers fed diets formulated by following different feeding standards (35-50 weeks of age)

Variables	Dietary Treatments					Level of significance
	Control	NRC	INSP	ISI	BSTI	
Feed intake, (g/bird/day)	94.8^{ab} ± 4.38	96.5^{ab} ± 3.62	97.7^{ab} ± 3.47	93.1^{b} ± 4.79	105.6^{a} ± 3.50	P<0.05
Change in body weight, (g)	+13.80 ± 23.95	-30.50 ± 29.33	-17.60± 23.51	-34.90± 37.34	+4.30 ± 26.24	NS
Egg production, hen-day %)	66.1^{ab} ± 3.21	64.2^{b} ± 4.26	72.5^{ab}± 1.27	63.8^{b} ± 4.20	73.9^{a} ± 1.13	P<0.05
Egg weight, (g/egg)	61.8^{a} ± 0.48	59.8^{b} ± 0.29	59.3^{b} ± 0.37	60.4^{b} ± 0.42	60.2^{b} ± 0.37	P<0.01
Egg mass, (g/hen/day)	41.0^{ab} ± 2.24	38.6^{b} ± 2.72	43.0^{ab}± 0.96	38.7^{ab}± 2.79	44.5^{a} ± 0.70	P<0.05
FCR	2.40 ± 0.089	2.32 ± 0.074	2.36± 0.089	2.28± 0.066	2.51 ± 0.157	NS
Livability, (%)	99.70 ± 0.26	100.00 ± 0.00	97.70± 0.26	100.00± 0.00	100.0 0± 0.00	NS

Control, Shaver 579, 1997; NRC, National Research Council, 1994; INSP, International Nutrition Standards for Poultry, 1983; ISI, Indian Standard Institute, 1992 and BSTI, Bangladesh Standard Institute, 1988.
Values indicate ± Standard Error; mean sharing no common superscripts differ significantly; NS, Non-significant.

Table 4: External quality traits of eggs from layers fed diets of different nutrient specifications

Variables	Nutrient specifications					Statistical Results
	Control	NRC	INSP	ISI	BSTI	
Egg sample weight, g	65.4 ±2.79	61.90± 0.09	61.10± 0.51	61.60± 0.77	63.30± 1.33	NS
Egg length, cm	5.8^{a}±0.02	5.6^{b} ± 0.05	5.6^{b} ± 0.01	5.6^{ab}± 0.02	5.7^{ab}± 0.01	P<0.01
Egg width, cm	4.00±0.43	4.30± 0.01	4.30± 0.02	4.30± 0.03	4.40± 0.08	NS
Egg shape index	0.70±0.075	0.76± 0.005	0.74± 0.015	0.77± 0.010	0.77± 0.010	NS
Shell weight, g	6.20±0.39	6.00± 0.12	6.10± 0.09	6.20± 0.08	6.40± 0.07	NS
Shell thickness, mm	0.46±0.000	0.41± 0.045	0.45± 0.025	0.46± 0.020	0.46± 0.030	NS
Membrane weight, g	0.18±0.015	0.18± 0.010	0.15± 0.025	0.16± 0.010	0.18± 0.010	NS
Membrane thickness, mm	0.034±0.0085	0.033± 0.008	0.029± 0.0075	0.027±0.007	0.031± 0.0085	NS
Breaking strength, kg	2.30±0.08	2.20± 0.00	2.10± 0.01	2.20± 0.02	2.20±0.04	NS
Shell surface area, cm^{2}	76.20± 2.22	73.10± 0.08	72.40± 0.44	72.90± 0.64	74.20± 1.10	NS

Control, Shaver 579, 1997; NRC, National Research Council, 1994; INSP, International Nutrition Standards for Poultry, 1983; ISI, Indian Standard Institute, 1992 and BSTI, Bangladesh Standard Institute, 1988.
Data are reported as average of two measurements (41 and 50 weeks of age);Values indicated ± Standard Error; means sharing no common superscripts differ significantly; Si NS, Non-significant.

all treatments faced a common stress, the heat stress. The average temperature during that period was 30 ^{0}C. The results on egg mass, followed a trend, more or less similar to egg production. Feed conversion value was poorest in BSTI and data obtained from other diet groups were close to each other and therefore, showed no significant differences among treatments. There was little mortality only in control and INSP treatments, which were independent of diet effect. So, the over all livability result was satisfactory.

Egg quality characteristics: Table 4 shows the external characteristics of eggs. The only difference in external trait was noted for egg length, which was significantly decreased in NRC and INSP as compared to the control ($P<0.01$) but not from ISI or BSTI. All other external quality traits remained unaffected in spite of variations among treatments with regard to nutrient specifications. The weight of dry albumen was found to be significantly reduced in ISI treatment ($P<0.01$) while the values in NRC, INSP and BSTI remained statistically similar to the control group (Table 5). Other internal quality characteristics did not differ significantly across treatments.

Discussion

The significant increase in feed intake in BSTI group compared with the ISI might be due to low energy concentration of the diets. Birds fed according to BSTI standard consumed 200 kcal (6.89%) less energy per kg diet as compared to those maintained on ISI treatment (Table 2). That the birds on low energy diets generally consume more feed than those on high-energy diets is well documented. There are also recent reports supporting this view (Yong *et al.*, 1994; Yong *et al.*, 1997; Harms *et al.*, 2000). Except the energy content, the amounts of other nutrients in BSTI diet were either close to or more than those maintained for NRC and ISI treatments. This probably caused a significant increase in egg production in BSTI group ($P<0.05$) but such a nutrient status failed to show any difference from the control or INSP group. The reason might be that both these groups had better nutrient status than NRC or ISI that brought their egg production to the levels of nonsignificant differences from the BSTI group (Table 3). The significant increase in egg weight in the control group in comparison with other treatment groups ($P<0.01$) could be attributed to high linoleic acid concentration of the diet. The linoleic acid concentration in the control diet was highest during the study period with the exception that during phase II, the concentration was only close to INSP (Table 2). The increased concentration of linoleic acid in the diet, an essential fatty acid, has a direct role in increasing egg weight in layers (March and McMillan, 1990). Reports are also available which state that linoleic acid level in layer diet above 10.2 g/kg has no beneficial effect (Grobes *et al.*, 1999; Harms *et. al*, 2000). However, the result on egg weight in the control group, was close to the performance objective of the strain (average 62.7g during 20 to 70 weeks of age) used in this study. The weekly egg weight data were also encouraging in the same diet group during the study period (Fig. 1). In spite of showing a highest egg production, the birds on BSTI treatment because of highest feed consumption, had the poorest feed conversion ratio although this result did not differ significantly from any of the treatment groups.

Although egg length was significantly increased in the control group in comparison with NRC group, it did not affect values of shape indices and therefore had no effect on shell quality. Similarly, an increase in the amount of dry albumen did not affect albumen quality traits. The results on external and internal quality characteristics clearly demonstrated that in spite of some variations in the amount of nutrients in different standards, it did not, in any way, affect qualities of albumen, yolk and eggshell. Previous reports indicated that egg qualities were not affected much by the amount of protein and energy levels in the diets (Qudratullah and Eshwaraiah, 1991; Acosta Iraida and De Acosta Iraida, 1990). The current study was conducted between March and July months in Bangladesh when, the temperature ranged from 26 to 34.5 ^{0}C. Therefore, over all feed intake, egg production, FCR, body weight did not reach up to the levels as claimed by the breeders (Shaver 579, 1997). Nevertheless, it did

Table 5: Internal quality traits of eggs from layers fed diets of different nutrient specifications

Variables	Nutrient specifications					Statistical results
	Control	NRC	INSP	ISI	BSTI	
Albumen height,(mm)	11.10 ± 1.63	10.70 ± 0.87	9.70 ± 1.93	9.80± 0.81	8.60 ± 0.92	NS
Albumen diameter,(cm)	7.40 ± 0.12	7.40 ± 0.16	8.10 ± 0.68	7.60± 0.06	7.8 0 ± 0.25	NS
Fresh albumen weight, (g)	40.80 ± 2.33	37.50 ± 0.05	36.60 ± 0.19	37.00± 0.08	37.90± 1.90	NS
Dry albumen weight, (g)	5.5^{a} ± 0.20	4.9^{ab} ± 0.04	4.8^{ab} ± 0.08	4.7^{b} ± 0.11	5.0^{ab} ± 0.29	P<0.01
Albumen index	0.15 ± 0.02	0.14 ± 0.015	0.12 ± 0.035	0.13± 0.01	0 .11 ± 0.015	NS
Yolk height, (mm)	18.90 ± 1.11	19.10 ± 0.85	19.40 ± 0.64	19.50± 0.57	19.00± 0.77	NS
Yolk diameter, (cm)	3.60 ± 0.27	4.00 ± 0.06	4.00 ± 0.04	4.00± 0.03	4.10 ± 0.11	NS
Fresh yolk weight, (g)	14.50 ± 0.56	14.30 ± 0.32	14.50 ± 0.13	15.20± 0.12	15.20± 0.17	NS
Dry yolk weight, (g)	8.00 ± 0.09	8.10 ± 0.22	7.7 0± 0.00	8.20± 0.18	8.10 ± 0.00	NS
Yolk color score	3.10 ± 1.44	3.40 ±1.48	3.60 ± 1.83	2.70± 1.54	3.5 0 ± 1.45	NS
Yolk index	0.49 ± 0.04	0.47 ± 0.15	0.48 ± 0.02	0.48± 0.01	0.46 ± 0.030	NS
Haugh unit	102.60 ± 6.14	102.00 ±3.58	97.40 ± 9.44	98.00± 3.86	91.8 ± 4.50	NS

Control, Shaver 579, 1997; NRC, National Research Council, 1994; INSP, International Nutrition Standards for Poultry, 1983; ISI, Indian Standard Institute, 1992 and BSTI, Bangladesh Standard Institute, 1988.
Data are reported as average of two measurements (41 and 50 weeks of age); Values indicated ± Standard Error. Means sharing no common superscripts differ significantly; NS, Non-significant.

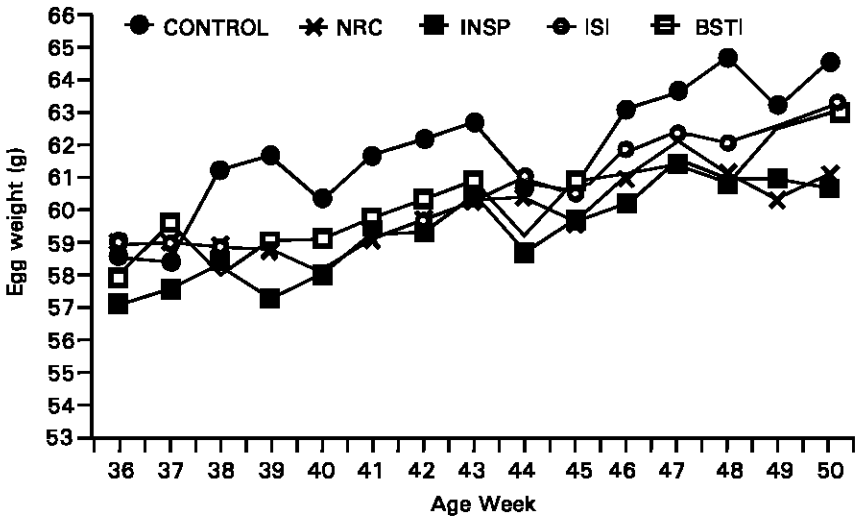

Fig. 1: Weekly egg weight responses of layers fed diets of different nutrient specifications

allow some comparisons to make during hot weather condition. Although, some variations in results among different feeding standards were observed due to variations in nutrient concentrations, the impetus behind the study was neither to determine the effect of specific nutrient/nutrients nor their specific interactions. Rather, it was aimed at investigating responses in terms of cumulative effects of the nutrients suggested by different standards. The results of the current study clearly revealed that responses of laying birds fed diet based on formulation following Shaver 579, 1997 (control in this experiment), were at least equal to or better than those of other standards (NRC, INSP, ISI and BSTI) during March to July months under Bangladesh conditions. The next logical steps would be to carry out experimentations covering other seasons of the year to investigate performance and egg quality and determine nutrient concentrations that are excreted through fecal materials. The latter is important to reduce effect on environment by following an appropriate standard under local conditions.

Acknowledgement

The authors are grateful to Mr. S.C. Das and Mr. M. A. Hoque, Departments of Poultry Science and Animal Breeding and Genetics respectively of Bangladesh Agricultural University for their assistance during the course of the experiment. Dr. A. Kamyab from Tehran Agricultural University in Iran deserves special thanks for his kind comments on an initial draft.

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Pakistan Journal of Nutrition 1 (3): 132-136, 2002

Effect of Milk Pretreatment on the Keeping Quality of Domiati Cheese

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Abstract: This study compared the usage of raw milk, heat treated milk and pasteurized milk in the manufacture of Egyptian soft Domiati cheese. The physico-chemical composition of the manufactured cheese was different. Soluble nitrogen, salt as well as pH values were high in raw milk cheese in comparison with the heat treated and pasteurized milk cheese. Considerable changes had occurred in raw milk cheese during the storage period more than heat treated and pasteurized milk cheese. The obtained results suggest that, pasteurization greatly improves the keeping quality of soft Domiati cheese and increase its shelf life.

Key Words: Milk, domiati cheese, pasteurized milk,

Introduction

Domiati cheese is the most type of pickled white soft cheese in Egypt. It is consumed either fresh or after pickling for few months. The microbial quality and safety of Domiati cheese is the major area of concern for producers and consumers. It depends on the types of microorganisms introduced from raw milk, efficiency of processing and the hygienic practice applied in dairy plant. Handling of milk during cheese manufacture plays an important role in the proliferation of microbial flora and consequently impair its utility and render the product unfit for human consumption (Sveum *et al.*, 1992; Yousef *et al.*, 2000; Leuschner and Boughtflower, 2002).

Due to the high microbial load present in raw milk, pasteurization is commonly used to eliminate all pathogenic and most of non-pathogenic organisms before its further processing into cheese. Moreover, pasteurization of milk for cheese making is intended mainly to reduce microbial load, ensure greater yield, standard level of quality and ripening at higher temperature (Ordonez *et al.*,1999; Blumenthal, 2002).

Although, cheese production should employs the full pasteurization process, there is a long standing tradition of making cheese from raw or heat treated milk. Heat treatment is defined as heating milk at time temperature less rigorous than pasteurization referred as sub-pasteurization. It has no international standards and could destroy most of pathogenic microorganisms with partial inactivation of indigenous enzymes, and other biological components initially present in raw milk (Zottola and Smith, 1993; Schaffer *et al.*, 1995; Elein *et al.*, 1999; Sofos, 2002).

In Egypt, the information about the involvement of Domiati cheese in human illness and economic losses are unknown. Therefore, this investigation was aimed to study the effect of milk pretreatment on the organoleptic, chemical, and microbiological quality of Domiati cheese during manufacturing and storage. Finally to suggest the control measures for microbial hazard to safe guard the consumer health.

Materials and Methods

Cheese manufacture: Domiati cheese was manufactured with some modifications according to Abou-Donia (1986) as follow: Fresh buffalo's milk was collected from dairy farms at El- fayoum district then standardized to 6% & 8.5% fat and solid not fat respectively. The salt was added at rate of 7% (w/w) to all milk before renneting using commercial animal rennet (Hansen Company, Denmark). The standardized milk was used in three experimental trials. The first trial used raw milk warmed at 40°C, at which rennet was added. The second trial used heat treated milk (H), the milk was heated at 65°C for 15 sec then warmed to 40°C. The third trial used pasteurized milk (P), the milk was heated at 72°C for 15 sec followed by sudden cooling at 5°C then warmed at 40°C which rennet was added. The manufactured cheese was stored at 10°C in soldered tins, filled with boiled salted whey (7%) and analyzed when fresh and after 15, 30, 45, 60, 90 and 120 days of storage.

Table 1: Organoleptic examination of Domiati cheese samples

Storage time/day	Cheese trials	Organoleptic scores			
		Flavor (50)	Body& Texture (35)	Appearance & Color (15)	Total scores (100)
0 (fresh)	R	46	35	13	94
	H	43	33	14	90
	P	43	32	15	90
15	R	46	35	13	94
	H	43	33	14	90
	P	43	32	15	90
30	R	45	33	12	90
	H	42	32	13	87
	P	42	30	14	86
45	R	45	33	12	90
	H	42	32	13	87
	P	42	30	14	86
60	R	47	34	11	93
	H	44	32	12	88
	P	43	31	14	88
90	R	47	34	11	92
	H	44	32	12	88
	P	43	31	14	88
120	R	48	35	11	94
	H	44	33	12	89
	P	43	32	13	88

R = cheese made from raw milk
H = cheese made from heated milk
P = cheese made from pasteurized milk

Organoleptic examination: The cheese samples were organoleptically scored using score card for flavor (50 points), body and texture (35 points) and appearance & color 15 points). The scores were averaged by five panelists according to Nelson and Trout (1981) and Hassan *et al.* (1983).

Chemical analysis: All cheese samples were chemically examined for pH using pH meter (model SA 720); titratable acidity according to AOAC (1990). Moisture; salt content; fat, cheese yield; total nitrogen (T.N.) and soluble nitrogen content (S.N.) according to Kuchroo and Fox (1982) and Guinee and Fox (1993).

Microbiological examination: The cheese samples were prepared for microbiological examination according to ICMSF, 1996. The treated cheese samples were examined for total colony count (TCC); aerobic spore former count; total proteolytic count; *Coliform* (MPN) count; *Staphylococcal* count and total mold and

Table 2: Chemical composition of Domiati cheese samples

Storage period /day	Cheese trials	Chemical composition							
		Moisture %	Fat%	F/D	Acidity %	pH	Salt % In water phase	SN/TN %	Cheese Yield kg/100kg
0(fresh)	R	59.65	18.20	45.11	0.22	6.45	7.46	9.58	29.30
	H	60.75	17.90	45.61	0.20	6.45	7.40	8.72	29.88
	P	61.40	17.60	45.65	0.22	6.42	7.24	9.40	30.95
15	R	58.91	18.65	45.38	0.33	6.05	7.60	11.26	28.15
	H	59.48	18.55	45.78	0.28	6.15	7.65	10.29	28.75
	P	59.95	18.25	45.57	0.25	6.32	7.51	9.78	29.05
30	R	57.90	19.30	45.84	0.43	5.88	7.85	11.38	27.35
	H	59.35	19.10	45.86	0.32	6.05	7.96	10.70	27.89
	P	58.80	18.90	45.87	0.28	6.19	7.83	9.87	28.15
45	R	57.41	19.55	45.89	0.49	5.65	7.98	12.11	26.90
	H	58.09	19.28	46.00	0.38	5.90	8.01	11.13	27.35
	P	58.55	19.05	45.96	0.31	6.08	7.94	9.92	27.90
60	R	58.15	19.88	46.39	0.55	5.35	8.14	13.05	26.40
	H	57.88	19.45	46.18	0.43	5.80	8.12	11.82	26.95
	P	58.22	19.25	46.07	0.37	5.98	7.99	10.07	27.75
90	R	56.75	20.18	46.66	0.62	5.15	8.19	14.06	25.85
	H	57.25	19.85	46.43	0.48	5.75	8.30	12.75	26.28
	P	57.75	19.50	46.15	0.42	5.88	8.14	10.44	27.22
120	R	55.90	20.75	47.05	0.73	4.90	8.41	16.19	25.30
	H	57.18	20.05	46.82	0.48	5.80	8.43	13.95	25.97
	P	57.55	19.05	46.76	0.40	5.95	8.25	10.83	26.95

* S.N. / T.N. = soluble nitrogen/total nitrogen% *F/D = fat / dry matter%

Table 3: The mean total colony count (cfu/g) of Domiati cheese

Storage period/days	R	H	P
0(fresh)	$1.9x10^8$	$2.8 x10^4$	$5.0 x10^3$
15	$3.0x10^8$	$5.3x10^4$	$3.1x10^3$
30	$5.1x10^8$	$7.4x10^4$	$5.2x10^3$
45	$6.0x10^8$	$8.0x10^4$	$9.7x10^3$
60	$7.3x10^8$	$8.9x10^4$	$8.0x10^3$
90	$1.5x10^8$	$6.6x10^4$	$4.4x10^3$
120	$4.1x10^7$	$1.3x10^4$	$2.8x10^3$

Table 4: Total aerobic spore former count (cfu/g) of Domiati cheese

Storage period/days	R	H	P
0 (fresh)	$3.0x10^5$	$3.4x10^4$	$2.4x10^2$
15	$4.9x10^5$	$5.8x10^4$	$3.0x10^2$
30	$5.1x10^5$	$6.6x10^4$	$4.9x10^2$
45	$6.0x10^5$	$7.1x10^4$	$7.0x10^2$
60	$7.2x10^5$	$8.2x10^4$	$8.9x10^2$
90	$4.0x10^5$	$1.3x10^4$	$3.2x10^2$
120	$1.6x10^5$	$9.0x10^3$	$1.0x10^2$

Table 5: Total proteolytic count of Domiati cheese

Storage period /days	R	H	P
0 (fresh)	$3.5x10^5$	$1x10^3$	$1.1x10^2$
15	$5.0x10^5$	$2.1x10^3$	$3.0x10^2$
30	$6.4x10^5$	$3.9x10^3$	$4.1x10^2$
45	$8.3x10^5$	$4.5x10^3$	$6.1x10^2$
60	$9.7x10^5$	$6.9x10^3$	$2.0 x10^2$
90	$6.8x10^5$	$2.7x10^3$	$1.5x10^2$
120	$1.2x10^5$	$1.2x10^3$	9.0x10

Table 6: The mean total *coliform* count (MPN/g) of Domiati cheese

Storage period /days	R	H	P
0 (fresh)	$1.2x10^5$	$1x10^2$	ND
15	$3.0x10^5$	$2.9x10^2$	ND
30	$4.2x10^5$	$4.8x10^2$	ND
45	$6.0x10^5$	$6.6x10^2$	ND
60	$2.2x10^4$	$3.0x10^2$	ND
90	$1.3x10^4$	1.0x102	ND
120	$2.0x10^3$	ND	ND

*ND = Not detected

yeast count/g, according to American public health Association (APHA, 1992). All experiments were repeated in triplicate and each analysis in duplicate.

Detection and determination of aflatoxin M_1 : Aflatoxin M_1 was detected in all type of cheese at zero time and during the storage period by Enzyme linked immunosorbent assay method (ELISA) according to Scott, (1999). The detection limit was 20 ng/kg

Results and Discussion

Organoleptic properties: Data illustrated in Table 1 showed the organoleptic total score of fresh and refrigerated stored cheese made from raw, heat treated and pasteurized milk. The flavor in all types of cheese was improved during storage period. The flavor of raw milk cheese had the highest total score compared to heat treated and pasteurized cheese respectively. This may be due to the natural flora initially present in raw milk which participate in flavor production (Law, 1980).

Chemical analysis: The effect of pretreatment of milk on the most important parameters in the manufactured Domiati cheese as moisture content, fat %, salt/water % and cheese yield were recorded in Table 2. Heat treated milk and pasteurized milk cheese revealed higher moisture than raw milk cheese. The moisture content also decreased in all cheese types throughout the storage period. The fat % was slightly lower in heat treated and pasteurized milk cheese than in raw milk cheese, while it increased during storage period as a result of the decrease in moisture content. Concerning the salt /water %, the higher salt water content was detected in raw milk cheese than the other types of cheese either fresh or during storage. Cheese yield also affected by heat treatment and pasteurization. It was noticed that the highest cheese yield was obtained in pasteurized milk cheese either fresh or during the storage period. This may be attributed to the effect of pasteurization on kappa casein forming complex with B lactoglobulin which increase clotting time and subsequent cheese yield (Kanka *et al.*,1989; Schaffer *et al.*,1995; Elein *et al.*,1999). As shown in (Table 2 & Fig.1) cheese made from heat treated milk and pasteurized milk had pH values higher than raw milk cheese.

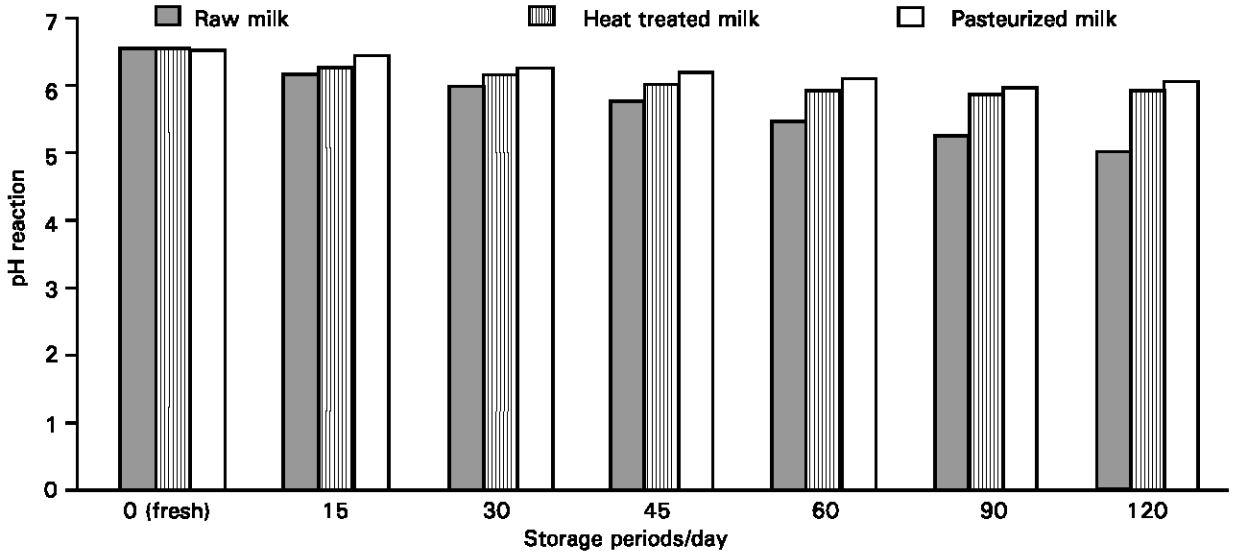

Fig. 1: pH reaction of Domiati cheese during storage

Table 7 Mean total *Staphyloccal* count of Domiati cheese

Storage period /days	R	H	P
0 (fresh)	$5.9x10^4$	$3.8x10^2$	ND
15	$3.2X10^4$	$5.1x10^2$	ND
30	$1.0X10^4$	$2.0x10^2$	ND
45	$6.5X10^3$	ND	ND
60	$5.1X10^3$	ND	ND
90	$4.0X10^3$	ND	ND
120	$1.9X10^3$	ND	ND

On the other hand, pasteurized milk cheese had the highest pH value. This trend was observed till reach the minimum pH at the end of storage period. This may be attributed to the high microbial content of raw milk cheese and the greater utilization of lactic acid leading to low pH value, while pasteurized milk cheese contained the lowest bacterial content owing to the effect of pasteurization (Ghosh *et al.*, 1999). Nearly similar finding were reported by Abd El-Salam *et al.*, 1992; Elein *et al.*, 1999.

The data presented in Table 2 showed the lowest titratable acidity (T.A.) in pasteurized milk cheese than those made from raw and heat treated milk. During cheese ripening, the T.A. increased in all types of cheese. This may be attributed to lactic acid bacteria initially present in raw milk and destroyed by pasteurization. Nearly similar finding were obtained by Omer and Elshibiny (1985); Abd El-Salam *et al.*, 1992; Marth and Steele, 2001.

The data illustrated in Table 2 and Fig. 2 show the effect of pretreatment of milk on total nitrogen (T.N.) and soluble nitrogen (S.N.) content of the manufactured cheese. Pasteurized milk cheese showed the lowest total nitrogen (T.N.%). During storage period, T.N.% increased in all types of cheese. The highest values of S.N./T.N. % were recorded with the raw milk cheese either fresh or during storage followed by heat treated and pasteurized milk cheese respectively. The lower rate of ripening in heat treated milk cheese may be due to the destructive effect of heat treatment on the natural flora and milk enzymes which in turn affect fat and protein degradation (Ghosh *et al.*, 1996; Elein *et al.*, 1999).

Microbial profile

Total colony count (T.C.C.): Results presented in Table 3 showed an increase of T.C.C. in the cheese of the three manufacture trials at refrigerated storage. The T.C.C. of cheese in all manufactured trials gradually increased until 60 days of refrigerated storage. This increase can be explained by the sufficient change in the environmental conditions which happen during cheese storage and allow the growth and multiplication of microorganisms (Hamed *et al.*, 1992). It could be noticed that T.C.C. of pasteurized milk cheese was less than other trials. This was probably due to the destruction of bacteria by milk pasteurization and rapid cooling of milk at 5°C before renneting which drastically reduce the growth rate of microorganisms than raw and heat treated milk cheese (Rehman *et al.*, 2000; Johnson, 2001; Carlos, 2002).

Aerobic spore former count: As shown in Table 4, gradual increase in aerobic spore former count of all manufactured cheese trials was demonstrated up to 60 days of refrigerated storage. The results showed that pasteurized milk cheese contained less aerobic spore former than other trials. Nearly similar finding were reported by El-Sissi and Neamat Allah (1996). Growth of aerobic spore former in raw milk produces extracelleular lipase enzyme which absorb on milk fat globules and concentrated in the manufactured cheese. During storage, the enzyme causes bitter flavor by hydrolysis of fats into fatty acids and glycerides. The enzyme could be inactivated by pasteurization while heat treatment could not destroy it. So raw and heat treated milk cheese may subjected to rapid spoilage than pasteurized milk cheese (Kroll, 1995; Beresford *et al.*, 1998).

Total proteolytic count : As shown in Table 5 the total proteolytic count of cheese was increased in all manufactured trials up to 60 days and then decreased until the end of 120 days of storage. Pasteurized milk cheese demonstrated significant decrease in total proteolytic count than raw and heat treated milk cheese ($P < 0.01$). At the end of 120 days refrigerated storage, pasteurized milk cheese showed the lowest values of proteolytic organisms. Nearly similar finding was recorded by Hamed *et al.*, 1992; Urbach, 1993; Ordonez *et al.*, 1999.

Proteolysis is the most important process happens during cheese storage. It contributes to cheese off-flavor, off odor and abnormal texture through the break down of the released proteolytic products as amino acids and peptides into amines and acids. Their growth in cheese leading to production of protease enzyme which affect on the plasmin and plasminogen of the casein micelle leading to slow cheese making and low cheese yield. The enzyme could not affected by heat treatment but may be destroyed at 70°C for 15- 30 sec. This explain the relationship between the high proteolytic count and the low cheese yield in raw and heat treated milk cheese (Bastian *et al.*, 1993; Kroll 1995).

Total *coliform* count: From the data summarized in Table 6 it could be seen that *Coliform* counts markedly decreased with heat

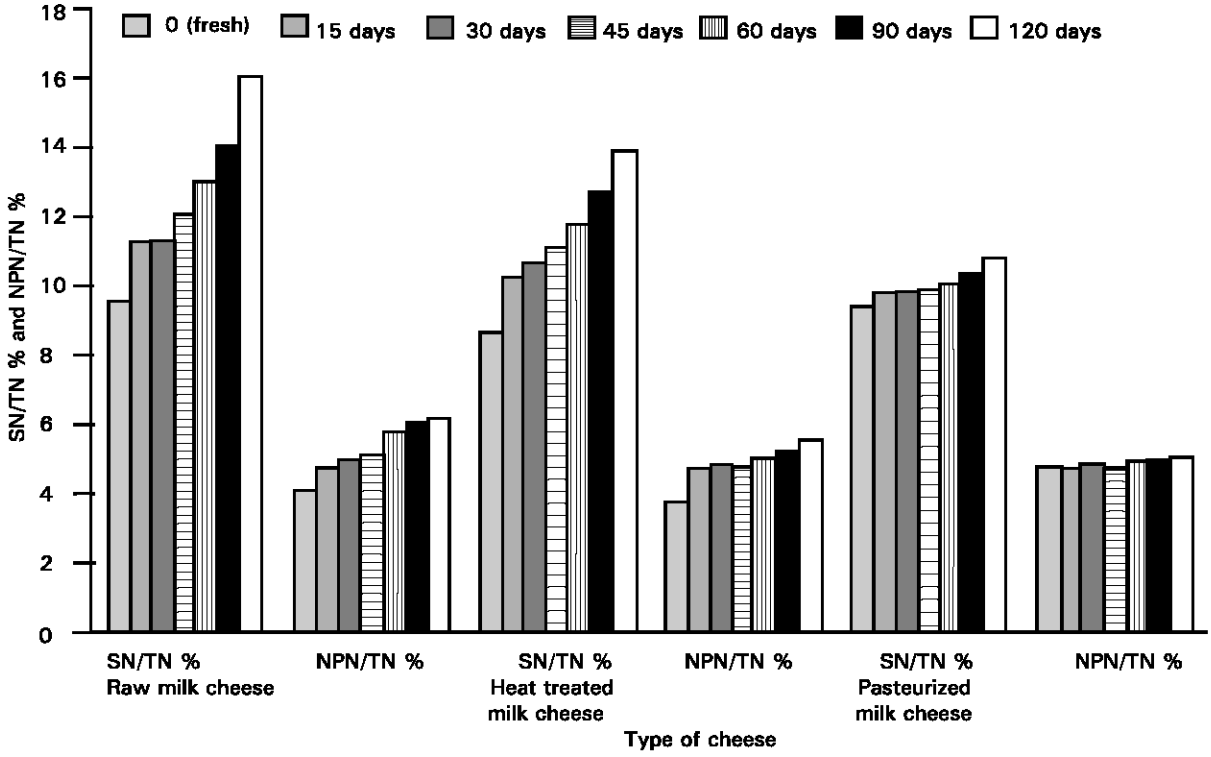

Fig. 2: SN/TN % and NPN/TN % of Domiati cheese

Table 8: Mean total mold and yeast count of Domiati cheese

Storage period /days	R	H	P
0 (fresh)	$6.2x10^6$	$3x10^3$	$5.2x10^2$
15	$6.6x10^6$	$3.9x10^3$	$6.1x10^2$
30	$7.9x10^6$	$4.2x10^3$	$6.9x10^2$
45	$9.3x10^6$	$5.6x10^3$	$7.1x10^2$
60	$9.8x10^6$	$6.0x10^3$	$9.4x10^2$
90	$2.1x10^6$	$3.1x10^3$	$5.0x10^2$
120	$1.9x10^6$	$2.6x10^3$	$1.9x10^2$

treatment and completely disappeared in cheese made from pasteurized milk. Nearly similar finding was reported by Shehata *et al.*, 1984. The obtained results can explain the blowing defects which may appear in cheese made from raw milk due to gas production by *Coliform* (Hamed *et al.*, 1992; Elein *et al.*, 1999; Moatsou 2001).

Total *Staphylococcal* count: In regards to the *staphylococcal* count it behaved as coliform. During storage period, its count decreased and was not detected at 90 and 45 days in raw milk cheese and heat treated milk cheese respectively. Pasteurized milk cheese has no staphylococcal growth during storage period (Table 7). Nearly similar finding were reported by Rashed *et al.*, 1992; Zottola and Smith, 1993.

The descending growth of *Staphylococcal* count in raw and heat treated milk cheese may be attributed to the high salt content and low pH values during storage period as well as absence of aerobic condition required for their growth (Kanka *et al.*, 1989; Quintanilla and Pena, 1991).

Total mold and yeast count: The total mold and yeast count were significantly ($P<0.01$) higher in cheese made from raw milk in comparison with the heat treated and pasteurized milk cheese (Table 8). This increase may be correlated to the higher acidity of raw milk cheese which may improve their growth. Nearly similar finding were reported by Hamed *et al.*, 1992. *Yeast and mould* are considered as spoilage organisms resulting in flavor and textural deterioration including softening, discoloration and slime formation (Besancon *et al.*, 1992). Aflatoxin M_1 could not be detected in all manufactured cheese trials either fresh or during storage period. Nearly similar finding were reported by Robinson and Tamime, 1991.

As the international microbial legislation for soft cheese should not exceed 10^2-10^3 cfu/g with their freedom from all pathogenic microorganisms (Law, 1999), raw milk cheese is more likely to serve as a vector for food borne illness.

In conclusion, pasteurized milk cheese has high quality and safety, free from pathogenic microorganisms, better acid during manufacture and storage and with high cheese yield. The disadvantage may be increase in cost and the flavor development is slower and not as that of raw milk cheese. The advantages of pasteurized milk cheese are strongly outweighing the disadvantages. On the other hand, in spite of the better flavor and high quality of heat treated milk cheese, some pathogenic and spoilage contaminants may survive the sub-pasteurization process leading to economical and public health hazard to the producer and consumer (Kanka *et al.*, 1989; Quintanilla and Pena, 1991).

So, in cheese factories where hundreds of thousands of litres of milk may be processed in a single day, it is imperative that milk is pasteurized to maintain Domiati cheese production with high quality, safety and premium grade.

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Pakistan Journal of Nutrition 1 (3): 137-142, 2002

Breast Feeding Practices in Pakistan

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Abstract : The beneficial effects of breast feeding, both for the mother and child, are well-known. However, there is evidence that breast feeding is on the decline in many developing countries. In 1991-92, a nationwide survey was conducted in Pakistan to collect baseline health information on a variety of maternal and child health issues. Several of the survey questions addressed breast feeding practices. Survey results indicate that fewer mothers are breast feeding their children, and that mothers who do breast-feed often supplement breast milk unnecessarily and/or stop breast feeding earlier. Undesirable breast feeding practices were found to be associated with urban residence, younger mother's age, and higher educational attainment. Possible explanations for the decline in breast feeding are explored and implications for the development of more effective breast feeding promotion campaigns are discussed.

Key Words: Breast feeding, behavioral determinants, urban/rural differentials

Introduction

The beneficial effects of breast feeding are well-known to health care professionals around the world. It is generally believed that breast feeding directly promotes the overall health of the child and results in decreased childhood morbidity and mortality. Breast feeding is an important determinant of the nutritional status of the child, which in turn influences growth and development (El~Zanaty *et al.*, 1992). In addition, breast feeding protects the child from diarrheal diseases by decreasing exposure to pathogens. Early initiation of breast feeding also impacts on the health status of the child because the first milk, colostrum, contains antibodies that will protect the child from disease. Prolonged breast feeding also benefitsthe child because mature breast milk contains additional compounds which can further strengthen the child's immune system and resistance to infection. Despite its obvious advantages, breast feeding in many developing countries is on the decline, especially in urban areas (Boerman *et al.*, 1991). An increasing number of women are supplementing breast milk with formula and/or cease breast feeding earlier (Boerman *et al.*, 1991). This trend has been attributed to a variety of factors including: Western influence, urbanization, and increased economic power combined with the increased availability of commercial milk substitutes. Many problems are associated with bottle feeding in the developing world, especially in areas where sanitation is inadequate. Because bottle feeding requires an uncontaminated water supply, both to mix the formula and to sterilize the bottle, there is a higher risk of childhood morbidity and mortality attributed to bottle feeding. A study conducted in the Philippines demonstrated that even small amounts of contaminated water can drastically increase the risk of diarrhea (VanDerslice *et al.*, 1994). Another problem is that bottle feeding does not provide the child with the immunological protection that breast milk does, placing the child at greater risk of infection.

Unsupplemented breast feeding works as an effective postpartum contraceptive for the mother, allowing longer intervals between pregnancies (Kennedy *et al.*, 1989). Birth spacing benefits both the mother and the child because the mother is able to breast-feed longer and she remains healthier in general by postponing pregnancy.

Previous surveys in Pakistan have concluded that breast feeding is still highly prevalent, but that mothers stop breast feeding early or begin to supplement breast milk when the child is still young (Ashraf *et al.*, 1993). Given the aforementioned benefits of unsupplemented breast feeding, it is essential to find out more information regarding knowledge, attitudes, beliefs and practices in order to develop more effective breast feeding promotion campaigns.

In 1991 and 1992, a nationwide survey was conducted to obtain information regarding the knowledge, attitudes, and practices of mothers concerning child health care in Pakistan. The purpose of the survey was to collect baseline data on a variety of issues, in order to develop effective health education program and evaluate ongoing ones. Several of the survey questions addressed breast feeding. The survey results provide valuable information regarding breast feeding practices in Pakistan. Further analysis of the results provide direction for the development of a breast feeding promotion campaign in Pakistan.

Materials and Methods

The Pakistan Health Education Survey (PHES) was conducted throughout the entire country during October 1991 through February 1992. The major objective of the survey was to collect information on health-related knowledge, attitudes, and maternal and child health concerning practices of women with children under two years of age. This information will provide baseline data on which to initiate new programs as well as to evaluate ongoing health education and service delivery activities.

The design for this survey is a stratified, clustered and systematic sample of households. The universe consists of all urban and rural areas of the four provinces of Pakistan and Azad, Jammu and Kashmir (AJK), defined as such by the 1981 Population Census. The universe excluded military restricted areas, areas of D.G. Khan District, Kohistan, Chitral and Malakand Districts as well as the Federally Administered Tribal Areas (FATA). The population of these excluded areas constitute approximately 4 percent of the total population. The population of the survey covers mothers with children 2 years of age or less and is estimated to be between 6-7 percent of the total population of 120 million. A complete description of the sampling methodology is presented in an article (Morisky *et al.*, 1995). Briefly, a total sample size of 5,400 eligible respondents (women having children equal to or less than 2 years of age) was expected to provide valid reliable estimates at national level of key variables with a +/- 5% coefficient of variability at the 95% confidence level. Table 1 presents the number, percent of primary sampling areas and the total sample interviewed from each of the five provinces by urban and rural areas.

Questionnaire: The PHES questionnaire was developed by a multi-disciplinary team of experts from the Ministry of Health, including health education experts, members of the Federal Communication Advisory Group, program managers of various categorical programs (TB Control, Expanded Program on Immunization, Center for Diarrheal Disease, etc.), international agencies, and technical experts. The questionnaire was translated into the

Table 1: Number and Percent of Primary Sampling Areas (PSUs) and Total Sample from Each Province

Province/Area	Urban	Rural	Total	Sample Size
Punjab	48	72	120	2400
Percent	40	60	45	
Sindh	30	30	60	1200
Percent	40	60	22	
N.W.F.P.	16	24	40	800
Percent	40	60	15	
Balochistan	12	18	30	600
Percent	40	60	11	
AJK	8	12	20	400
Percent	40	60	7	
TOTAL	114	156	270	5400
Percent	42	58	100	

Table 2: Social Demographic Characteristics

Demographic Data	N	Percent
TOTAL	5433	100.0%
Province		
Punjab	2406	44.3%
Sindh	1176	21.6%
N.W.F.P.	812	14.9%
Balochistan	640	11.8%
AJK	399	7.3%
Residence		
Rural	3167	58.3%
Urban	2266	41.7%
Age		
15 - 19	196	3.6%
20 - 24	1063	19.6%
25 - 29	1842	33.9%
30 - 34	1303	24.0%
35 - 39	740	13.6%
40 - 44	236	4.3%
45 - 49	53	1.0%
Education		
None	3953	72.8%
Primary	574	10.6%
Middle	298	5.5%
> =Secondary	608	11.2%
Monthly Income		
< RS 1,000	1727	31.8%
RS 1,000 - 1,999	1613	29.7%
RS 2,000 - 3,499	1018	18.7%
RS 3,500 - 4,999	346	6.4%
> RS 5,000	293	5.4%
Don't Know	436	8.0%

national language, Urdu, and pre-tested prior to its implementation. The content areas of the questionnaire included background socio-demographic characteristics, breast feeding practices, knowledge, attitudes and practices concerning diarrhoea, immunization, malaria and smoking. Questions concerning knowledge about AIDS, and its routes of transmission were also asked of each respondent. The following breast feeding questions were asked of each respondent and directed to the youngest child: "What milk do you give to your youngest child"?; "Did you ever breast feed your youngest child"?; "When did you begin to breast feed your youngest child"?; How long did you breast feed your youngest child"?; "Why did you stop breast feeding your youngest child"?; How long did you exclusively breast feed your youngest child without supplementing with other foods or liquid"?; "At what age did you begin to add solid food for your youngest child"?. The same set of questions were asked to mothers with an additional child under two years of age.

Results

Socio-demographic Characteristics: Table 2 presents the frequency and percentages of various socio-demographic characteristics of the surveyed population. A total of 5433 women were interviewed throughout the five provinces. The largest surveyed area was Punjab with 44.3% of the total sample. The respondents are not proportionate to the population of provinces and area of residence (Urban:Rural). The sample was drawn by the Federal Bureau of Statistics, keeping in view the homogeneity and heterogeneity of populations in different provinces and areas of residence. The urban population represented approximately 60% of the sample and the rural population represented approximately 40%. The mean age of the population was 26.5 with the largest number of respondents (33.9%) falling into the age group 25-29 years followed by 24% belonging to the age group 30-34. A total of 72.8% of the respondents have no education, 10.6% have attained primary education, 5.6% have gone to middle level and 11.2 % secondary and above. Thirty-two percent of the households have less than Rupees 1000 monthly income which can be considered below the poverty line.

Breast Feeding Behaviors : Of the women surveyed, 95.4% reported ever breast feeding their youngest child. Only 3.9% of mothers had never breast fed their youngest child. Table 3 indicates that the behavior "ever having breast fed your infant" appears to be unrelated to province of residence, education level, or income. These results confirms the findings of previously conducted surveys which have concluded that 87-98% of mothers still breast-feed their infants (National Nutrition Survey, 1988).

Breast feeding patterns by type of milk: The breast feeding patterns, when examined by type of milk, presents a different story. When asked how they were currently feeding their youngest child, 73.8% of mothers were breast feeding exclusively, 21.6% were both breast and bottle feeding, and 3.9% were bottle feeding only." Comparison of the feeding patterns between provinces indicated that fewer mothers were breast feeding in the more developed provinces 69.8% in Punjab and 66.7% in AJK compared to 75.4% in the province of Sindh and 81.4% in NWFP and 80.9% in Balochistan. Use of combined breast and bottle feeding is also more popular in urban, better educated and upper income groups as displayed in Table 4.

Breast feeding initiation: Breast feeding should be initiated as soon as possible after birth to give the child the full immunological benefits of the colostrum. However, many women wait before beginning to breast feed. Only 36% of mothers began breast feeding the day they gave birth, 30.7% started on the 2nd day, and 34% started on the third or fourth day. There were significant differences in breast feeding practices among provinces, rural and urban residents, educated and non-educated, and income, as indicated in Table 5. For example, 40% of urban residents initiated breast feeding on the first day, compared with only 32.9% of rural residents.

Punjab had the lowest rate of early breast feeding initiation, only 20%. One possible explanation for the late initiation of breast feeding in Punjab may be 'that many' mothers do not believe that colostrum is milk, and they think they must wait two or three days until the milk becomes available.

Exclusive breast feeding : A child who is being breast fed does not require any additional food or liquids for the first 4-6 months of life, yet many mothers supplement breast milk with additional liquids or small quantities of food. Aside from being unnecessary, early weaning can be dangerous because it exposes the child to disease agents and may deprive the child of essential nutrients. Also, the earlier a mother begins to supplement her breast milk, the earlier she tends to stop breast feeding.

The survey indicated that 62% of mothers reported starting supplementary feeding of their children before 5 months of age. Some of the supplementary foods that were mentioned include:

Table 3: Breast Feeding Behavior of Pakistani Mothers by Demographic Characteristics, Pakistan National Health Education Survey 1991-92

Demographic Data	Ever Breast-Feeding			Total	
	Yes (N=5,183)	No (N=211)	DK * (N=39)	%	Valid N
PAKISTAN (Total Percent)	95.4%	3.9%	0.7%	100.0%	5,433
Province					
Punjab	95.4%	4.3%	0.3%	100.0%	2,406
Sindh	95.2%	4.1%	0.8%	100.0%	1,176
N.W.F.P.	96.7%	2.2%	1.1%	100.0%	812
Balochistan	93.9%	4.2%	1.9%	100.0%	640
AJK	96.0%	3.5%	0.5%	100.0%	399
Residence					
Rural	96.6%	2.7%	0.8%	100.0%	3,167
Urban	93.8%	5.6%	0.6%	100.0%	2,266
Age					
15-19	94.9%	4.1%	1.0%	100.0%	196
20-24	94.7%	4.2%	1.0%	100.0%	1,063
25-29	94.8%	4.6%	0.6%	100.0%	1,842
30-34	96.2%	3.1%	0.8%	100.0%	1,303
35-39	96.4%	3.2%	0.4%	100.0%	740
40-44	97.5%	2.5%	0.0%	100.0%	236
45-49	90.6%	5.7%	3.8%	100.0%	53
Education					
None	96.5%	2.7%	0.8%	100.0%	3,953
Primary	94.3%	4.9%	0.9%	100.0%	574
Middle	93.3%	6.0%	0.7%	100.0%	298
> =Secondary	90.3%	9.4%	0.3%	100.0%	608
Monthly Income					
"RS < 1,000"	95.8%	3.1%	1.1%	100.0%	1,727
"RS : 1,000 - 1,999"	95.7%	3.6%	0.7%	100.0%	1,613
"RS : 2,000 - 3,499"	95.2%	4.3%	0.5%	100.0%	1,018
"RS : 3,500 - 4,900"	94.5%	5.2%	0.3%	100.0%	346
"RS > 5,000"	92.8%	6.1%	1.0%	100.0%	293
DK *	95.6%	4.4%	0.0%	100.0%	436

* DK : Do Not Know

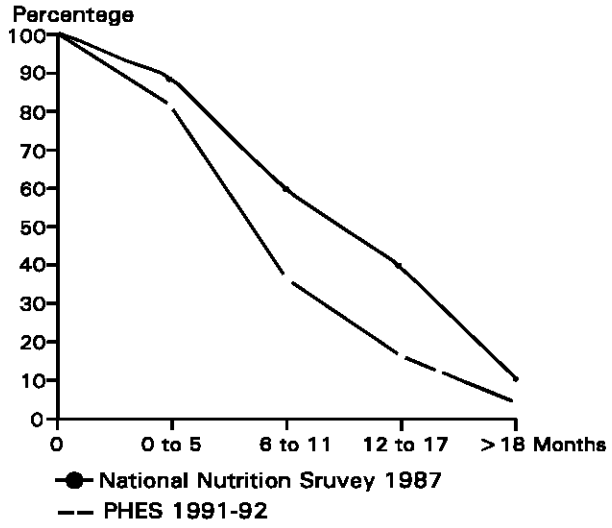

Fig. 1: Duration of breast feeding of youngest child: National Nutrition Survey 1987 and Pakistan Health Education Survey 1991-92

ghutti (a mixture of honey, butter, sugar and liquid), homemade liquids, bread, and boiled potatoes. Early supplementation of breast feeding was associated with urban, more educated, wealthier, women. Late initiation of supplementation was more common among rural, less educated, poorer mothers.

Duration of breast feeding : At the time of the interview, 79.2% of mothers were still breast feeding their youngest child. Further analysis revealed that more rural, less educated, poor, mothers were breast feeding at the time of the interview. For those mothers who had stopped breast feeding, 12% reported that they breast fed for two months, 17.6% up to five months, 35.6% up to 12 months, and 29.2% beyond one year. Fig. 1 compares the duration of breast feeding for the youngest child for the 1987 National Nutrition Survey and the present PHES 91-92 Survey. As indicated, the duration of breast feeding has declined considerably between the two surveys, with 58% of mothers breast feeding between 6-11 months for the 1987 survey compared to less than 40% breast feeding for this duration in the PHES Survey.

Reasons for discontinuation of breast feeding: Among women who had stopped breast feeding their youngest child, 34.5% of women stated pregnancy as the reason for stopping. The second and third most common reasons were "milk dried up" and "child refused", for 19.1% and 12.1% of women respectively. Some less common reasons for stopping breast feeding were: the child reached weaning age, the mother became ill, or the child became ill.

Breast Feeding Patterns for the Older Child: A total of 288 mothers have two children over 2 years of age. These mothers were asked the same set of questions about breast feeding of the older child. A total of 81.6 percent of mothers reported ever breast feeding their older child compared to 95.4 percent for the youngest child. These results indicate a significant intergenerational increase in the proportion of mothers initiating breast feeding practices among their youngest child.

Table 4: Types of Milk Given to Youngest Child by Demographic Characteristics,"Pakistan National Health Education Survey 1991-92

	Breast Only n=4,011	Breast + Bottle n=1,172	Bottle Only n=211	DK* n=39	Valid N
PAKISTAN (Total Percent)	73.8%	21.6%	3.9%	0.7%	5,433
Province					
Punjab	69.8%	25.6%	4.3%	0.3%	2,406
Sindh	75.4%	19.7%	4.1%	0.8%	1,176
N.W.F.P.	81.4%	15.3%	2.2%	1.1%	812
Balochistan	80.9%	13.0%	4.2%	1.9%	640
AJK	66.7%	29.3%	3.5%	0.5%	399
Residence					
Rural	77.2%	19.3%	2.7%	0.8%	3,167
Urban	69.1%	24.7%	5.6%	0.6%	2,266
Age					
15-19	82.1%	12.8%	4.1%	1.0%	196
20-24	74.7%	20.0%	4.2%	1.0%	1,063
25-29	73.5%	21.3%	4.6%	0.6%	1,842
30-34	72.7%	23.5%	3.1%	0.8%	1,303
35-39	72.6%	23.8%	3.2%	0.4%	740
40-44	76.3%	21.2%	2.5%	0.0%	236
45-49	73.6%	17.0%	5.7%	3.8%	53
Education					
None	77.5%	19.0%	2.7%	0.8%	3,953
Primary	67.1%	27.2%	4.9%	0.9%	574
Middle	75.8%	17.4%	6.0%	0.7%	298
> =Secondary	55.3%	35.0%	9.4%	0.3%	608
Monthly Income					
RS < 1,000	78.2%	17.6%	3.1%	1.1%	1,727
RS:1,000-1,999	74.0%	21.8%	3.6%	0.7%	1,613
RS:2,000-3,499	71.5%	23.7%	4.3%	0.5%	1,018
RS:3,500-4,900	68.2%	26.3%	5.2%	0.3%	346
RS > 5,000	64.5%	28.3%	6.1%	1.0%	293
DK *	72.2%	23.4%	4.4%	0.0%	436

* DK : Do Not Know

Multivariate Analysis for Breast Feeding: Those variables which were found to significantly relate to exclusive breast feeding practices were entered into a multivariate analysis using the variable "feeding pattern" as the dependent variable. This was categorized as 1 = exclusive breast feeding and 0 = to all other responses. For categorical dependent variables, logistic regression analysis was used to predict the best model that fit the data. Model Chi Square and goodness of fit tests are used to evaluate the efficiency of the model. The parameters of the model are estimated using the maximum likelihood method, For each independent variable, the coefficient and its standard error are calculated. The Wald test and its significance are used to test if the coefficient of the independent variable is different from zero. For each independent variable, the odds ratio is calculated by obtaining the exponent for the coefficients of the independent variables. Table 6 presents the logistic regression model to predict the feeding pattern of the mother. It is apparent that the mother's area of residence, age, education, number of consultations for child health, having a radio, TV or reading the newspaper, age at which she began to breast feed, and age of the child are significant predictors of exclusive breast feeding. These seven variables significantly predict 76.8% of the feeding pattern of the mother. Mothers living in urban areas are 10% less likely to exclusively breast feed their youngest child. Lower levels of education are also significant predictors with mother's with primary education 89% less likely to exclusively breast feed and mothers with no education are 2.56 times less likely to exclusively breast feed. Finally mothers who have a radio, TV or read the newspaper are 8% less likely to exclusively breast feed their infant.

Discussion

Breast feeding is on the decline in the more developed areas of Pakistan. Urban, more educated, wealthier women are more likely to stop breast feeding earlier, whereas rural, less educated and poor women tend to breast feed for longer. This pattern has been documented in other Middle Eastern countries including the United Arab Emirates and Saudi Arabia (Shahraban *et al.*, 1991; Serenius *et al.*, 1988). One reason for the rural-urban differential in Middle Eastern nations may be that urban, more educated, wealthier women are more subject to Western influence and have increased access to milk substitutes. Although the vast majority of women do breast feed their children for a time, they often cease breast feeding exclusively too early to give the child maximum benefit. Another problem is identifying the proportion of mothers who exclusively breast feed their infants. A prospective study conducted in Lahore, Pakistan found that only 9% of mothers were exclusively breast feeding, and that 23-73% of mothers were giving water in addition to milk (Ashraf *et al.*, 1993). This distinction is important because some studies have shown that unsupplemented breast feeding is much more strongly associated with decreased child mortality than supplemented breast feeding (Shahidulla, 1994). In any case, supplementing breast milk with formula and/or ghutti is unnecessary during the first 4-5 months, and it poses health risks to the child. Furthermore, early supplementation with breast feeding often leads to early stopping of breast feeding. It is difficult to explain the late initiation of breast feeding in Punjab, although cultural beliefs and attitudes appear to be a contributing factor. For example, many mothers in Punjab do not believe that colostrum is milk. They believe that milk only becomes available two to three days after birth. Other studies have shown that mothers believe the child should receive ghutti during the first day which consists of honey, butter mixed with sugar, glucose and other liquids.

The mothers also believe that ghutti has a dual purpose: it gives nutrition to the child until the mother's milk becomes available and it also cleans the intestines of the new born infant. commercial

Table 5: Initiation of Breast Feeding by Demographic Characteristics, Pakistan National Health Education Survey 1991-92

	Immediately After Birth N = 1,844	2nd Day After Birth N = 1,581	3rd Day After Birth N = 1,570	4th Day After Birth N = 143	DK* N = 45	Valid N**
PAKISTAN (Total Percent)	35.6%	30.5%	30.3%	2.8%	0.9%	5,183
Province						
Punjab	19.7%	30.2%	45.4%	3.8%	1.0%	2,295
Sindh	63.6%	23.7%	10.5%	1.6%	0.5%	1,119
N.W.F.P.	35.0%	38.9%	24.2%	1.4%	0.5%	785
Balochistan	36.8%	35.8%	23.3%	3.2%	1.0%	601
AJK	48.3%	26.9%	21.1%	1.8%	1.8%	383
Residence						
Rural	32.6%	30.0%	33.9%	2.8%	0.7%	3,058
Urban	39.8%	31.3%	25.0%	2.7%	1.1%	2,125
Age						
15-19	36.0%	30.1%	27.4%	4.8%	1.6%	186
20-24	36.4%	30.5%	29.7%	2.2%	1.2%	1,007
25-29	35.4%	31.5%	29.3%	2.9%	0.9%	1,746
30-34	38.1%	28.4%	31.0%	2.4%	0.2%	1,253
35-39	32.4%	31.1%	32.4%	2.9%	1.1%	713
40-44	30.4%	33.0%	30.9%	4.3%	1.3%	230
45-49	29.2%	29.2%	39.6%	0.0%	2.1%	48
Education						
None	32.0%	31.5%	33.1%	2.6%	0.8%	3,815
Primary	39.2%	29.2%	27.4%	3.5%	0.7%	541
Middle	48.2%	27.7%	21.2%	1.4%	1.4%	278
> =Secondary	50.5%	26.4%	18.2%	3.6%	1.3%	549
Monthly Income						
RS < 1,000	28.4%	30.8%	37.6%	2.5%	0.7%	1,654
RS:1,000-1,999	33.9%	31.3%	31.4%	2.7%	0.6%	1,544
RS:2,000-3,499	38.3%	30.3%	26.3%	3.6%	1.4%	969
RS:3,500-4,900	45.9%	30.9%	19.0%	2.8%	1.5%	327
RS > 5,000	53.3%	26.5%	17.3%	1.5%	1.5%	272
DK **	44.1%	29.3%	23.7%	2.9%	0.0%	417

* DK : Do Not Know # Valid N : Only 5,183 respondents ever breast fed their youngest child

Table 6: Logistic Regression Analysis for Feeding Pattern for Children, Pakistan National Health Education Survey 1991-92

Variable	B	S.E.	Wald test	Significance	R	Exp(B)
Area of residency	-0.10	0.074	1.93	0.165	0.00	0.90
Mother's age	-0.02	0.006	13.63	0.0002	-0.05	0.98
Mother's education *						
- no education	0.94	0.112	71.15	0.000	0.11	2.56
- primary education	0.64	0.124	26.29	0.000	0.07	1.89
Number of consultants for child health	-0.17	0.037	21.10	0.000	-0.06	0.84
"Having radio, TV, newspaper"	-0.08	0.039	4.67	0.030	-0.02	0.92
Age to begin breast-feeding	-0.29	0.039	55.38	0.000	-0.10	0.75
Age of child (month)	-0.07	0.005	160.16	0.000	-0.17	0.93
Constant	2.96	0.245	146.07	0.000		

Model Chi Square = 367, Significance = .000, Prediction = 76.8%

* Mother's education : using the secondary education category as a reference category

ghutties are available in the market. Young mothers are informed about ghutti from older women, traditional birth attendants, midwives, mothers-in-law and in some cases, health care providers. The pressure to use ghutti prevents a mother who is often young and uneducated to initiate the early practice of breast feeding and use of colostrum.

In general, rural, poor, and less educated women are more likely to continue breast feeding for longer durations, but they are also more likely to initiate breast feeding later. Traditional practices and cultural beliefs appear to be an important factor in the decision to delay breast feeding. For example, mothers in some areas consider that colostrum is "stale" milk and that fresh milk will arrive on the third day. (Ashraf *et al.*, 1993). During this interim period, the child is usually given traditional and/or ceremonial foods such as honey, animal milk, rosewater, or cardamom water (Ahsraf *et al.*, 1993). These practices much be considered dangerous since there is a high potential for contamination.

Conclusion: Pakistan is a society in transition; traditional practices are being abandoned in favor of a more.' westernized lifestyle. Since there is evidence that socioeconomic factors influence early supplementation and bottle use, breast feeding is likely to decline even further as Pakistan continues to develop and urbanize. Now more than ever, women need to be better educated about breast feeding.

A targeted health education campaign should stress the many advantages of breast feeding. Specifically, messages should promote breast feeding as the optimal and most nutritious food for the child, an effective means of preventing disease, and an excellent contraceptive for child spacing. Mothers should be encouraged to continue breast feeding for as long as possible.

Since it is stated in the Holy Koran that mothers should breast feed their children for two years, there is potential to involve religious leaders in a breast feeding campaign.

A breast feeding campaign should also target specific behaviors,

such as late onset of breast feeding. There is a particular need to communicate this message to residents of poor and rural areas. Messages should stress the protective benefits of colostrum and encourage mothers to begin breast feeding immediately after birth. Potentially hazardous practices, including pre lacteal feeding, should be discouraged.

Another issue that needs to be addressed is early supplementation of breast feeding. Mothers should be educated that the child does not require anything ,in addition to breast milk during the first five months. It should be made clear that breast milk contains everything the child needs for proper nutrition and that even water is unnecessary.

Some mothers discontinue breast feeding because their milk production slows. Thus, it is important to teach mothers that they can increase their milk production by feeding the child on demand, as often as the child needs. It is widely accepted that breast feeding saves infants' lives. six million infant deaths from diarrhea and acute respiratory infections alone are prevented annually through breast feeding and it is estimated that if more women were to breast-feed optimally (i.e., exclusively from birth through the first 4 to 6 months and for one year or longer), an additional two million infant deaths could be averted annually (Huffman *et al.*, 1991). It is essential that these beneficial effects of breast feeding be disseminated to all mothers and promote breast feeding for as long as possible as advocated in Islam.

Acknowledgement

The research presented in this article was conducted by Donald E. Morisky and Snehendu B. Kar, WHO short-term consultants to the Ministry of Health, Division of Health Education, Islamabad, Pakistan 1990-1991. The views and opinions of authors expressed herein do not necessarily state or reflect those of the World Health Organization

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Pakistan Journal of Nutrition 1(3): 143-150, 2002

Update on ORS Usage in Pakistan: Results of a National Study

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Abstract: Diarrhea disease continues to rank as one of the leading causes of child mortality throughout the world. It is estimated that 1 billion episodes of diarrhea occur in young children each year, The World Health Organization has recommended the use of oral rehydration solution (ORS) for the treatment of dehydration associated with diarrhea. Numerous studies have documented the effectiveness of ORS in treating diarrhea and reducing mortality. Diarrheal disease in Pakistan has been identified as the major cause of child mortality in Pakistan, accounting for an estimated 200,000 - 300,000 deaths each year. This paper reports the results of a nationwide survey conducted in Pakistan to obtain information regarding the practices of mothers concerning child health care and factors that influence these practices. The purpose of the survey was to collect baseline data on a variety of issues, in order to develop effective health education programs and evaluate ongoing ones. Within the context of two theoretical models (diffusion of innovation and stages of change), adoption practices of the population with respect to use of ORT treatment are described and assessed. These results pose new challenges to health care professionals in their ability to influence and persuade adoption of effective public health practices. Recommendations are provided as how to modify the misconceptions of mothers with young children in the treatment of diarrhea.

Key Words: ORS, diarrhea, theory, behavioral determinants

Introduction

Diarrhea is one of the leading causes of child mortality in many developing countries. It is estimated that diarrheal diseases cause 3.3 million deaths annually among children under the age of five in the developing world (Bern *et al.*, 1992), accounting for 23% of child mortality worldwide. An estimated 1 billion episodes of diarrhea occur in young children each year, equivalent to 2.6 episodes per child per year (Bern *et al.*, 1992; Gadomski *et al.*, 1988).

Diarrhea in developing countries is caused by a variety of bacterial, viral, and parasitic pathogens; some of the most common are rotavirus, *E. coli*, campylobacter, shigella, and salmonella (Taylor, 1993). The health consequences of frequent or persistent diarrhea can be severe, including malnutrition and impaired growth and development (Chen and Scrimshaw, 1983). Moreover, diarrhea can be viewed as both a cause and an effect of malnutrition since it can be difficult to determine whether diarrhea precedes malnutrition of vice-versa in individual cases (Walker-Smith, 1993). Diarrheal episodes can lead to a dangerous spiral of illness because diarrhea impedes growth and malnutrition increases the frequency of diarrhea (Guerrant *et al.*, 1992).

The World Health Organization recommends the use of oral rehydration solution (ORS) for the treatment of dehydration associated with diarrhea (WHO, 1991). Numerous studies have documented the effectiveness of ORS in treating diarrhea and reducing mortality (WHO, 1997; Varavithya *et al.*, 1991; Richards *et al.*, 1993). ORS is particularly appropriate for the treatment of diarrhea in the developing world because it is inexpensive and can be administered in the home.

Improved use of ORS in conjunction with appropriate feeding practices could markedly reduce the morbidity and mortality associated with diarrhea. For this reason, ORS education and distribution programs have been implemented throughout the world, increasing the availability, accessibility and afford ability of ORS (Merson, 1986). Yet despite the widespread availability of ORS, many mothers continue to use alternative therapies to treat childhood diarrhea or they use ORS incorrectly (Merson, 1986; Hudelson, 1993; Mull *et al.*, 1988).

Many factors contribute to the failure of some populations to adopt ORS, but one of the most important is a paucity of social support for the behavior. Cultural beliefs and practices may encourage the use of traditional therapies, and disagreement among health care providers about which treatment is best may further impede the adoption of oral rehydration therapy in some societies. The inappropriate use of antibiotics to treat diarrhea has become commonplace in many developing countries because doctors continue to prescribe them unnecessarily, many women believe this is the only appropriate treatment for illness (Hudelson, 1993).

Modern pharmaceuticals are readily available without a prescription in many developing countries. A study in the Philippines demonstrated that most childhood illness were treated without the advice of a physician, yet half of these treatments involved the use of pharmaceuticals (Hardon, 1987). Another study in India showed that, even when physicians were consulted, prescription practices were inappropriate and sometimes dangerous (Greenhalgh, 1987). Paredes *et al.*, 1996 identified physician prescribing practices for Peruvian mothers who brought their children to the health center for diarrheal management. Most physicians reported that family members usually expect to receive a prescription when they visit a physician. If a prescription is not given, the physician would be considered to lack experience or to 'know nothing about treating diarrheal disease'. Furthermore, mothers who reported receiving 'only' ORS left the consultation often unhappy or frustrated. They reported that this was because they did not receive a prescription but only ORS (Paredes *et al.*, 1996).

ORS promotion in Pakistan: Diarrheal disease in Pakistan has been identified as the major cause of child mortality in Pakistan, accounting for an estimated 200,000 - 300,000 deaths each year (Lambert, 1986). Nearly 50% of child hospital admissions are related to diarrhea. Furthermore, a recent survey found that 14.5% of children under the age of 5 years had experienced an episode of diarrhea during the preceding 24 hours (UNICEF, 1991). Rates were highest among children under one year of age, and declined steadily with age. The need to promote appropriate treatment of diarrhea, especially in young children, is clear.

In 1984, the Government of Pakistan launched a program to control diarrheal diseases (CDD). The program has promoted the use of ORS through an intensive public health education campaign. Previously conducted surveys have indicated that knowledge concerning ORS has been steadily rising, from 38% in 1984 to more than 85% in 1987 (ORS-KAP Survey, 1987). However, while

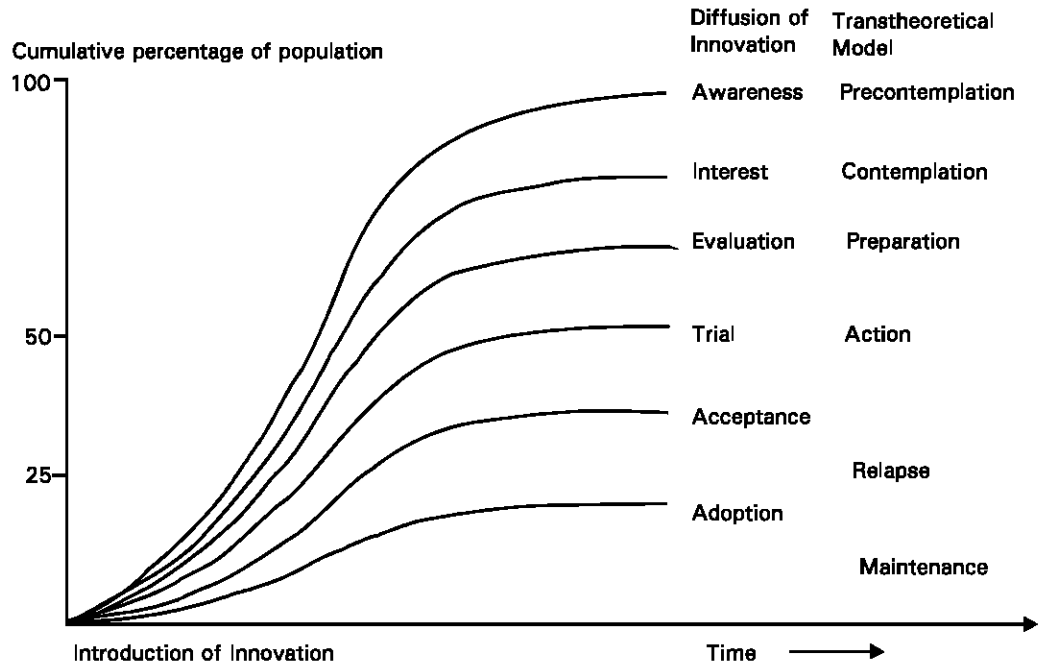

Fig.1: An integrative model of diffusion of innovation and the transtheoretical model

awareness of ORS remains high, ORS use lagged significantly behind. A nationwide survey of about 5000 Pakistani households commissioned by the Pakistan Ministry of Health in 1987 found that 90% of those interviewed knew that a 1-liter packet of ORS should be mixed with 1 liter of water. However, detailed questions regarding the preparation and administration process were not asked. Mull and Mull (1988) state that WHO's current estimate that ORS is being used `effectively' by 80% of diarrhea cases is much too optimistic, at least for Pakistan.

Theoretical framework: The Diffusion of Innovation Model provides a useful framework for examining the adoption of ORST in Pakistan. This model categorizes individual behavior change into five stages: awareness, interest, persuasion, decision, and adoption (Rogers, 1971). Individuals pass through these stages and adopt new behaviors at different rates. The model classifies these different rates of adoption by dividing the population into five groups: innovators, early adopters, early majority, late majority, and laggards (Rogers, 1971). When a new behavior is introduced into a population, the cumulative curve follows an S-shaped rate of adoption, as more individuals reach the fifth stage and adopt or internalize the new behavior.

The Diffusion of Innovation Theory is closely linked to the Transtheoretical Model of behavior change (Prochaska *et al.*, 1984). Prochaska and DiClemente have identified five stages of change through which an individual must pass before achieving behavior change precontemplation, contemplation, preparation, action, and maintenance. Both Diffusion of Innovation Theory and the Transtheoretical Model are based on the assumption that individuals pass through several stages before successfully achieving behavior change. The major distinction between these two models is that the Transtheoretical Model limits examination of behavior change to the individual level, while Diffusion of Innovation goes beyond the individual to describe behavior change in a population. The Transtheoretical Model has traditionally been used to analyze the cessation of addictive behavior from a psychological perspective. However, if the Transtheoretical Model is expanded and viewed from a community/public health perspective, the two theories appear to have many shared components. Rogers has conceptualized the characteristics of an innovation, as perceived by the members of a social system, which determine its rate of adoption. These factors include relative advantage, compatibility, complexity, trial ability and observ ability. A more recent health education and health promotion diagnostic framework (PRECEDE/PROCEED) developed by Green and Kreuter, 1999 addresses these issues and identifies specific factors in the adoption process, including various predisposing, enabling and reinforcing factors which can help explain why mothers do not translate their knowledge of ORS into taking action. Fig. 1 presents the comparative features of these two theoretical constructs and potential linkages of stages of behavioral change in the models. The current study presents an opportunity to link and test these two classical models with empirical data.

Background of Pakistan: Pakistan is the seventh most populous country in the world, with an estimated population of 125 million in 1995. The country is divided into five provinces. Punjab is the most densely populated with 55% of the population, followed by Sindh with 22% of the population. The two least populous provinces are North West Frontier Province (NWFP), with 13% of the population, and Balochistan, with 5%. Approximately 70% of the population is rural, and 90% is Muslim.

In 1991 and 1992, a nationwide survey was conducted in Pakistan to obtain information regarding the practices of mothers concerning child health care and factors that influence these practices. The purpose of the survey was to collect baseline data on a variety of issues, in order to develop effective health education programs and evaluate ongoing ones. Several of the survey questions addressed knowledge, attitudes, beliefs and practices regarding ORS. Several other questions were designed to gather information about the respondents' sources of health information. Taken together, these two components of the survey can help to direct the development of an anti-diarrhoeal treatment/ prevention program in Pakistan.

Materials and Methods

The Pakistan Health Education Survey (PHES) was conducted throughout the entire country during October 1991 and February 1992. The major objective of the survey was to collect

Table 1: Number and Percent of Primary Sampling Areas(PSUs) by Urban and Rural Areas and Total Sample Size

	Urban	Rural	Total	Total Sample Size
Province/Area				
Punjab	48	72	120	2400
Percent	40	60	45	
Sindh	30	30	60	1200
Percent	40	60	22	
N.W.F.P.	16	24	40	800
Percent	40	60	15	
Balochistan	12	18	30	600
Percent	40	60	11	
AJK	8	12	20	400
Percent	40	60	7	
TOTAL	114	156	270	5400
Percent	42	58	100	

information on health-related knowledge, attitudes, and maternal and child health concerning practices of women with children under two years of age. This information will provide baseline data on which to initiate new programs as well as to evaluate ongoing health education and service delivery activities.

The design for this survey is a stratified, clustered and systematic sample of households. The universe consists of all urban and rural areas of the four provinces of Pakistan and Azad, Jammu and Kashmir (AJK), defined as such by the 1981 Population Census. The universe excluded military restricted areas, areas of D.G. Khan District, Kohistan, Chitral and Malakand Districts as well as the Federally Administered Tribal Areas (FATA), because of governmental restrictions and safety of visiting health nurses. The population of these excluded areas constitute approximately 4 percent of the total population. The population of the survey covers mothers with children 2 years of age or less and is estimated to be between 6-7 percent of the total population of 120 million.

Sampling frame-urban domain: The sampling frame for the urban domain consists of lists of enumeration blocks provided by the Federal Bureau of Statistics. Each city or town had been divided into a number of small areas called Enumeration Blocks. Each Enumeration Block is a compact area consisting of 200 to 225 households on the average with well-defined boundaries recorded on the prescribed forms. Each Enumeration Block is demarcated on the map with physical features describing the locality and physical features.

Sampling frame-rural domain: The sampling frame for rural domains consists of all mouzas/dehs/villages prepared by the Population Census Organization as a result of the 1981 Population Census. A mouza/village/deh is the smallest revenue estate identified by its name.

Several factors were considered in determining the sample size of the survey, including: the main objectives of the survey, level of estimate, acceptable level of error in the estimate, proportionality of study population, strata/sub-strata requirements, minimum number of observations, time and resource constraints, and coverage problems. Table 1 presents the distribution of samples in the urban and rural domains of the four provinces and AJK according to the Primary Sampling Area (PSU). Approximately 20 households were surveyed within each PSU.

The total sample size of 5,400 eligible respondents (women having children equal to or less than 2 years of age) was expected to provide valid reliable estimates at national level of key variables with a +/- 5% coefficient of variability at the 95% confidence level.

Stratification Plan: In consideration for the level of estimates desired and required heterogeneity in the population, stratification has been done according to self-representing cities, by urban and rural areas. Cities having populations of 500 thousand and greater according the 1981 population census, namely Karachi, Lahore, Faisalabad, Gujranwala, Rawalpindi, Multan, Hyderbad and Peshawar have been taken as self-representing cities. Islamabad, the national capital and Quetta, a provincial capital have been specially considered as a self-representing city (SRC). Each of the SRC's constitute an independent or explicit stratum. After excluding the population of SRCs from the respective districts of a province, the remaining urban population in each division of the Punjab, Sindh, N.W.F.P. and Balochistan Provinces have been grouped together to form another stratum in all the four provinces of Pakistan. Each SRC was further divided into three sub-strata according to low, middle and high income groups based on the information collected from each Enumeration Block at the time of demarcation and updating of the urban sampling frame. Rural populations of each District in the Punjab, Sindh, and N.W.F.P. Provinces have been grouped to form a stratum. For Balochistan Province, each division has been considered as a stratum.

Sample Design: A two-stage stratified sampling design was adopted for the survey. The sample PSUs from each urban stratum were selected with a probability proportional to the number of households. The sample PSUs from each rural stratum were selected with a probability proportional to the population enumerated in the 1981 census. The second stage of sampling consisted of selection of households in the selected cluster, done on a random basis. Standardized sampling procedures were used to identify households in each cluster. The interview team went into the middle of the cluster and through a randomized procedure determined the starting quadrant for the first household. The first house was selected by the first digit of a currency note. Thereafter, each door was approached to find an eligible respondent.

Questionnaire: The PHES questionnaire was developed by a multi-disciplinary team of experts from the Ministry of Health, including health education experts, members of the Federal Communication Advisory Group, program managers of various categorical programs (TB Control, Expanded Program on Immunization, Center for Diarrheal Disease, etc.), international agencies, and technical experts. The questionnaire was translated into the national language, Urdu, and pre-tested prior to its implementation. The content areas of the questionnaire included background socio-demographic characteristics, breast feeding practices, knowledge, attitudes and practices concerning diarrhoea, immunization, malaria and smoking. Questions concerning knowledge about AIDS, and its routes of transmission were also asked of each respondent.

Recruitment, Training and Fieldwork: Health education supervisors from each of the four provinces and AJK were trained during a three-day session in the Ministry of Health, Islamabad. These 17 individuals were instructed in the techniques of interviewing, probing, and monitoring. Regional training was conducted in five areas of the country, in which health education supervisors trained a total of 64 lady health visitors who conducted the interviews with the mothers. All interviewers were female. The fieldwork began in November 1991 and concluded in February 1992. A 10% random sample of interviews were field checked by the health education supervisor following each days work. Throughout the survey, health education staff in Islamabad monitored closely all 16 teams by direct communication and spot checking. All questionnaires were transcribed to data coding sheets by field supervisors and express mailed to Islamabad for data entry. Coding sheets were also randomly checked with the original questionnaires and incorrect entries were less than 0.1%.

Data Entry and Cleaning: All data were entered an IBM personal computer using an SPSS-PC Data Entry II software program

Table 2: Demographic Characteristics of the Sampled Population

Demographic Data	N	Percent
TOTAL	5433	100.0%
Province		
Punjab	2406	44.3%
Sindh	1176	21.6%
N.W.F.P.	812	14.9%
Balochistan	640	11.8%
AJK	399	7.3%
Residence		
Rural	3167	58.3%
Urban	2266	41.7%
Age		
15 - 19	196	3.6%
20 - 24	1063	19.6%
25 - 29	1842	33.9%
30 - 34	1303	24.0%
35 - 39	740	13.6%
40 - 44	236	4.3%
45 - 49	53	1.0%
Education		
None	3953	72.8%
Primary	574	10.6%
Middle	298	5.5%
> =Secondary	608	11.2%
Monthly Income		
< RS 1,000	1727	31.8%
RS 1,000 - 1,999	1613	29.7%
RS 2,000 - 3,499	1018	18.7%
RS 3,500 - 4,999	346	6.4%
> RS 5,000	293	5.4%
Don't Know	436	8.0%

which allows the identification of each variable, its value, and a value range to signal incorrect entries. Frequency distributions of all variables identified outlier which were confirmed by examining the original questionnaire. Logic checks were performed on all meaningful variables to identify incorrect entries. Data were analyzed using a mainframe IBM computer, with SPSS statistical software programs.

Results

Socio-demographic Characteristics: Table 2 presents the frequency and percent of various socio-demographic characteristics of the surveyed. A total of 5433 women were interviewed throughout the five provinces population, 33 individuals more than the minimal sample requirements. The largest surveyed area was Punjab with 44.3% of the total sample. The respondents are not proportionate to the population of provinces and area of residence (Urban:Rural) in order to maximize the homogeneity and heterogeneity of populations in different provinces and areas of residence. The urban population constituted approximately 40% of the sample and the rural population constituted approximately 60%. The mean age of the population was 26.5 with the largest number of respondents (33.9%) falling into the age group 25-29 years followed by 24% belonging to the age group 30-34. A total of 72.8% of the respondents have no education, 10.6% have attained primary education, 5.6% have gone to middle level and 11.2 % secondary and above. Thirty-two percent of the households have less than Rupees 1000 monthly ($40) income which can be considered below the poverty line.

Awareness of ORS: Knowledge is often considered to be a necessary but not sufficient condition for behavior change. Shea and Basch's review of five major community cardiovascular disease prevention programs highlighted the significance of knowledge transfer and innovation diffusion as the most important link in the causal chain of adoption behavior (Shea and Basch, 1990).

Table 3: Percent of Best Treatment for Diarrhea, by Province, Residence, Age and Education

	Home Liquids	ORS	Home liquids and ORS	Drugs	Don't know
PAKISTAN	8.2	40.5	7.3	39.7	4.3
Province					
Punjab	9.0	28.9	9.9	47.5	4.7
Sindh	5.7	42.9	4.0	44.0	3.4
N.W.F.P.	4.8	50.5	11.7	29.8	3.2
Balochistan	14.2	35.9	2.2	39.4	8.3
AJK	7.5	90.5	0.0	0.5	1.5
Residence					
Rural	9.8	38.5	5.1	41.2	5.4
Urban	5.8	43.4	10.2	37.6	3.0
Age					
15-19	7.7	41.3	10.7	30.1	10.2
20-24	7.5	39.7	9.9	38.3	4.6
25-29	7.5	43.3	6.2	39.0	4.0
30-34	8.5	41.4	5.0	41.2	3.9
35-39	9.5	35.8	7.4	43.4	3.9
40-44	10.2	33.5	11.0	40.3	5.0
45-49	9.4	34.0	13.2	35.8	7.6
Education					
None	8.9	37.3	6.2	43.3	4.3
Primary	5.7	49.1	8.4	32.9	3.9
Middle	6.0	49.0	11.4	29.9	3.7
> =Secondary	6.9	49.5	11.2	29.3	3.1

Awareness of ORS was high for most respondents in the survey. Overall, 91% of mothers responded that they had heard of ORS. There was some variation by province 81.4% of respondents had heard about ORS in Balochistan compared with 97.7% in AJK. Education, income, and urban residence were all positively associated with awareness of ORS. This is a significant increase from 1984 when only 38% of mothers had heard about ORS (National Nutrition Survey 1985-1987, 1988).

Knowledge of Treatment for Diarrhea: Table 3 presents comparisons of knowledge of treatment for diarrhea by education, income, and residence. When asked about the best treatment for childhood diarrhea, 56% of mothers identified oral rehydration therapy (ORT), while 39.7% stated that drugs were the best treatment. Variability between provinces was significant, with 90.5% of respondents in AJK answering correctly and only 28.9% responding correctly in Punjab. The higher level of knowledge among AJK residents is attributed to recent information campaigns conducted in this area. These results indicate a significant gap between awareness and knowledge. Although 91% of mothers know about ORS, only 57% believe it is the best way to prevent diarrheal dehydration.

Use of ORS: Actual use of ORS is even lower than knowledge. Only 34.7% of mothers gave ORS to their infants during their last episode of diarrhea. Another 22.9% responded that their infant was treated in the hospital. Other responses included antibiotics (11%), other pills or syrups (9.2%), and home remedies (6.8%). These results support the findings of previous studies in Pakistan which have found that practice of ORT has consistently lagged behind knowledge.

Availability of ORS in the home: Mothers who reported knowing about ORS were asked if they had a packet of ORS at home. Only 27.5% of these mothers responded in the affirmative. Income, education, and age of the mother were all predictive of having ORS in the home, with wealthier, more educated, and younger women being more likely to have it. To verify their responses, the mothers who indicated that they had ORS at home were asked to show the ORS packet to the interviewer. Over 90% of these women were able to produce the ORS packet.

Table 4: Respondents who continued giving food when child had diarrhea by Province, Residence, Age, Education, Income level

	Food given when child in diarrhea					
Demographics	Continued N = 4,487	Discontinued N = 318	Reduced N = 335	DK N = 293	% valid	N
PAKISTAN (Total Percent)	82.6%	5.9%	6.2%	5.4%	100.0%	5,433
Province						
Punjab	82.6%	3.7%	4.7%	9.1%	100.0%	2,406
Sindh	78.9%	8.1%	12.4%	0.6%	100.0%	1,176
N.W.F.P.	79.3%	8.3%	6.2%	6.3	100.0%	812
Balochistan	83.7%	9.8%	4.1%	2.3%	100.0%	640
AJK	98.2%	1.3%	0.3%	0.3%	100.0%	399
Residence						
Rural	84.2%	4.6%	5.3%	6.0%	100.0%	3,167
Urban	80.4%	7.6%	7.4%	4.6%	100.0%	2,266
Age						
15-19	77.0%	5.1%	4.1	13.8%	100.0%	196
20-24	81.0%	6.3%	6.2	6.5%	100.0%	1,063
25-29	83.3%	5.6%	5.9	5.1%	100.0%	1,842
30-34	83.3%	6.3%	6.0	4.5%	100.0%	1,303
35-39	82.7%	5.8%	7.2	4.3%	100.0%	740
40-44	84.7%	4.2%	7.6	3.4%	100.0%	236
45-49	81.1%	3.8%	5.7	9.4%	100.0%	53
Education						
None	82.7%	6.1%	6.0%	5.2%	100.0%	3,953
Primary	81.7%	5.2%	6.1%	7.0%	100.0%	574
Middle	81.5%	7.7%	5.0%	5.7%	100.0%	298
> =Secondary	82.9%	4.1%	7.7%	5.3%	100.0%	608
Monthly Income						
RS < 1,000	82.5%	4.1%	6.3%	7.2%	100.0%	1,727
RS:1,000-1,999	83.3%	5.7%	6.4%	4.6%	100.0%	1,613
RS:2,000-3,499	83.8%	5.7%	6.0%	4.5%	100.0%	1,018
RS:3,500-4,900	85.8%	4.9%	6.9%	2.3%	100.0%	346
RS > 5,000	88.1%	3.1%	4.8%	4.1%	100.0%	293
DK *	71.6%	16.5%	5.5%	6.4%	100.0%	436

* DK : Do Not Know

Feeding Practices During Diarrhea: Studies conducted during the early 1980s found that the majority of mothers stopped giving food and/or liquids to the child during diarrheal episodes. For example, before the CDD campaign, only 40% of mothers continued giving food and liquids during diarrhea. Failure to provide food and liquids is an extremely dangerous practice because it accelerates dehydration and denies the child essential nutrients. As part of the CDD program, a massive public health campaign was launched to encourage mothers to continue feeding during diarrheal episodes. An evaluation of the CDD program undertaken in 1988 indicated that an increasing proportion of mothers - 59% - were continuing to give food and liquids to their children during diarrhea. These results are confirmed by the PHES survey.

In response to a question about feeding practices during diarrhea, 82.6% of mothers indicated that they continued to provide food for the child. Feeding practices differed between provinces 98% of mothers in AJK reported continuing food, while lower levels of 79% were found in Punjab and Balochistan. Feeding practices did not differ significantly with respect to income, education, or age of the mother. Table 4 provides a more detailed description of the factors found to be associated with feeding practices.

Since dehydration is the biggest risk associated with diarrhea, the continuation of liquids is essential to recovery. This study found that most mothers, 90.8%, continue to give liquids when their child has diarrhea. The urban-rural differential was small, but there was significant variability according to age. Young mothers, 15-19, were least likely to continue providing liquids compared to older mothers.

Multi variate analysis: Several variables were found in the bivariate analyses to relate significantly with the use of ORT as the best treatment of diarrheal disease. These variables were entered into a logistic regression model with the dependent variable being "perceived best way to prevent diarrheal dehydration" (1 = ORS + both ORS and home remedy and 0 = others). These independent variables included the number of individuals consulted for child health, mother's education, mother's age (continuous variable), number of household appliances (continuous variable), residence (rural or urban), and income (< = RS 1999 = 0; > RS 2000 = 0). Together, these six variables accounted for 62.6% of the variability in the identification of ORS as the best way to prevent diarrheal dehydration. Table 5 presents the results of the logistic regression. All independent variables except number of health consultants were found to be significantly associated with ORS as the best treatment. Mothers with more appliances in the household (such as radio, television, etc.) are 30% more likely to perceive ORS as the best way to prevent diarrheal dehydration. Mothers whose household income < = RS 1999 are 40% more likely to perceive that ORS and home remedy are the best ways to prevent diarrheal dehydration in comparison to those from higher income.

A second logistic regression model was used to assess the influence of several variables on "mother used ORS during the last episode of diarrhea" Independent variables entered into the model included mother-in-law as a consultant for child's health, doctor as a consultant for child health, residence, mother's age, reading newspaper, and income level. Together, these six variables accounted for 62.6% of the variability in using ORS during the last episode of diarrhea. Mothers who indicate that the mother-in-law was identified as a consultant for the child's health are 42% more likely to use ORS during the last episode of diarrhea compared to individuals who do not use a mother-in-law as a consultant. Also, mothers who read the newspaper are 32% more likely to use ORS

Table 5: Logistic Regression Analysis for Feeding Pattern for Children, Pakistan National Health Education Survey 1991-92

Variable	B	S.E.	Wald test	Significance	R	Exp(B)
Area of residency	- 0.10	0.074	1.93	0.165	0.00	0.90
Mother's age	- 0.02	0.006	13.63	0.0002	- 0.05	0.98
Mother's education *						
- no education	0.94	0.112	71.15	0.000	0.11	2.56
- primary education	0.64	0.124	26.29	0.000	0.07	1.89
NO. of consultants for child health	- 0.17	0.037	21.10	0.000	- 0.06	0.84
"Having radio, TV, newspaper"	- 0.08	0.039	4.67	0.030	- 0.02	0.92
Age to begin breast-feeding	- 0.29	0.039	55.38	0.000	- 0.10	0.75
Age of child (month)	- 0.07	0.005	160.16	0.000	- 0.17	0.93
Constant	2.96	0.245	146.07	0.000		

Model Chi Square = 367, Significance = .000, Prediction = 76.8%
* Mother's education : using the secondary education category as a reference category

Table 6: Multiple Regression Analysis for Predicting Mother's Adherence To Beneficial Surrounding Diarrhea

Variable	B (unstand.)[1]	S.E. (stand.)[2]	B	T	sign.	Adj. R^2
Mother-in-law as a consult for child's health	0.09	0.02	0.05	3.8	0.002	0.268
Doctor as a consultant for child's health	0.05	0.02	0.03	2.2	0.030	
Have ORS at home	1.04	0.03	0.49	41.9	0.000	
Having radio, TV, newspaper	0.06	0.01	0.07	5.4	0.000	
Perceived diarrhea as a major childhood Disease	0.09	0.02	0.05	3.9	0.001	
Constant	2.15	0.03		77.9	0.000	

[1] unstand. = unstandardized regression coefficient [2] stand. = standardized regression coefficient

than those who do not read the newspaper.

A multiple linear regression stepwise method was used to determine the effects of several independent variables on the number of positive practices mothers use for treatment of diarrhea (such as continue providing solid food, liquids, ORS, etc). Independent variables regressed in this analysis consisted of whether ORS was at home, having a radio, TV or read newspaper, perceived diarrhea as a major childhood disease, mother-in-law used as a consultant for child health, and doctor was used as a consultant for child's health. Table 6 identifies the unstandardized and standardized regression coefficients with respective levels of significance. Overall, the model explained 26.8% of the variability in number of positive treatment practices. The strongest predictor was whether or not ORS was available at home.

Discussion

The results of this survey indicate that the CDD program and media and other influences has been successful in increasing knowledge regarding ORS. However, actual use of ORS continues to lag far behind knowledge while 91% of mothers have heard of ORS, only 34.7% adminisd it during their child's last episode of diarrhea. This gap between knowledge and practice suggests that ORS education programs are effectively reaching the target population, but that the messages are failing to change behavior. Clearly, there is a need to change the direction of the program and focus on encouraging mothers to translate their knowledge into action.

By linking diffusion of innovation and interpersonal change theories together, it becomes clear that public health education programs must address the specific needs of both individuals and communities to be effective. One implication of this is that health educators must determine where individuals and communities lie along the behavior change continuum before designing interventions; otherwise, program may not be matched to the needs of the target audience. Thus, health education programs must have two objectives: (1) to provide behavior change programs sequentially to match the individual's stage of change, and (2) ensure that individuals in the target population are moving through the stages of behavioral change (i.e., that the behavior is diffusing through the population).

The results of this study provide evidence that the population is moving along a continuum of behavior change 91% are aware of ORS. This is the precontemplation/awareness stage in the two theories of behavior change described above. A total of 56% believe it is the best way to prevent diarrheal dehydration (contemplation/persuasion), 34.7% used it during the last episode (action), and 27% keep ORS is the home (maintenance). Some individuals and communities have moved along the continuum quickly; others, for one reason or another, are detained at one stage and must be convinced to move to the next stage. The stages of change provide points of intervention for future health education efforts; for example, it would be beneficial to determine what prevented 20% of mothers who believe ORS is the best treatment from actually practicing it, and to concentrate on removing those barriers.

The knowledge-practice gap suggests that current messages and channels have been successful in raising awareness of ORS, but unable to persuade mothers to use it. Knowing that the target population is aware of the product suggests that ORS promotion messages should begin to focus less on providing information and more on persuasive communication. At this middle stage of behavior change, mothers need advanced education regarding ORS; for example, they must be convinced that ORS is more effective than other methods to treat their child's diarrhea, and they must be taught to mix and administer it properly. Research by Prochaska demonstrates that an individual's evaluation of the pros and cons of a specific behavior were linked to their decision to perform that behavior, and subsequent maintenance of the behavior (Prochaska, 1994). In the case of ORS, this means identifying perceived advantages and barriers to using ORS.

According to the Diffusion of Innovation Model, those individuals in Pakistan most likely to adopt ORS have already done so; the next challenge is to reach the remaining individuals. More research is needed to determine the exact characteristics which separate the early adopters from the late adopters in each community. The need for more research in this area is underscored by a study in Haiti which found that the most important predictors of ORT knowledge and practice were the attributes of the individuals studied (Coreil *et al.*, 1988) . More information about the target population will help health professionals identify the resources in the community which are more closely matched to the individual and community stage of change.

The Diffusion of Innovation Model demonstrates that the acquisition of new information does not necessarily correspond to subsequent behavior change. In Pakistan, 91% of mothers have reached the first stage of change, awareness of ORS, but only

34.7% have reached the fifth stage and adopted ORS use. This underscores the need to better understand what motivates individuals to move through the stages of change. Some of the differentiation can be explained by predisposing characteristics some individuals are simply more ready to change than others or are more likely to defy social norms. These characteristics are associated with other socio-demographic characteristics. For example, it is generally believed that early adopters tend to be more educated, wealthier, and more urban-dwelling than late adopters a belief that is further supported by the results of this survey.

Due to these predisposing characteristics, early adopters have more access to the mass media, and they tend to rely on it as a source of health information. As a result, early adopters are more easily influenced by the mass media. Late adopters less educated, poor, and rural-dwelling individuals pose a challenge to health educators because they cannot be as easily reached or influenced by mass media channels. Late adopters rely more on interpersonal communication channels for information, and may be distrustful of other sources. This suggests that some Pakistani women may have heard about ORS through communication channels that are not likely to influence them. Health education campaigns, especially in the later stages, need to consider which communication channels will be most effective at persuading this audience.

Social influence is another important determinant of behavior. As the diffusion curve begins its ascent, precedent and support for the new behavior is developing simultaneously. Early adopters of the behavior can be used to influence late adopters both by serving as an interpersonal communication channel and providing an example.

Cultural belief systems may have a significant impact on the adoption or rejection of ORT within a community, yet many ORT promotion campaigns have been implemented with little understanding of the local cultures and dominant belief systems into which they are introduced. (Mull and Mull, 1988; Weiss, 1988). Mull found that remarkably little is known about how Pakistani women perceive ORT, resulting in culturally inappropriate messages (Mull and Mull, 1988). An improved understanding the issues and beliefs surrounding child health care will drastically improve the chances that messages will be received by the population.

A carefully selected communication channel can increase the salience and appeal of health education message. In Pakistan, where the mass media has already reached the early adopters, ORS promotion campaigns should use the existing social networks to channel information. For example, a poor or rural mother may be more receptive to receiving health information from other mothers, especially ones she knows. For this reason, a mother-to-mother dialogue should be actively encouraged. Where the existing social networks are inadequate, health outreach workers should be trained and dispersed to provide health education in the community and to build support for ORS.

The survey results indicate that many mothers continue to treat diarrhea with antibiotics and/or other drugs. In most cases, this is entirely unnecessary and potentially dangerous. Mothers must be convinced that ORS is the most effective way to prevent diarrheal dehydration. Promotion campaigns should also stress the added advantages of ORS, i.e. inexpensive, widely available, and does not require a visit to a doctor.

Research by Coreil and Genece in Haiti documented the pivotal role of medical institutions can play in dissemination of information about ORT (Coreil, 1988). However, current prescription practices in Pakistan are not supportive of ORS use. Health professionals sometimes view ORS as an adjunct approach to prevent diarrheal dehydration, to be used as a supplement to antibiotic therapy. Health professionals also need to be better educated about ORS and encouraged to prescribe it in place of antibiotics when appropriate. When health providers continue to recommend antibiotics to their patients, even in conjunction with ORS, it is reinforces the belief in the community that ORS is not an effective way to prevent diarrheal dehydration. Furthermore, when consulted about childhood diarrhea, health professionals should take advantage of this teachable moment to educate the mother about ORS instead of prescribing only antibiotics. Broader policy issues regarding the availability of drugs in Pakistan must also be addressed.

Conclusion: ORT is not the final solution for childhood diarrhea. Since diarrheal diseases are caused in part by social and environmental conditions that facilitate their transmission, the most significant improvements in child health will come as a result of improved sanitary conditions, personal hygiene, and living conditions. To be optimally successful, ORT promotion must be incorporated into an overall program of social and economic development. The combination of these two approaches, including targeted interpersonal modeling will be an important stimulus to move the remaining 65 percent of the population into the trial and adoption stages of behavioral change.

Acknowledgement

The research presented in this article was conducted by Donald E. Morisky and Snehendu B. Kar, WHO short-term consultants to the Ministry of Health, Division of Health Education, Islamabad, Pakistan 1990-1991. The views and opinions of authors expressed herein do not necessarily state or reflect those of the World Health Organization

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Pakistan Journal of Nutrition 1(3): 151-152, 2002

Isolation of *Escherichia Coli* from Raw Milk and Milk Products in Relation to Public Health Sold under Market Conditions at Tandojam

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Abstract: Hundred raw milk and sixty milk product samples namely Gulabjamun, Mawa and Dahi were randomly collected from different localities/sources of Tandojam for the isolation of *E.coli*, a notorious contaminant. All the samples were inoculated on different bacteriological media and a number of biochemical tests were performed for the confirmation of the isolate. The results revealed that out of 100 milk samples 57% showed growth of *E. coli*. The highest number of milk samples contaminated with *E. coli* were recorded in milk samples obtained from milk vending shops and houses. Among the 60 milk product samples 31(51.66%) showed growth of *E.coli*, the highest rate of contamination was found in Mawa/Khoa samples.

Key words: Raw milk, milk product, *E.coli*, public health

Introduction

The quality of milk is determined by aspects of composition and hygiene. Due to its complex biochemical composition and high water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. Therefore in the processing of milk, some of them may produce undesirable effects and some micro-organisms produce food infections which can either carry the pathogens that will increase the likelihood of infection of the consumer`s food. The contamination of milk and milk products is largely due to human factor and unhygienic conditions. Usually milk is contaminated with different kinds of microorganisms at milk collecting places. Milk is a major part of human food and plays a prominent role in the Pakistani diet. Approximately 50 percent of the milk produced, is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as Ice cream, Butter, Khoa, Paneer, Rabri, Kheer, Burfi and Gulabjaman (Anjum *et al.*,1989). The manufacture of these products is based on traditional method without any regard to the quality of raw material used and/ or the hygienic quality of the products. Under such conditions many microorganisms can find access to the milk products. Among all micro-organisms *Escherichia coli* is frequently contaminating organism, and is reliable indicator of fecal pollution generally in insanitary conditions of water, food, milk and other dairy products (Diliello,1982). Martin *et al.*, (1986) reported two cases of hemolytic uraemic syndrome which provide evidence that raw milk may be a vehicle of transmission of *E.coli* O157: H7, both affected person consumed raw milk. Recovery of *E. coli* from food is an indicative of possible presence of enteropathogenic and/or toxigenic micro-organism which could constitute a public health hazard. Enteropathogenic *E. coli* (EEC) can cause severe diarrhoea and vomiting in infants and young children (Anon, 1975). In 1971 USA faced out break of food poisoning in which 387 persons were suffered with Enteropathogenic *E.coli* due to the consumption of imported French cheese (Marrier, 1973).

E.coli was isolated from milk products like Mawa/Khoa, Cream, Dahi, Cheese, Butter and Gulabjaman (Bhat *et al.*, 1948; Kumar and Sinha, 1989; Kulshrestha, 1990).

Considering the above facts the present study was designed to isolate the *E. coli* from milk and milk products sold under market conditions at Tandojam.

Materials and Methods

All the samples were collected in sterilized container at random from different localities of Tandojam town, and were brought to Dairy Technology Laboratory, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, for the isolation of *E. coli*. All the samples positive for *E. coli* contamination were confirmed using Gram's staining, cultural and bio-chemical examinations. The samples were inoculated on MacConkey Agar (Difco laboratories, USA) and incubated aerobically at 37 °C for 24 hours. The plates were observed for the growth of *E. coli*. A single, isolated colony was picked and sub-cultured again on MacConkey agar for purification of the isolate. Simultaneously another single colony with similar characters was picked for the preparation of smear and stained with Gram's stain for the examination of staining and morphological characters of the isolate using bright field microscope. The cultural characteristics of the isolates were confirmed by inoculating the pure colonies on Blood Agar, Nutrient Agar, Nutrient Broth and Violet Red Bile Agar (Table 1). Biochemical tests were performed to confirm the *E. coli* using catalase test, Simmon's Citrate Agar, sugar fermentation on Triple Sugar Iron Agar, Gelatin liquefaction, Indole Production, Nitrate reduction, Urease production, Voges proskaur, Methyl red and Presumptive test (Table 2).

Table 1: Culture characteristics of *E.coli* on different media

Media used	Culture character
Mac Conkey Agar	Smooth, circular pink colonies with spreading growth.
Blood Agar	Non hemolytic, grey white moist, glistening, opaque, circular, convex colonies with entire edge.
Nutrient Agar	Colorless and yellowish white, circular, smooth colonies with entire edge.
Nutrient Broth	Organism showed uniform turbidity.
Violet Red Bile Agar	Small, circular pink colonies.

Table 2: Biochemical reactions of *E.coli*

No. of Isolates	Biochemical Test	Reaction
88	Catalase	+ve
	Simmon's citrate	-ve
	TSI	A/A+gas
	Gelatin liquefaction	-ve
	Indole Production	+ve
	Nitrate Reduction	+ve
	Urease	-ve
	Voges Proskaur	-ve
	Methyl Red	+ve
	Presumptive test	+ve

Results

The results of the present study are summarized in the Table 3. According to these results the highest *E. coli* contamination was recorded from the samples of raw milk obtained from milk vending shops and houses in 13 out of 20 samples each (65%), followed by raw milk samples obtained from milk vendors on donkey that

Table 3: Summarizes the over all percentages of *E. coli* contamination in milk samples

Source/locality	No. of samples collected	No. of samples found positive	(%)
Dairy farm	20	9	15
Milk vendors on cycle	20	10	50
Milk vendors on Donkey	20	12	60
Milk vending shops	20	13	65
Houses	20	13	65
Dahi	20	11	55
Gulabjamun	20	8	40
Mawa	20	12	60
Total	160	88	

is 12 out of 20 (60%), raw milk obtained from milk vendors on cycle showed 10 out of 20 samples (50%) and raw milk collected from dairy farms showed 9 out of 20 samples (45%) contaminated with *E.coli*. Where as in case of 60 milk product samples 51.66% were found positive for *E. coli* contamination. However the highest number 12 (60%) of Mawa/Khoa samples were contaminated with *E.coli* in contrast to dahi 11(55%)followed by Gulabjaman samples 8 (40%).

Discussion

The literature reviewed in the present study provided evidence that *Escherichia coli* is frequently occurring organism in milk. The methods of production, transportation, handling and sale of milk are entirely unhygienic. The milk sold in raw form poses a great hazard to public health without adopting hygienic measures because of possibilities of contamination with *E.coli*. The raw milk falls on an easy prey to bacterial contamination because of high ambient summer temperatures of Pakistan. The results of milk samples showed that 57 out of 100 samples were contaminated with *E.coli* are in agreement with the results of Naqvi, 1972; Martin *et al.*, 1986; Hanjra *et al.*, 1989; Ahmed and Sallam, 1991; Sharma and Joshi, 1992; Adesiyun, 1994.

The results of the milk product samples revealed that highest percentage of Mawa/Khoa samples were contaminated with *E. coli* as compared to Gulabjaman samples. However, both milk products (i.e Mawa/Khoa and Gulabjaman) available at Tandojam market were highly contaminated with *E. coli*. Indeed, indigenous sweet products are commonly manufactured and consumed in Pakistan; the method of production, handling, transportation and marketing of these products are entirely depend upon traditional system. Such system could pose favourable environment for bacterial contamination. The unclean hands of worker, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk products and the post manufacturing contamination.(Bhat *et al.*,1948; Marrier, 1973; Tariq Masud *et al.*, 1988; Kumar and Sinha, 1989; Grewal and Tiwari, 1990; Kulshrestha, 1990). Where as the traditionally made dahi available in the market of Tandojam was also found contaminated with *E.coli*. The results of the study are supported by Tariq Masud *et al.* (1988). Although *E.coli* is frequently occurring organisms in milk and its products, the incidence of the species of *E. coli* itself in milk and milk products as a possible cause of food borne disease is insignificant because *E. coli* normally is a ubiquitous organisms (Hahn, 1996). Important, however, is the occurrence of pathogenic strains of *E. coli* in milk products which could be hazardous for consumers.

Conclusion: The results obtained in this study concluded that milk and milk products available to the consumer have a high *E.coli* contamination. Thus, the results of the present study warn the need for more strict preventive measures. For this, regular sterilization of dairy equipment, washing of utensils, milker's hands, udders, eradication of diseased animals, pasteurization/boiling of milk is required before collection and distribution for consumption and product making. In this respect immediate cooling to 5 °C and/or pasteurization of milk could be more effective. The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of milk and milk products to the point of consumption and at all intermediary levels.

Thus present study suggest to isolate and characterize the different strains of *E.coli* which may cause the pathogenicity in milk products and also to investigate the HACCP of such strains in milk products.

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Pakistan Journal of Nutrition 1(3): 153-155, 2002

Rice Straw , It's Quality and Quantity as Affected by Storage Systems in Bangladesh

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Abstract: The effects of current storage practices on the nutritive quality and the level of mycotoxin contamination of rice straw were studied in four selected villages of Mymensingh district of Bangladesh. Farmers were allowed to store the same variety of rice straw in both improved and traditional systems. Straw samples were collected from both the stores and analysed for nutrient composition and *in vitro* digestibility. Chemical analysis of rice straw showed that storage methods had no significant ($P > 0.05$) effect on OM, CP and ME contents of rice straw. However, improved storage method significantly ($P < 0.01$) increased nitrogen free extract, *in vitro* dry matter digestibility and *in vitro* organic matter digestibility of rice straw compared with those of traditional storage method. Chemical analyses for mycotoxin content in the rice straw of traditional storage showed lower level of Fumonisin and no detectable level of Aflatoxins (B_1, B_2, G_1 and G_2). The results suggest that improved storage system is essential since it increases the quality of rice straw in respect of nutrient composition as well as *in vitro* digestibility. A definite conclusion regarding the mycotoxin level can only be made after two or three years of monitoring.

Key words: Rice straw, storage method, nutrient composition, *In vitro* digestibility, mycotoxin

Introduction

Ruminant livestock are mainly fed on low quality roughage based diets in Bangladesh. About 70% of the roughage available is crop residue and rice straw constitutes about 87% of the dry roughage available for cattle and buffaloes (Tareque, 1991). Due to inadequate production of green grasses, rice straw has become the major feed resource for livestock production. In some areas of the country rice straw constituting over 90% of dry matter intake due to lack of alternative feed resources. However, heavy rainfall during wet season leads to serious losses in the quantitative and qualitative availability of straw. Post harvest losses of rice straw during that season due to spoilage is a major contributing factor to the subsequent feed shortage. Losses of nutrients during storage have been described by Tripathi *et al.* (1995) which varies from the shattering loss of leaves, leaching of soluble nutrients by rain, potentially large losses due to mold damage and bleaching by exposure to sunshine. In Bangladesh it has been estimated that about 7.7 million tons of rice straw dry matter is being rotten during the monsoon (Chowdhury and Huque, 1996). It has been reported that overall 21% of rice straw is lost due to spoilage as a result of faulty storage and heavy rainfall in Bangladesh (M. A. Akbar, personal communication). Moreover, damp feed residues are susceptible to mold attack with associated mycotoxin contamination (Coker,1979). Rice straw is more frequently contaminated by mycotoxin producing organisms of the genera *Aspergillus, Penicillium, Fusarium, Alternaria, Stachybotrys* and *Cladosporium* (Phillips and Wareing, 1993; Phillips *et al.*, 1996). Among the genera *Aspergillus parasiticus* produces Aflatoxins B_1, B_2, G_1 & G_2 and *Fusarium moniliforme* produces Fuminisin B_1 and ingestion of these mycotoxins can be carried over from the diet into milk and results in toxic and carcinogenic effect (Jones and Coker, 1994). Feeding of moldy straw has adverse effects on animal health and milk consumers. Therefore, there is an urgent need to develop an improved post harvest storage method to reduce nutrient loss and mycotoxin contamination of rice straw and of milk, which will have a positive impact on human health. Considering the above points the present research was undertaken to evaluate the effects of current practices of storage on the quality of rice straw and its comparison with those of the improved storage methods. The other objective was to determine the level of mycotoxin contamination of rice straw in the traditional system of storage.

Materials and Methods

Keeping in view the objectives of the study, PRA was conducted in experimental areas. Four villages in Mymensingh District were selected purposively. Among 4 villages Rajpur and Garaikuti are in Muktagachha Thana, and Mothbari and Bhoradoba are in Trishal Thana. The government officials of respective Thana offices helped in selecting these areas. The experimental period lasts from January to October, 2001.

Climatic condition of the experimental sites: The climatic condition of the experimental sites is characterized by light rainfall during April- May and heavy rainfall during June- September. The soil is silt loamy to silty clay loamy and gray brown to dark gray. The agro-climatic condition of the experimental areas is shown in Table 1.

Construction of improved storage: Eight improved stores were constructed, four in each site. Out of four, two stores were built in each village, one for small farmers and other for medium farmers. The improved store houses were gable type tin shed with raised slate about $1^1/_2$ft height from the ground. The size of the storage was length- 22.5 ft., height- 9ft., and width- 13ft.

Sampling of rice straw: After construction, same rice straw was stored in both improved and traditional stores so that the comparison of the effectiveness of storage technique could be made irrespective of farm size, in regard to nutritive value, *in vitro* digestibility, metabolisable energy content and mycotoxin level in the rice straw. Rice straw was randomly sampled from both improved and traditional stores in two occasions. Ten straw samples of 1 kg was randomly collected from different locations of improved and traditional storage. Straw samples were kept in polyethylene bag and was labeled properly for future use.

Preparation of straw sample : After collection from the stack, straw was chopped to 3-4 cm size with the help of sickle. After that the chopped straw was exposed to sun drying in order to facilitate grinding. Then the chopped straw was ground to 2-3 mm size for chemical analyses. After grinding of each 10 kg straw from one stack, it was mixed properly, piled, flattened and quartered until it was reduced to 2.5 kg. Then 0.5 kg was kept for proximate analysis and *in vitro* digestibility and 2.0 kg of mixed sample was sent to Natural Resources Institute (NRI), UK. for analysis of mycotoxins. For *In vitro* study the ground sample was again oven dried and ground to 1.0mm mesh size with the help of a micro grinder.

Chemical Analyses of rice straw: The chemical analyses was done at the Bangladesh Agricultural University, Animal Nutrition Laboratory following the methods of AOAC (1990). Straw sample was analyzed for dry matter (DM), crude protein (CP), crude fiber

Table 1: Agro-climatic conditions of Trishal and Muktagachha

Experimental area	Agro-ecological zone	Soil texture and colour	Physiography	Climate		Drainage/ Flooded
				Annual rainfall (mm)	Mean annual temp. (°C)	
Trisal and Muktagachha (Mymensingh)	9b	Silt loamy and silty clay loamy. Gray brown to dark gray	Most areas have broad ridges and basins. Relief is irregular. The difference in alleviation between ridge tops and basin centers usually is 2-5 meters.	2000-4000 ($\bar{x}$ = 2966)	25.3 (<15->40)	Shallowly flooded. Early and rapid flooding by run off from adjoining higher land when heavy pre-monsoon or early monsoon rainfall occurs locally. Other time flood level are controlled by flood level in the Jamuna.

Source: FAO-UNDP, 1988.

Table 2: Effect of storage method on nutrient composition, *In vitro* digestibility and Metabolizable Energy (ME) contents of rice straw

Parameters	Storage method		SED	Statistical significance
	Improved	Traditional		
DM (g/100 g sample)	91.68 ± 0.88	91.88 ± 1.09	0.440	NS
Nutrient composition (g/100 g DM):				
OM	85.94 ± 0.52	85.40 ± 0.67	0.254	NS
CP	4.06 ± 0.23	3.85 ± 0.21	0.102	NS
CF	33.59 ± 0.40	34.03 ± 1.28	0.363	NS
EE	1.81 ± 0.27	1.83 ± 0.29	0.159	NS
NFE	46.48 ± 0.84	45.62 ± 1.07	0.177	**
Ash	14.06 ± 0.52	14.65 ± 0.67	0.262	NS
In vitro digestibility (g/100 g DM):				
IVDMD	45.27 ± 0.84	42.81 ± 0.93	0.399	**
IVOMD	48.31 ± 1.02	46.07 ± 1.06	0.473	**
ME (MJ/kg DM)	5.96 ± 0.11	5.64 ± 0.12	0.319	NS

^{NS}Not significant; $^{**}P < 0.01$; ^{SED}Standard error of difference

(CF), ash, ether extract (EE) and nitrogen free extract (NFE).

***In vitro* digestibility:** *In vitro* digestibility of rice straw, collected from improved and traditional storage was done using the procedure of Hohenheim Gas Test (Menke and Steingass, 1988).

Mycotoxins Analyses: Twenty four samples of rice straw were used for mycotoxin analyses in duplicate. Finally duplicates were again combined to give 24 samples. Mycotoxins assayed were done for Fumonisin B_1 and Aflatoxins B_1, B_2, G_1 and G_2 following the standard methods described below:

Fumonisin: The NRI standard operating procedure for Fumonisin analysis, MT_SOP209, with immuno-affinity clean-up (VICAM Fumonitest), quantification by high performance liquid chromatography using a Spherisorb ODSI reverse phase column, pre-column derivatisation with o-phthaldialdehyde, fluorescence detection using 335 nm excitation and 440 nm emission wavelengths has been used.

Aflatoxins: The NRI standard operating procedure for Aflatoxin analysis, MT_SOP, (Bradburn *et al.*, 1990) with quantification by high performance liquid chromatography using a Spherisorb ODSI reverse phase column, post column derivatisation by means of a KOBRA electrochemical cell and fluorescent detection at 360 nm excitation and 440 nm emission wavelengths has been used. Extraction is performed by adding acetone:water (80:20) and shaking for 45 minutes and the clean-up uses a phenyl-bonded SPE column (Varian). The method has a limit of detection for Aflatoxin B_1 of less than 1 μg/kg. Due to the high absorbtivity of this matrix, the sample size was reduced to 25 g and the meal to solvent ratio for extraction was increased from 5 to 10.

Statistical Analyses: Data for nutrient composition, *in vitro* digestibility, and metabolizable energy content and mycotoxin level in rice straw kept in traditional and improved storage were analyzed statistically using simple t-test (Steel and Torrie, 1980) and significant difference between the techniques was recorded.

Results and Discussion

Findings of the present study are reported in this chapter with elaborate discussion through citation of literatures wherever appropriate.

Nutrient composition of rice straw: The nutrient composition (g/100gDM) of rice straw stored under different conditions (improved storage and traditional storage) is shown in Table 2. Storage method (improved vs traditional) had no significant ($P > 0.05$) effect on dry matter (91.60 vs 91.88), organic matter (85.94 vs 85.40), crude protein (4.06 vs 3.85), crude fiber (33.59 vs 34.03), ether extract (1.81 vs 1.83) and ash (14.06 vs 14.65) contents of rice straw. However, nitrogen free extract (NFE) content of rice straw was significantly ($P < 0.01$) higher in rice straw kept in improved storage condition compared with that in the traditional storage condition. Although not significantly, OM and CP contents of rice straw from improved storage were slightly higher and CF and ash contents were slightly lower than those of the traditional one. The nutrients OM and CP were expected to be higher in straw of improved storage than traditional one, however, it did not happen, which might be due

Table 3: Level of mycotoxins in rice straw stored in traditional system in two experimental sites

Parameters	Traditional storage method		SED	Statistical significance
	Muktagachha	Trishal		
Fuminisin (ppb) #	31.82 ± 11.42	21.01 ± 13.73	10.31	NS
Aflatoxins B_1, B_2, G_1, G_2 (ppb) ##	0	0	0	

[NS]Not significant ; [SED]Standard error of difference #Limit of detection = 20 ppb; ##Limit of detection = 0.2 ppb

to the reason that the straw under traditional storage was not rotten enough to make significant difference in these nutrients compared to that of improved storage. It is fact that the rainfall of Bangladesh during wet season varies between the years as well as different regions in one particular year and it is also true that the degree of damage of straw depends on the degree of rainfall. As has been expected, the significantly ($P<0.01$) higher NFE content of straw of improved storage compared with that of traditional storage clearly indicates the beneficial effect of improved storage on straw quality. The nutrient NFE contains mostly soluble carbohydrates and which is quite likely to be washed out by the rain water when straw is exposed to rainfall in traditional storage system. It has been supported by Tripathi *et al.*(1995) who reported that during the traditional system of storage, leaching of soluble nutrients by rain is an important loss of nutrients from straw.

***In vitro* digestibility of rice straw sample:** *In vitro* DM digestibility (IVDMD), *In vitro* OM digestibility (IVOMD) and metabolizable energy(ME) contents of rice straw samples stored under different storage conditions (improved and traditional) are presented in Table 2. The values indicated that the storage method had apparently no significant ($P>0.05$) effect on ME content of rice straw, although it is clear that the ME value for straw of improved storage was higher than that for straw of traditional storage. However, the improved storage method significantly ($P<0.01$) increased IVDMD (45.27 vs 42.81%) and IVOMD (48.31 vs 46.07%) of rice straw compared with those of the traditional storage. The significantly higher values for IVDMD and IVOMD of improved storage compared to traditional may be due to the following reasons. In traditional storage condition nutrients of rice straw might be lost by the damaging action of the predators such as rats, birds, anjona, chicken, mongoose etc. Certain portion of nutrients might also be lost by the successive rainfall to which the straw is exposed. In case of traditional storage system excessive surface heat might have been another reason for reduced IVDMD and IVOMD of straw in comparison with improved storage. The results for IVDMD and IVOMD of rice straw stored in traditional storage systems are very close to the results reported by Huque and Akbar (1988) and the ME contents is almost similar to the value observed by Balch (1977).

Level of mycotoxins in rice straw : Table 3. shows the level of mycotoxins (Fumonisin and Aflatoxin B_1, B_2, G_1 and G_2 in rice straw kept in traditional storage in two different areas (Muktagachha and Trishal) of Mymensingh district. The results showed that the experimental area had no significant ($P>0.05$) effect on the level of Fumonisin in rice straw. It is also observed that no detectable levels of Aflatoxins (B_1, B_2, G_1 and G_2) were found in the rice straw samples. The low levels of mycotoxins in rice straw might be due to the reason that the straw was not damaged by excessive rainfall and the moisture level did not permit sufficient mold growth in the straw. It was reported by Phillips *et al.* (1996) that there was no significant amounts of Aflatoxins in rice straw of Bangladesh. The level of mycotoxins in rice straw of improved storage was not determined as there was neither detectable level of Aflatoxin nor the harmful level of Fumonisin even in straw of traditional storage, which was exposed to rain water and there was every possibility of being contaminated with mycotoxin. It was assumed that the level of mycotoxins would be low in rice straw stored in improved condition than that in traditional condition since straw was better stored in improved storage system where it was not exposed to wet and dampness allowing mold growth.

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Pakistan Journal of Nutrition 1(3): 156-160, 2002

A Comparative Study on the Quality of Rasogolla Made in Laboratory and Collected from Local Markets of Mymensingh, Bangladesh

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Abstract: Rasogolla is one of the most important pleasant and charming foods to most of the people of Bangladesh. In most of the markets of Bangladesh rasogolla are more or less available, but the quality of rasogolla varies from place to place. Sometimes manufacturers add some ingredients that decrease the quality of rasogolla. That is why, this research work was carried out to study the quality of rasogolla available in markets and to compare them with rasogolla prepared in the laboratory and also to investigate both the physical and chemical characteristics. Four rasogolla samples were collected from local markets of Mymensingh district and another sample was prepared in the laboratory to conduct the experiment with the above theme. Then the rasogolla were judged by a panel of expert judges for organoleptic test and also analyzed for chemical qualities. Considering the physical and chemical properties of both the samples of rasogolla, the results indicated that the laboratory made rasogolla was significantly better in quality than market rasogolla. As the laboratory made rasogolla was prepared with special care whereas market rasogolla might have the adulterated during preparation. It may be suggested that to obtain the better quality rasogolla proper method, proper composition of the ingredients, and also the strict hygienic and sanitation measures should be followed that will also gives the consumers satisfaction.

Key words: Rasogolla, physical quality and chemical quality

Introduction

Bangalies consume various daintily foods, among them rasogolla is the very important one. That is why, the rasogolla is acceptable to Bangali and also to others. Not only Bangali but also Indian people are fond of this product. Kuila *et al.* (2000) in their review paper discussed the manufacture and characteristics of milk sweets in eastern India. These include sandesh, rasogolla, cham-cham, pantooa, rasmalai, khir mohan, kalojam, chhanar jilipi, babri, sitabhog, sharpuria, sharbhaja, chhana poda, burfi, peda, kalakand, kheer kadamba and misti doi (dahi). It is concluded that the industry offers employment to approximately 200,000 people per year and has tremendous export potential. Bhattacharya and Raj (1980) also defined rasogolla as famed chhana based sweet-meats of Bengal, being made from milk curd which is kneaded into small balls that are boiled in clarified sugar syrup. Chhana is the residue obtained after the liquid portion is drained off from fermented boil milk and rasogolla is prepared by boiling chhana (rolled in to small ball) in 40-60% sugar syrup.

Actually when the rasogolla first prepared was not investigated but it is more or less obvious that the product was first introduced in the Indian subcontinent. It is reported that about 10% of the total milk produced in Bangladesh are used for the preparation of chhana and finally for sweet making (Forth plan study, No. 3, 1968).

In Bangladesh, more commonly rasogolla is made from cow milk. But adequate quantity of milk is not available round the year. The average milk production of local cow is very low. Moreover, the production and supply of cow milk is not satisfactory, specially in the months from July to November due to the seasonal effect the production goes to a minimal level. The scarcity of milk hampers the production of sweetmeat, which contributes in the rise of price. The daily requirement of milk and its deficits in Bangladesh are given in Table 1.

Rasogollas are obtained most of all markets of Bangladesh because from birth to death in each sphere of life rasogolla have occupied a significant place in our society. On each and every occasion like Eid, Puja, Birthday, Marriage, funeral ceremonies, religious festivals and even in guest entertainment, rasogolla inevitable. In shortly it may said that, there is no such ceremony and festival which goes out of rasogolla. Rasogolla is one of the most important pleasant and charming foods to most of the people of Bangladesh. Rasogolla are extensively used, chiefly alone with other foods due to their flavour and high food value. They are also easily digested. However, rasogolla is chhana based food product, it is very vital to health because of its fairly high protein and fat content, minerals, specially calcium and phosphorus content and also fat soluble vitamins, particularly, vitamin A and D content. The food value of rasogolla largely depends upon the chhana which is prepared from milk. The average chemical composition of chhana are Moisture-55.37%; Fat-23.52%; Protein-17.26%; Lactose-2.21%; Ash-1.66%; and Sucrose-29.86%; (Ravichandra *et al.*, 1997). Sweetmeats are nature's most important contribution to civilization. The first pre-requisite for producing excellent quality of sweetmeats is the availability of high quality chhana. Efforts have been made to manufacture rasogolla from buffalo milk with only limited success and market specimens do not possess the desired body texture (Kanwal *et al.*, 1980). Cow milk is exclusively used for chhana preparation by halwais as it yields a superior and most acceptable quality product suitable from rasogolla making (Suguna Rao *et al.*, 1989).

In most of the markets of Bangladesh rasogollas are more or less available but the quality of rasogolla of various places varies due to varieties in manufacturing procedure followed by persons of different places. Kanwal *et al.* (1980) showed the variation in rasogollas composition which obtained from laboratory and market. The compositional differences which were obtained are given in Table 2.

The persons involved in manufacturing rasogolla, all are not honest. There are some people among them who add some ingredients that gives unsatisfactory quality of rasogolla. Among the factors which affect the quality of rasogolla it is one of them. In the laboratory, the scientific methods are followed to manufacture the rasogolla. That is why, the quality of laboratory made rasogolla are generally superior. Not only laboratory but also have some manufacturer who tries to maintain the quality and also try to develop their quality to keep their goodwill. Considering the above stated facts, the present experiment was undertaken with the following objectives:

i. To study the quality of laboratory made and local market rasogolla
ii. To inform the consumers about the food value of laboratory made and local market rasogolla.
iii. To suggest an appropriate method of rasogolla preparation.

Table 1: Daily requirement of milk and its deficits in our country

Content deficits	Per day requirement and availability		Total requirement and availability		Total deficits	Average
	Requirement	Production	Requirement	Production		
Milk	250 ml	34 ml	9.90 million ton	1.40 million ton	8.02 million ton	86.20 %

Average of five years production (1986-87 to 1990-91), Source: Alam (1994) B.L.R.I. Savar, Dhaka.

Table 2: Composition of rasogolla

Constituents	Rasogolla			ISI. Standards
	Laboratory made	Market samples		
		Yamunanagar	Karnal	
Moisture	37.0	35.2	30.9	55.0(max)
Total solids	63.0	64.8	69.1	-
Sucrose	51.9	53.6	55.1	45.0 (max)
Protein	6.8	6.6	8.0	5.0 (min)
Fat	4.2	4.6	5.7	-

Materials and Methods

This experiment was conducted at the Bangladesh Agricultural University, Dairy Science Laboratory during the period from 26th February to April 12, 2001, chemical analysis was done at the Dairy Science Laboratory and Food Technology Laboratory of Bangladesh Agricultural University, Mymensingh.

For this experiment four samples of market rasogolla from four different markets of Mymensingh district, surrounding areas were chosen. One sample from each market was taken and three replications were made for each sample.

Similarly sample was prepared in the Laboratory under strict hygienic condition and designated as 'L' rasogolla. Market prepared rasogolla were brought to the Dairy Technology Laboratory and were kept in refrigerator under 4 ^{0}C for organoleptic evaluation and chemical analysis.

Preparation of rasogolla in the laboratory: For making chhana, cow milk was collected from Bangladesh Agricultural University (BAU) Dairy Farm. Before making chhana milk sample was analyzed in the laboratory to now their fat and SNF content. It was found that cow milk contains 43 g/kg (4.3%) fat and 84 g/kg (8.4%) SNF respectively.

Physical tests (sensory and organoleptic evaluation): To judge the physical parameter Flavour score, Body and texture, Colour and appearance, Taste score, Total physical score were carried out.

Chemical tests: To investigate the chemical characteristics Moisture contents (g/kg), Total solids content (g/kg), Protein contents (g/kg), Fat contents (g/kg), Carbohydrate contents (g/kg), Ash content (g/kg), Acidity percentage, and pH were determined

Statistical analysis: Data collected from different parameters were subjected to statistical analysis. Analysis of Variance test (ANOVA) was done to find out the statistical difference between different treatments. In this experiment all experimental materials were completely homogenous and for this reason data were analyzed by using one way analysis of variance test (CRD) as per MSTAT statistical programme. The differences among sample means were compared by calculating LSD value with the help of a Least Significant Difference test. (Gomez and Gomez, 1984).

Results and Discussion

Flavour score: Flavour score of rasogolla L was 41.76 ± 1.15, rasogolla M_1 was 37.82 ± 1.58, rasogolla M_2 was 39.74 ± 0.92, rasogolla M_3 was 39.15 ± 1.06, and rosogolla M_4 was 37.94 ± 1.36 respectively. The statistical analysis showed significant difference

Milk
↓
Heated to boiling temperature
↓
Cooling
↓
Adding whey water at 70^{0}C
↓
The lumps of casein formed and allowed to stay for few minutes for complete coagulation of chhana
↓
Filtering through muslin cloth
↓
Chhana (Coagulum)
↓
Grinding, kneading and cutting into small pieces
↓
Kneading of chhana with flour of subsequent preparation into ball
↓
Preparing and boiling of sugar syrup
↓
Dropping of chhana balls (10-15 pieces) in the boiling syrup
↓
Cooking for 10-15 minutes (during cooking sprinkling of cold water
↓
Swelling of chhana balls after 10-15 minutes
↓
Appearance of desirable colour
↓
After 30 minutes rasogolla was ready to serve and consumption

Fig. 1: Schematic diagram for the preparation of rasogolla

($P < 0.05$) between laboratory and market rasogolla (Table 3). Flavour score of market rasogolla samples were almost similar but laboratory rasogolla showed highest score The result indicates that the laboratory made rasogolla were superior from site of flavour. Bhattacharya and Raj (1980) indicated that flavour of rasogolla was enhanced by cooking. Katra and Bhargava (1994) said that flavour was adversely affected by the addition of soyamilk and Chakrabarti and Gangopadhyay (1990) also said that flavour of soy-bean could considerably be overcome with the use of rose flavour. Flavour may differ with source of milk. Joshi *et al.* (1991) revealed that, chhana from cow and buffalo milk had acceptable flavour where as that from goat milk had acidic flavour.

Body and texture score: The body and texture score for L, M_1, M_2, M_3 and M_4 sources rasogolla were 28.10 ± 0.62, 25.12 ± 1.28, 24.99 ± 0.43, 27.19 ± 1.00 and 26.46 ± 1.55 respectively (Table 3). Statistical analysis showed that there were significant difference ($P < 0.05$) within the body and texture score of different sources of rasogolla samples (Laboratory made rasogolla and market rasogolla). According to Gupta *et al.* (1993) textural quality of market rasogolla was significantly correlated with moisture, fat, protein and calcium contents and Bhattacharya and Raj (1980) said that use of high fat milk leads to higher fat contents in rasogolla

Table 3. Comparison of average score of various physical parameters (organoleptic characteristics) of different sources of rasogolla

Source of variation	Sources of rasogolla					LSD value	Level of significance
	L	M_1	M_2	M_3	M_4		
Flavour score (45)	$41.76^{a} \pm 1.10$	$37.82^{ab} \pm 1.58$	$39.74^{ab} \pm 0.92$	$39.15^{b} \pm 1.06$	$37.94^{b} \pm 1.36$	2.25	*
Body and texture (30)	$28.10^{a} \pm 0.62$	$25.12^{b} \pm 1.28$	$24.99^{b} \pm 0.43$	$27.19^{a} \pm 1.00$	$26.46^{ab} \pm 1.55$	1.93	*
Colour& appearance (15)	$14.01^{a} \pm 0.56$	$11.83^{b} \pm 0.95$	$12.11^{ab} \pm 0.35$	$10.98^{b} \pm 0.61$	$10.27^{b} \pm 1.13$	1.99	**
Taste score (10)	$8.93^{a} \pm 0.09$	$6.79^{b} \pm 0.78$	$7.34^{b} \pm 1.04$	$6.65^{b} \pm 1.20$	$7.56^{ab} \pm 0.59$	1.52	*
Total physical score (100)	$92.80^{a} \pm 1.54$	$81.56^{b} \pm 2.19$	$84.18^{b} \pm 0.94$	$83.97^{b} \pm 3.56$	$82.22^{b} \pm 2.27$	5.89	**

** = Significant at 1% level ; * = Significant at 5% level; L = Laboratory made rasogolla ; M_1 =Rasogolla collected from Trisal market; M_2 = Rasogolla collected from Muktagacha market; M_3 = Rasogolla collected from Sodeshibazar; M_4 = Rasogolla collected from Sutiakhalibazar

which softness the body and improve the texture. Body and texture of rasogolla may vary with various factors. Joshi *et al.* (1991) observed that chhana prepared from buffalo milk had hard body and coarse texture. Cow and goat milk produced chhana with soft body and smooth texture. Texture also decreased with increased temperature and length of storage (Arora *et al.*, 1996). The body and texture of rasogolla was better in laboratory made rasogolla than the market rasogolla and it may due to the above factors mentioned by the different author.

Colour and appearance score: The colour and appearance score of L, M_1, M_2, M_3 and M_4 sources rasogolla were 14.01 ± 0.56, 11.83 ± 0.95, 12.11 ± 0.35 and 10.98 ± 0.61 respectively, which is shown in the (Table 3). Statistical analysis showed that there was significant difference ($P < 0.01$) within the colour and appearance score of different sources of rasogolla samples. The highest mean value (14.01) of colour and appearance was recorded for laboratory made rasogolla than other rasogolla samples collected from markets. The variation of colour and appearance were probably due to fat% in milk. According to Mini *et al.* (1995) rasogolla prepared from whole milk score higher than the skim milk for colour and appearance. Tambat *et al.* (1992) showed effect of fat and maida levels gives acceptable colour and appearance when rasogolla were prepared. Some times cooking time might enhanced the colour of rasogolla (Bhattacharya and Raj, 1980).

Taste score: The average mean value for laboratory made rasogolla (L) and markets rasogolla (M_1, M_2, M_3 and M_4) were observed 8.93 ± 0.09, and 6.79 ± 0.78, 7.34 ± 1.04, 6.65 ± 1.20 and 7.56 ± 0.59 respectively (Table 3). Statistically the differences within the values were highly significant ($P < 0.01$). Highest score was noticed for laboratory made rasogolla and market rasogolla score were more or less similar. During preparation of rasogolla in laboratory all of the ingredients used as standard levels. Actually taste of rasogolla depends on the ingredients used for. Soni *et al.* (1979) reported that the rasogolla contained 37% moisture, 51.9% sucrose, 6.8% protein and 4.2% fat. The rasogolla prepared in laboratory was better than the market rasogolla.

Total physical score: Total physical score showed in Table 3 for rasogolla were 92.80 ± 1.54 for laboratory made (L) and 81.56 ± 2.19, 84.18 ± 0.94, 83.97 ± 3.56 and 82.22 ± 2.27 for market rasogolla, M_1, M_2, M_3 and M_4 respectively. As the all physical parameters for laboratory made rasogolla were higher than the market rasogolla that is why total physical score gone highest. And this highest value indicated that the laboratory made rasogolla was superior than the market sources. Total score for market rasogolla were near for each other, as all physical parameters score, were near. The difference between the laboratory made rasogolla and market rasogolla were highly significant ($P < 0.01$). Total physical score of rasogolla may vary with different factors. According to Puranik *et al.* (1997) for pure recombined milk chhana was not acceptable and according to Mini *et al.* (1995) milk sources (whole milk, skim milk, coconut milk) were responsible for overall quality of rasogolla and in this case they showed control rasogolla gave higher score than the others. Tarafdar *et al.* (1988) obtain that overall quality scores of rasogolla prepared with mechanically kneaded chhana were 7.5% lower than those market rasogolla.

Chemical parameters:

Moisture content: The average amount of moisture of rasogolla samples L, M_1, M_2, M_3 and M_4 were 525.80 ± 9.60, 433.63 ± 8.56, 450.03 ± 12.04, 487.00 ± 8.07 and 479.40 ± 15.25 g/kg respectively. Statistically there were significant differences ($P < 0.01$) between the moisture of different sources of rasogolla (Table 4). Higher moisture content was noticed in laboratory made rasogolla whereas market rasogolla samples noticed lesser amount of moisture. Bhattacharya and Raj (1980) reported that acceptable quality rasogolla contain 49.85 to 53.80% moisture. The higher amount of moisture indicate good quality rasogolla and sometimes it may give good flavour. Tewari and Sachdeva (1991) observed good flavour in the products whereas chhana containing 62.5 and 63.5 % moisture. Gupta *et al.* (1993) said overall textural quality was significantly correlated with moisture. Hardness of rasogolla also influenced by moisture contain and this type of comments was drown by Ravichandra *et al.* (1997). So that it may expressed that laboratory made rasogolla was superior than market rasogolla.

Total solids content: The total solids content for L, M_1, M_2, M_3 and M_4 sources of rasogolla were 474.25 ± 9.56, 566.33 ± 8.62, 549.97 ± 12.04, 513.00 ± 8.07 and $520.60 \pm 60 \pm 15.25$ g/kg respectively (Table 4). Statistical analysis showed that there were significant differences ($P < 0.01$) within the total solids content of laboratory made rasogolla and market rasogolla. Sur *et al.* (1999) reported standard total solids for rasogolla 44.83% which was more or less similar with the laboratory made rasogolla (474.25 g/kg). The total solids contents of market rasogolla were higher than laboratory rasogolla indicating inferiority of the sources. Kanwal *et al.* (1980) also showed total solids of 63.0% for laboratory made rasogolla and 64.8% for market rasogolla which also indicated higher percent of total solids content in market rasogolla. So the results obtained by the scientists are more or less agreed with the result got this research.

Protein content: Protein contents of different sources of rasogolla are presented in Table 4. From this table it was found the mean protein content of rasogolla samples were 59.50 ± 1.87, 54.33 ± 4.36, 52.77 ± 2.96, 52.37 ± 2.32 and 51.37 ± 1.86 g/kg for L, M_1, M_2 , M_3 and M_4 sources sample respectively. Statistical analysis showed that protein content of rasogolla samples varies significantly ($P < 0.05$). Laboratory made rasogolla contain higher protein level as compare to market rasogolla though all this sources (Laboratory made rasogolla and market rasogolla) content protein level according to BSTI. As per the Bangladesh Standard Testing Institute (BSTI, 1993) specification of minimum protein content of rasogolla should be 5%. Higher protein percent increase the quality of rasogolla. Sur *et al.* (2000) stated that protein percent 6.62 and Desai *et al.* (1993) also observed 6.7% protein in better quality of spongy rasogolla. Kanwal *et al.* (1980) revealed that laboratory rasogolla and market rasogolla content 6.8 and

Table 4. Comparison of average chemical composition of different sources of rasogolla

Source of variation	Sources of rasogolla					LSD value	Level of significance
	L	M_1	M_2	M_3	M_4		
Moisture (g/kg)	$525.80^{a} \pm 9.60$	$433.63^{c} \pm 8.56$	$450.03^{c} \pm 12.04$	$487.00^{b} \pm 8.07$	$479.40^{b} \pm 15.25$	28.53	**
Total solids (g/kg)	$474.25^{c} \pm 9.56$	$566.33^{a} \pm 8.62$	$549.97^{a} \pm 12.04$	$513.00^{b} \pm 8.07$	$520.60^{b} \pm 15.25$	28.54	**
Protein (g/kg)	$59.50^{a} \pm 1.87$	$54.33^{b} \pm 4.36$	$52.77^{b} \pm 2.96$	$52.37^{b} \pm 2.32$	$51.37^{b} \pm 1.86$	5.15	*
Fat (g/kg)	$49.30^{a} \pm 0.66$	$41.07^{b} \pm 1.20$	$42.87^{b} \pm 4.08$	$40.63^{b} \pm 1.18$	$40.53^{b} \pm 3.04$	6.25	**
Carbohydrate (g/kg	$357.07^{c} \pm 10.98$	$461.90^{a} \pm 11.87$	$445.23^{a} \pm 13.03$	$408.60^{b} \pm 2.35$	$417.27^{b} \pm 10.84$	27.24	**
Ash (g/kg)	$8.37^{b} \pm 0.42$	$9.16^{a} \pm 0.43$	$9.13^{a} \pm 0.13$	$9.26^{a} \pm 0.33$	$9.45^{a} \pm 0.41$	0.656	*
Acidity (%)	0.82 ± 0.10	0.86 ± 0.21	0.85 ± 0.17	0.90 ± 0.23	1.01 ± 0.13		NS
PH	6.5 ± 0.10	6.3 ± 0.26	6.4 ± 0.30	6.23 ± 0.57	6.10 ± 0.32		NS

** = Significant at 1% level; * = Significant at 5% level; NS = Not significant

6.6% protein respectively. Whereas Gangopadhyay *et al* (1996) indicated laboratory made rasogolla content 7.0-7.2% protein. This study also showed higher amount of protein level in laboratory made rasogolla than market rasogolla like the results drown by varies scientists given above.

Fat content: The amount of mean fat contents of L, M_1, M_2, M_3 and M_4 sources rasogolla sample were 49.30 ± 0.66, 41.07 ± 1.20, 42.87 ± 4.08, 40.63 ± 1.18 and 40.53 ± 3.04 g/kg respectively which are demonstrated in Table 4. Differences were highly significant ($P < 0.01$) among those mean values (Table 4). From this result it was observed that laboratory made rasogolla had significantly highest amount of fat and market rasogolla had the lowest amount of fat (Table 4). Quality of rasogolla mainly influences by the quality of milk Bhattacharya and Raj (1980) reported in a study that use of high fat milk leads to a higher fat content in the rasogolla which softness the body and improve the texture. Kanwal *et al*. (1980) studied that laboratory made rasogolla and market rasogolla contain 4.2 and 4.6% had respectively. In another study Sur *et al*. (2000) showed that fat percent of rasogolla was 5.39 when rasogolla was prepared from buffalo milk. So the result obtained by this research was almost satisfactory in relation to fat content. As the fat content of laboratory made rasogolla was higher than market rasogolla, it gave the good score compared to market rasogolla.

Carbohydrate content: The average amount of carbohydrate of rasogolla L was 357.07 ± 10.98, M_1 was 461.90 ± 11.87, M_2 was 445.23 ± 13.03, M_3 was 408.60 ± 2.35 and M_4 was 417.27 ± 10.84 g/kg respectively (Table 4). Statistical analysis indicated that there were significant differences ($P < 0.01$) within the carbohydrate content of different sources of rasogolla samples. Market sample of rasogolla may have adulteration and most of the ingredients which are used in rasogolla as aduterated materials generally content higher amount of carbohydrate that give the market rasogolla higher levels of carbohydrate content. Adhikari *et al*. (1992) said that chhana content with higher percent of lactose contributed this higher percent of lactose within the rasogolla when rasogolla was prepared from that chhana. Kanwal *et al*. (1980) indicated that rasogolla prepared in laboratory had sucrose of 51.90% whereas market rasogolla had 53.60% sucrose. In another study Sur *et al*. (2000) noted 32.13% sucrose in rasogolla which were prepared from buffalo milk. Carbohydrate content of laboratory made rasogolla was obtained by this study was close to noted by Sur *et al*. (2000). As carbohydrate content of laboratory made rasogolla was lesser amount than market rasogolla, so laboratory made rasogolla regarded as superior than market rasogolla.

Ash content: Amount of ash of different rasogolla samples were 8.37 ± 0.42, 9.16 ± 0.43, 9.13 ± 0.13, 9.26 ± 0.33 and 9.45 ± 0.41 g/kg for L, M_1, M_2, M_3 and M_4 sources rasogolla sample respectively (Table 4). Statistical analysis showed that there were significant differences ($P < 0.05$) between the ash content of different sources of rasogolla samples. Laboratory made rasogolla had lower level of ash as compared to market rasogolla. Generally the rasogolla which content higher amount of total solids may have higher levels of ash. In Table 4 total solids of laboratory rasogolla was lower (474.25 g/kg) than market rasogolla (around 540.00 g/kg), so market rasogolla had higher levels of ash. Sur *et al*. (2000) mention that 0.33% ash in rasogolla prepared from buffalo milk. Katra and Bhargava (1990) said higher ash and total carbohydrate decreased the sponginess.

Acidity percentage: The acidity percentage of different sources of rasogolla samples are shown in (Table 4). It was found that the average acidity for L, M_1, M_2, M_3 and M_4 sources rasogolla were 0.82 ± 0.10, 0.86 ± 0.21, 0.85 ± 0.17, 0.90 ± 0.23 and 1.01 ± 0.13 respectively. Statistically there were no significant differences between the acidity of different sources of rasogolla. Acidity of market rasogolla samples were higher than that of laboratory prepared rasogolla. Haque (2000) showed the acidity of rasogolla were 0.75, 0.70 and 0.71% respectively which are prepared from cow, buffalo and equal mixture of cow and buffalo milk. Chanda (1999) observed acidity of rasogolla 0.60, 0.70, 1.10 and 1.40% respectively which are prepared from cow milk chhana, 10% soya chhana, 20% soya chhana and 30% soya chhana. The result obtained by this research work were more or less nearest with the result obtained by Haque (2000) and Chanda (1999). So the results were within the accepted level. Arora *et al*. (1996) said that the lactic acidity was increased during the storage. Laboratory made rasogolla were not stored whereas market rasogolla may be stored, that is why, acidity of laboratory made rasogolla was relatively lower in value than market rasogolla.

pH value of different rasogolla sample: The average pH value of different rasogolla samples are presented in Table 4. From the table it is observed that mean pH for L, M_1, M_2, M_3 and M_4 sources of rasogolla were 6.5 ± 0.10, 6.3 ± 0.26, 6.4 ± 0.30, 6.2-3 ± 0.57 and 6.1 ± 0.32 respectively. Statistical analysis showed that there were no significant differences within the pH value of different sources of rasogolla samples. The pH of laboratory made rasogolla and market rasogolla were within the accepted level whereas pH of laboratory made rasogolla was slightly higher than market samples. Haque (2000) reported pH of rasogolla made from cow and buffalo milk were 6.60 and 6.73 and Chanda (1999) reported the pH of rasogolla within the range of 5.92 to 6.36. So, in relation to pH of market rasogolla specially the laboratory made rasogolla were within the standard value and quality of rasogolla were good.

Considering the chemical properties the laboratory made rasogolla were superior to market rasogolla from any point of view. As the laboratory made rasogolla was prepared with special care, so, the factor of preparation contributed to make the laboratory made rasogolla good in quality.

Conclusion: The experiment was conducted in the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh with the facility available in the Dairy Technology Laboratory. The objective of the experiment was to compare physical and chemical characteristics of market rasogolla found in Mymensingh district with that of laboratory made rasogolla.

The experiment was conducted through collection of rasogolla from four local markets of Mymensingh district and with that of the prepared in the laboratory. The rasogolla, both commercially produced and laboratory made, were judged by a panel of expert judges for organoleptic test. The samples were also analyzed for chemical qualities. Data obtained were analyzed statistically using

Completely Randomized Design (CRD).

The total final score of physical parameters (consisting flavour, body and texture, colour and appearance, taste) of laboratory made rasogolla was 92.80 ± 1.54 and the same score of market rasogolla (Surrounding Mymensingh) were 81.56 ± 2.19, 84.18 ± 0.94, 83.97 ± 3.56, 82.22 ± 2.27 respectively. According to panelists, the highest score was obtained in laboratory made rasogolla and lower score was obtained in market rasogolla. Statistically significant differences ($P < 0.01$) were found among those mean values.

From chemical analysis it was observed that average moisture contents of L, M_1, M_2, M_3 and M_4 rasogolla samples were 525.80 ± 9.60, 433.63 ± 8.56, 450.03 ± 12.04, 487.00 ± 8.07 and 479.40 ± 15.25 g/kg respectively. On the other hand total solids content of the above samples in the same order were 474.25 ± 9.56, 566.33 ± 8.62, 549.97 ± 12.04, 513.00 ± 8.07 and 520.60 ± 15.25 g/kg respectively. Statistically the levels of moisture and total solids content differ significantly ($P < 0.01$) among the different samples.

The protein contents of L, M_1, M_2, M_3 and M_4 rasogolla samples were 59.50 ± 1.87, 54.33 ± 4.36, 52.77 ± 2.96, 52.37 ± 2.32 and 51.37 ± 1.86 g/kg respectively. There was significant difference ($P < 0.05$) among them in respect of protein content. The fat contents of L, M_1, M_2, M_3 and M_4 rasogolla samples were 49.30 ± 0.66, 41.07 ± 1.20, 42.87 ± 4.08, 40.63 ± 1.18 and 40.53 ± 3.04 g/kg respectively. Statistical analysis showed that there was significant difference ($P < 0.01$) among them.

The carbohydrate contents of rasogolla samples of L, M_1, M_2, M_3 and M_4 were 357.07 ± 10.98, 461.90 ± 11.87, 445.23 ± 13.03, 408.60 ± 2.35 and 417.27 ± 10.84 g/kg respectively. Significant difference ($P < 0.01$) was observed among the carbohydrate contents of various sources of rasogolla.

The ash content of L, M_1, M_2, M_3 and M_4 rasogolla samples were 8.37 ± 0.42, 9.16 ± 0.43, 9.13 ± 0.13, 9.26 ± 0.33 and 9.45 ± 0.41 g/kg respectively. Statistically the difference within the values were significant ($P < 0.05$).

The average acidity and pH value of rasogolla sample of L, M_1, M_2, M_3 and M_4 were 0.82 ± 0.10, 0.86 ± 0.21, 0.85 ± 0.17, 0.90 ± 0.23, 1.01 ± 0.13 % and 6.5 ± 0.10, 6.3 ± 0.26, 6.4 ± 0.30, 6.23 ± 0.57, 6.1 ± 0.32 respectively. No significant differences were found within the different samples.

From the results of all parameters (physical and chemical) it was observed that the laboratory made rasogolla was better than the market rasogolla. This may be attributed to addition of pure chhana obtained from fresh milk, optimum level of sugar, control heating and maintenance of strict hygienic measures during preparation of rasogolla in the laboratory. With higher total solids and carbohydrate content and with lower protein and fat level in market rasogolla indicated that the manufacturers might have adulterated their products. The possible adulteration may be addition of skim milk chhana, wheat flour and high level of sugar in the rasogolla formulation. Hence, it is concluded that to produce better quality rasogolla the following recommendations may strictly be followed:

i. Suggested appropriate technique for rasogolla making with specific proportion of ingredients should be followed.
ii. Should have a authorized organization who will try to control the quality of milk products (rasogolla) by checking the quality of products (physical and chemical point of view, if possible).
iii. Extension work may be done among the producers "To teach them to produce better quality rasogolla".
iv. Better quality rasogolla should have the following composition: The moisture content should be within the range of 500-550 g/kg; total solids 450-500 g/kg; protein 50-75 g/kg; fat 40-60 g/kg; carbohydrate 350-380 g/kg and ash content 8-9 g/kg level.
v. Hygienic and sanitary measures should strictly be followed during preparation of rasogolla.

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