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Occurrence of *Streptococcus Pneumoniae* Infections in Lambs from Andhra Pradesh

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Keywords:

Abstract

Streptococcus Pneumoniae; Bacteriological Isolation; Biochemical Studies; Antibiotic Sensitivity Test

The present study was carried out to isolate the etiological agent responsible for an outbreak of pneumonia causing mortality in young lambs. The outbreak was occurred during November 2017 in a sheep flock in Maddakkatta village of peddara mandal in Prakasam district from Andhra Pradesh. Affected animals were clinically examined, nasal swabs and blood samples collected from live animals, whereas heart, liver and lung tissues collected during postmortem. The etiological agent was isolated and identified as Streptococcus pneumoniae on conventional bacteriological and biochemical methods. The antibiogram of the Streptococcus pneumoniae isolates revealed that the isolates were sensitive to Norfloxacin followed by ceftriaxone erythromycin and resistant to penicillin, tetracycline, Enrofloxacin and ciprofloxacin etc. The serum biochemical profile resulted in decrease in serum concentration of albumin, A/G ratio and increased level of total protein, globulins, Blood Urea Nitrogen(BUN), creatinine and serum enzymatic activities of ALT and AST supports the pneumoniae of bacterial origin implicating different degrees of organs dysfunction caused by the disease condition

Introduction

Sheep play a vital economic role as they are raised mainly for lamb production followed by wool for large section of population especially in villages and desert areas. Respiratory disorders are facing serious problem in sheep rearing (Haltem et al., 2003). The importance of respiratory diseases of sheep depends on their prevalence, their effect on productivity, the value of animal and their international spread (Ali et al., 2009). Respiratory diseases are caused by interaction of various factors like host, etiological agent and environmental factors. (Amit Kumar et al., 2013, Brogden et al., 1998, Kumar et al., 2011 & 2012). The disease is a complex and multifactorial in which bacterial, viral, mycoplasmal and fungal infections combine with other factors such as stress of weaning, transportation, comingling and parasitism to produce acute respiratory diseases (Louis, 1996). Among respiratory tract infections, bacterial diseases have drawn attention due to their varied clinical manifestations, disease severity and emergence of strains resistant to a number of chemotherapeutic agents and a number of causative agents were responsible for the respiratory disease complex including streptococcal infection. Among various etiological agents streptococcus pneumonia, a commensal bacteria of the nasopharynx of animals and has been associated with high mortality and morbidity in young lambs due to pneumonia. *S.*

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pneumoniae is also a public health concern as sheep rearing community in India rear them within their premises.

The losses are due to reduced growth rate, mortality in lambs and significant economic impact interms of treatment costs and condemnations in abattoirs (Jones et al., 1982; Goodwin et al., 2004). The present study revealed *S.pneumoniae* as an etiological agent responsible for high morbidity and mortality in lambs in an outbreak occurred in Maddakkatta village of peddara mandal in prakasam district from Andhra Pradesh.

History and clinical signs

An outbreak with severe respiratory signs was occurred in flocks of sheep with below one year age group and in lambs at Maddalakatta village of peddaraveedu mandal of Prakasam district. Affected animals were showing pyrexia 105°F, dysnpnoea, coughing and nasal discharges, anorexia and death within three days.

Materials and Methods

Collection of samples

Nasal swabs (20) were collected from sick animals (lambs) for isolation of the etiological agent where as heart (20), liver (20), lung tissues (20) were collected aseptically during postmortem for the isolation of the etiological agent. Blood samples were also collected from the affected animals, and separated the serum for biochemical studies.

Bacteriological Examination

The nasal swabs were directly inoculated into nutrient broth, BHI broth and PPLO broth whereas heart, liver, lung tissues were cut into small pieces with sterile scissors and made into 10% suspension with sterile PBS and then inoculated into Nutrient broth, BHI broth and PPLO broth incubated at 370C for 12 to 24hrs. The samples were processed and methods were followed according to Carter et al., 1995; Quinn et al., 2002.

After 24 hrs of incubation a loopful of broth culture was streaked onto the sterile blood agar, Trypticase soy agar and Sabouraud dextrose agar plates and incubated at 37°C for 24 to 48hrs (Quinn et al., 2002) to check the presence of bacteria , Mycoplasma and fungus. The bacterial isolates were further confirmed biochemically using catalase test, optochin and bile susceptibility tests according to Carter et al., 1995; Quinn et al., 2002.

Antibiotic Sensitivity Test

Bacterial isolates were tested for antibiotic sensitivity testing by the disk diffusion method (Kirby Beur et al., 1968) following the NCCLS (2002) guidelines. The antibiotics used in the study were Amikacin ($30\mu g$), Amoxycillin-clavulanic acid ($20/10\mu g$), Ampicillin($10\mu g$), Ciprofloxacin ($5\mu g$), Gentamicin ($10\mu g$), Enrofloxacin ($10\mu g$), Tetracycline ($10\mu g$), Penicillin (10IU), Norfloxacin ($10\mu g$) and Streptomycin ($25\mu g$) respectively.

Serum Biochemical Studies

Serum samples were assayed for the concentrations of total protein (TP), albumin (Alb), blood ureanitrogen (BUN), creatinine (Cr) and serum enzymatic activities of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). Total globulin was determined by subtracting albumin from serum total protein and then A/G ratio was estimated. These parameters were determined by spectrophotometric method using commercially available test kits supplied by Biodiagnostics (Egypt) and following the manufacturer's instructions.

Results and Discussion

The outbreak was occurred during November 2017. On clinical examination, the lambs had mucopurulent nasal discharges, lacrimation, dyspnoea, pyrexia (T-105^o). At Necropsy, greenish red frothy exudates in the trachea, bronchi, congestion of mucous membranes and pleuritis were observed.

Microbiological examination of nasal swabs and tissues (heart, liver and lung) revealed no growth in PPLO Broth and on Sabouraud dextrose agar media indicative of negative to Mycoplasma and fungal infections. Presumptive identification of bacteria was made by traditional bacteriological and biochemical methods. After incubation at 37°C for 48 hrs the blood agar and trypticase soy agar plates were observed for colony morphology, presence and absence of haemolysis. On blood agar media partial or incomplete alpha hameolysis was noticed (Fig. 1). On trypticase soy agar under microaerophilic conditions dew drop like mucoid colonies were observed. On gram staining the colonies revealed pear shaped cocci with capsule in pairs (Fig 2).



Fig 1: Partial or Incomplete Alpha Haemolysis on Blood agar plate Streptococcus Pneumoniae



Fig 2: Grams staining - Streptococcus Pneumoniae

The bacteria isolates were positive to Catalase reaction, optochin (Ethyl hydrocupreine hydrochloride) and bile solubility tests indicative of positive to Streptococcus pneumoniae.

In this study, bacteriological examination of nasal swabs (20), heart (20), liver (20), lung tissues (20) from infected lambs and sheep with one year age groups was carried out. Out of 20 samples 14 samples were found positive for S. pneumoniae with percent positivity of 70%. Antibiotic sensitivity test results revealed that all the bacterial isolates were sensitive to norfloxacin followed by ceftriaxone, erythromycin and resistant to penicillin, tetracycline, enrofloxacin and ciprofloxacin etc.

Table 1: Details of Positivity of samples of pneumonic animals

S.No.	Total no. of samples	No. of samples	No. of samples Positive	Percent positivity
1.	Nasal Swabs	20	14	70%
2.	Liver	20	14	70%
3.	Heart	20	14	70%
4.	Lung	20	14	70%

Antibiotic sensitivity test results revealed that all the bacterial isolates were sensitive to norfloxacin followed by ceftriaxone, erythromycin and resistant to penicillin, tetracycline, enrofloxacin and ciprofloxacin etc.

Serum biochemical studies

The results of the serum biochemical changes were shown in Table 2. The results indicate that there was decrease in serum concentration of albumin and A/G ratio in affected animals but serum values of total proteins and globulins showed increased levels. Serum concentrations of blood urea nitrogen, creatinine and serum enzymatic activities of ALT and AST were also increased in the affected animals.

Table 2: Serum Biochemical profile of Pneumonic animals

Parameters (Group N=20)	Values	
Total Protein (g/dl)	7.8	
Albumin (g/dl)	2.2	
Globulin (g/dl)	5.6	
A/G ratio	0.39	
Creatinine (mg/dl)	41.20	
BUN (mg/dl)	1.5	
ALT (U/L)	30.84	
AST (U/L)	38.16	

Serum biochemical alterations in respiratory diseases were common and might display reasonably predictable changes in response to inflammation. In this regard, the results of the present investigation revealed that there was increase in serum values of total protein and globulin and decrease in serum concentrations of albumin.

Hypoalbuminemia could be due to anorexia and inability of liver to synthesize protein (El-Seidy et al., 2003). Certain bacteria or bacterial toxins increase capillary permeability and permit escape of plasma proteins in tissues hence osmotic pressure of proteins is increased in the tissue fluids and at the same time decreased in the blood (Omran et al., 2005). Albumin is also considered a negative acute phase protein and its value frequently and markedly declines during inflammation (Ceron et al., 2005 and Georgieva et al., 2011).

Data obtained from the present study implicated a high serum enzymatic activity of ALT and AST in the diseased lambs. These changes could be attributed to dysfunction of various organs including liver due to hepatic degenerative and necrotic changes caused by bacterial infection and toxins (Raghib et al., 2004; Talkhan et al., 2009 and Aytekin et al., 2011). Sheep are considered as the most important future growth of livestock, that play a vital economic role and support the survival of millions of people in our country (Hatem et al., 2003). Respiratory diseases of sheep particularly pneumonia continues to be a major problem commonly encountered in sheep flocks (Naveed et al., 1999).

The present study revealed S. pneumoniae as major and potent cause of mortality in the young lambs with percent positivity of 70%. Similarly Bekele et al., (1992) also isolated streptococcus species as major pathogens in association with other etilogical agents from pneumonia Garedow et al., (2010) reported that 35% of the pneumonia cases in sheep are due to streptococcus organism. Several authors Sasani et al., 1998; Ragie et al., 1999 and Kumar et al., (2000) have reported isolation of diverge bacterial species from sheep with respiratory diseases and also established the role of commensal organisms in pneumonia including S. Pneumoniae. Progress in understanding the pathogenesis of pneumonia has been slow because of its complex etiology and varied epidemiology (Woldemeskel et al., 2002)

Treatment

As per the antibiotic sensitivity test Norfloxacin was given to sheep @10ml /75-150kg body weight orally for 5days, Hepatal- DS syrup – 15ml per day. After 10 days of treatment the clinical signs were subsided without any mortality in the flocks.

Conclusion

S. pneumoniae infection in lambs was one of the predominant upper respiratory tract infections nowadays and appears to be an emerging zoonotic threat to humans especially to the shepherd. Further study revealed the multidrug resistance of *S. Pneumonia* infections to commonly used antibiotics viz, enrofloxacin, ciprofloxacin, penicillin and erythromycin made the choice of antibiotic agents to the infection. Further studies are required especially for associated pathogens of respiratory tract infections in sheep especially in young lambs causing mortality.

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References

- 1. Ali BA, El-Hanafy AA and Salem HH. Genetic biodiversity studies on IGFBP-3 gene in Egyptian sheep breeders. Biotechnology in Animal Husbandry. 2009;25(1-2):101-109.
- Aytekin İ, Mamak N, Ulucan A and Kalinbacak A. Clinical, hematological, biochemical and pathological findings in lambs with Peste des Petits Ruminants. Kafkas Univ.Vet. Fak. Derg., 2011;17(3): 349-55.
- 3. Bekele. T.T. Woldeab. A. Lablou-Kassi and J. Sherington. Factors affecting morbidity and mortality on-farm and on-station in Ethiopia highland sheep. Acta Tropica. 1992;52:99-109.
- Brogden, K.A. H.D. Lehmkuhi and R.C. Cutlip. Pasteurella haemolytica complicated respiratory infections in sheep and goats. Vet. Res. 1998;29:233-54.
- 5. Ceron JJ, Eckersall PD and Martinez-Subiela S. Acute phase proteins in dogs and cats:current knowledge and future perspectives. Vet. Clin. Path. 2005;34:85-99.
- 6. Garedew. L. G. AyeleR. Yilrna. A. Zeleke and A. Gelaye. Isolation of diverse bacterial species associated with maedi-visna infection of sheep in Ethiopia. Afr. J. Microbial. Res. 2010;4:14-21.
- Georgievia TM, Andonova MJ, Slavov EP, Dzhelebov PV, Zapranova D S and Georgivia IP. Blood serum protein profiles and lysozyme activity in dogs during experimental infection with Staphylococcus intermedius. Revue Méd. Vét. 2011;162(12):580-85.
- Goodwin KA, R. Jackson, C. Brown, PR Davies, RS Morris and NR Perkins. Pneumonic lesions in lambs in New Zealand: Patterns of prevalence and effects on production. N. Z. Vet. J., 2004;52:175-79.
- Hatem ME, Zaki, SM, Osman AH and El-Shabrawy M. Bacteriological, histopathological and Clinicopathological of respiratory affection in sheep and goat in Egypt. Egypt. Vet. Med. Assoc., 2003;63(1):97-109.
- 10. GE Jones, AC Field, JSGilmour, AG Rae, PF Nettleton and M. McLauchlan. Effects of experimental chronic pneumonia on bodyweight, feed intake and carcase composition of lambs. Vet. Rec. 1982;110:168-73.
- 11. Kumar A, AK Verma and A Rahal. Myco-plasma bovis, a multi disease producing pathogen: Anovenriew. Asian J. Anim. Vet. Adv. 2011;6:537-46.
- Kumar A, AK Verma, NK Gangwar and A Rahal. Isolation, characterization and antibiogram of mycoplasma bovis in sheep pneumonia. Asian J. Ainm. Vet. Adv. 2012;7:149-57.
- 13. Kumar R. RC Katoch and P Ghaar. Bacteriological studies on pnewnonic Gaddi sheep of Himachal Pradesh. Indian Vet. J. 2000;77:846-48.

- 14. Louis JP. Immunology and prevention of bovine respiratory diseases. Presentation at the Proc. Of a symposium held in conjunction with XIX World Bui. Atrics Congress, Scotland. 1996.
- Raghib MF, Said EA, Hassan MS and Al-Gharabawy BS. Effect of pasteurllosis on health performance and some hematological and blood serum constituents of dromedary camel. Menufiya Vet. J. 2004;3(2):385-95.
- 16. Raji MA, D. Rodgern, AJ Natala and PD Halle. Aswvey of the aerobic bacterial agents associated

with ovine pnewnonia in Zaria Kadwma state. Nig. Vet. J. 1999;20:175-81.

- 17. Sasani F, J Avaspoor, K Afshar and K Iranmanesh, Ovine pneumoma: Pathological and microbiological correlation. Indian J. Vet. Pathol. 1998;22:156-58.
- 18. Woldemeskel M, M Tibbo and LN Potgieter.Ovine progressive pnewnonia (Maedi-Visna): An emerging respiratory disease of sheep in Ethiopia. Dtsch. Tierarztl. Wochenschr. 2002;109:486-88.



Efficiency of *Citrus* Fruits to Improve Functional Properties of Livestock Products

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Keywords:	Abstract
Citrus Fruit; Milk Products; Meat Products; Functional Food.	<i>Citrus</i> fruits are flowering trees belonging to genus shrubs and <i>Rutaceae</i> family. These are beautiful and fragrant plants, spreaded to all continents of the India. <i>Citrus</i> fruits are rich source of vitamins C (ascorbic acid), dietary fiber and bioactive compounds, which plays important role in protection of human health. Livestock foods are nutritional foods, but it lacks in dietary fiber and bioactive compounds. Due to the existence of functional ingredients in the citrus fruits and its byproducts, these fruits can be used for designing livestock food. Hence, enrichment of milk and meat products with <i>citrus</i> fiber is a best alternative to increase functional quality of the product and cater to the consumer demand.

Introduction

Livestock products are distinctive foods due to its taste, nutritional value, digestibility and easy availability, however these foods lack in the vitamins C (ascorbic acid), dietary fiber and bioactive compounds. Recently consumers are demanding for healthy food due to increasing awareness with respect to diet as well as health. Healthy food can be prepared with incorporation of natural (Fruit and vegetable) healthy ingredients as functional compounds. Citrus fruits are the best source of functional compounds and there is a great opportunity for improving functional properties of livestock oriented food products with addition of citrus fruit as natural ingredients. Citrus fruit is a well known fruit, most abundantly distributed worldwide; however it mainly grown in the part of

Southeast Asia, Northeast India and China. Major citrus producing states in India are Karnataka, Pondicherry, Tamil Nadu, Andhra Pradesh, Maharashtra, Jammu Kashmir, Himachal Pradesh, Madhya Pradesh, Orissa, Jharkhand etc. Orange, Lemon, Grape Fruit and Mandarin fruits accounts approximately 98% of the total citrus production.

Varieties of Citrus Fruit:

Different varieties of citrus fruits are available in market for human consumption. Following varieties of citrus fruits are commonly found in India.

- *Citrus aurantifolia* (Lime or Kagzi Nimbu)
- Citrus reticulata (Orange or narangi)
- Citrus sinensis var. mosambi (Mosumbi)

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- *Citrus medica* (Lemon)
- Citrus sinensis var. sinensis (Malta)
- Citrus limon (Galgal)
- *Citrus limenta* (Sweet Lime, Meetha Nimbu)
- *Citrus grandis* (Shaddock or Pomello, Chakotra) and
- Citrus pardesii (Grape fruit)

Most commonly Oranges, Lemons and Grape Fruits are used for daily consumption. *Citrus medica* commonly known as *Lemon* grown for sale as fresh fruit, which is highly acidic fruit. *Citrus reticulate* also known as *Orange or Narangi or Santra*. It grows in all tropical as well as rain fed area of the country. Rind of oranges is easily separable from pulp portion, and pulp is sweet or acidic. *Grapefruits* (*Citrus pardesiilocally*) plants grow in tropical as well as hot-humid subtropical environment. These fruits are more acidic, contains small amount of juices and a thicker peel.

Citrus fruit are processed mainly to produce juice and most often the peel is discarded as a waste which is subdivided into epicarp or flavedo (coloured peripheral surface), mesocarp or albedo (white spongy soft middle layers). Waste material from citrus fruits accounts upto 50% of total fruit weight (Bocco et al., 1998). Fruits is composed of principal components such as H₂O soluble sugar, fibre, organic acids, amino acids, protein minerals, oils, lipids, vitamins as well as flavonoids, proportion of these components varies in different parts of the citrus fruits i.e. juice, albedo flavedo, pulp and seeds (Braddock, 1995).



Table 1: Nutritional characteristics of common citrus fruits

Per 100 g	Orange	Grapefruit	Lemon
Energy (kcal)	47	53	20
Carbohydrates (g)	11.75	10.66	9.32
Protein (g)	0.96	0.77	1.10
Total fat (g)	0.12	0.14	0.30
Cholesterol (g)	0	0	0
Dietary fiber (g)	2.40	1.60	2.80
Vitamin C / ascorbic acid (mg)	53.20	31.20	53

β-Carotene (µg)	71	686	3
α-Carotene (µg)	11	3	1

Source: USDA National Nutrient Database for Standard Reference, Release 24, 2011a.

Bio Active Compounds

Major bio active compounds are health promoting, which are present in the form of phytochemical. Fruit and vegetables are well loaded with bioactive compounds, fruits are particularly high in a class of phyto-chemical known as the limonoids (Hasegawa and Miyake, 1996).

Table 2:

Limoniods content of citrus essential oils		
Orange	91.40%	
Grape fruit	95.12%	
Lemon	65.44%	

In variety of fruit, citrus fruit is rich in bioactive compounds (flavonoids, vitamin C, carotenoids and phenolic compounds) with antioxidant properties, which may provide additional health-promoting effects (Marín et al., 2007). citrus byproducts is good sources of phenolic compounds, however major portion of phenolics is available in peel (Balasundram et al., 2006). Peel as well as leaves of Citrus sinesis and Citrus Auravtium are potent source of phytochemicals- Geranyloxy Ferulic (GOFA) and Boropinic Acid (Genovese et al., 2014), which has pharmacological effect against cancer, chemo preventive anti-inflammatory, neuroprotectitve and anti helicon bacter pylorigent. Orange, lime and lemon juice prevents kidney stone formation (Pak, 2004), grape fruit lowers blood pressure and interferes with calcium blockers (Sica, 2006).

Flavonoids are polyphenolic compounds and mostly found in pulp, peel and rag tissues. The hesperidine, narirutin, Naringin, erocitrin flavonoid mainly present in citrus fruit (Schieber et al., 2001). Another class of O- methylate deglycones and flavones such as nobiletin and tangeretion which are relatively common polymethoxylated flavones (PMFS) (Li et al., 2014). Citrus peel contains highest amount of PMFS compared to edible parts of fruit (Wang et al., 2014). Citrus flavonides plays role in modulating hepatic metabolism (Cha et al., 2001) as well as in prevention of lipid peroxidation (Frutos et al., 2002), such as orange juice prevents and modulates inflammatory processes (Assis et al., 2013), grape fruit have antigentoxic effect (Alvarez Gorazales et al., 2010).

Citrus bio active compounds helps in prevention of atherosclerosis, certain types of cancer as well as diabetes (Garcia et al., 2001). The phyto phenolics content also contributes in the mechanism of health promotion and disease prevention with pro-carcinogene deactivation, DNA repair, maintainance and suppression of N Nitrosamine formation as well as change of oestrogen metabolism (Shahid, 1997). Citrus fruits exert favorable effect on human health by prevention of degenerative disease due to the presence of phenols, amino acids, essential oils pectins, carotenoids, flavonoids and vitamin C (Wang et al., 2014) as well as wide range of promising biological properties due to their phenolic profile as well as anti oxidant properties. (Montanary et al., 1998).

Dietary Fibre

Citrus fruits encompass dietary fiber in addition to bio active compounds. Dietary fiber (DF) are of two types i.e. soluble DF and insoluble DF. The good fibers should have a ratio of 1:2 for SDF/IDF (Jaime et al., 2002). Dietary fibers plays a significant role in prevention, reduction and treatment of chronic diseases such as bowel, gastrointestinal discarders, obesity, diabetes, CVD, cancer, also enhances physiological function i.e. reduction in blood cholesterol maintain insulin level (Champ, 2003) and maintains human health. The daily requirement of dietary fiber in the diet for women is 21-25gm and for men is 30-35gm (food and Nutrition Board Institute of Medicine., 2001). Dietary fibers not only exhibit health benefits but it also improves technological properties during processing i.e. increases water holding capacity, oil binding properties, lowers viscosity and gel forming ability of product (Hyun Jung and Hyun-Dong, 2012).

Livestock products viz, milk and meat are important source of protein and essential nutrients, however these products lacks in the dietary fiber and bioactive compounds. In preview of consumer awareness regarding their diet, health along with demand for healthy food to prevent health hazards as well as Hippocrates statement "Let food be the medicine and medicine be the food" there is necessity of development of functional livestock products.

Functional food: A food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improvement of health status, wellbeing and/or reduction of disease risk. Citrus fruit and its byproducts contains non digestible carbohydrate (diet fiber) and bio active compound (Main et al., 2002) as a functional ingredient and which helps in designing food (functional food) livestock products such as fat replacer, enrichment of dietary fiber, antioxidant and alteration of microorganisms i.e. promotes health beneficial and reduce harmful bacteria growth as well as reduction residual nitrite content.

Dietary fiber enrichment in livestock products

Dietary fiber is one of the most important and commonly used functional ingredients in food products (Sanchez-Zapata et al., 2010). Functional properties of citrus ingrédients were prooved by many reserchers with assessment of citrus ingredients incorporated livestock products. existence of associated bioactive compounds, such as flavonoids, polyphenols and carotene in citrus fibers depict importance as are better quality fibers than other dietary fibers (Fernandez-Gines et al., 2003; Wolfe et al., 2003). Citrus fiber enrichment also helps to develop low cost and value added products, so that every individual can afford the consumption of products and facilitate to overcome the fibre deficit (Fernandez Gines et al., 2003). Garcia et al. 2002 stated that 1.5% of cereals and fruits fiber was final fiber content of reduced-fat, dry-fermented sausages, which improves nutritional properties and provides an acceptable sensory profile. Lemon albedo was added at different concentration (2.5% to 10%) to cooked sausages (Fernández-Ginés et al., 2004) and dry-cured sausages (Aleson-Carbonell et al., 2004) and perceived sensory properties of 2.5% to 7.5% lemon albedo added sausages similar to the conventional sausages. Fernandez- Lopez et al. (2004) developed cooked sausages with addition of lemon albedo (raw and cooked) as a good source of dietary fiber and concluded that it can also be employed as functional ingredient in other meat products. Saricoban et al. (2008) obtained better results with respect to emulsion on addition of 5 % lemon albedo as a potential source of dietary fiber in frankfurter-type meat products formulation. However Eda et al. (2015) determined the usability of lemon fiber (LF-2%, 4%, 6%) and carrot fiber (CF-2%, 4%, 6%) to produce low fat beef hamburgers and found best results for 2% fiber added products.

Fernandez Gines et al. (2003) observed that addition of orange fiber powder (0.5% to 2%) to cook sausages (bolognas) comprised good sensory score and improved the nutritional value of the sausage; however 2% citrus fiber content gives harder & springy texture. Fernandez- Lopez et al. (2007) also employed successfully different levels of orange fiber as potential functional ingredient in dry cured sausage without affecting its quality. Similarly Fernandez Lopez et al. (2008) did not found any negative effects on flavour of orange fiber (0, 1, 2%) added "salchichon" (Spanish dry-fermented sausages). Sendra et al. (2008) noted good acceptability for citrus fiber enriched fermented milk i.e. yougurt, however found more acceptability of orange fiber enriched fermented milks than the others.

Residual nitrite reduction

In meat industry, nitrite is especially employed to develop colour of the product and to inhibit outgrowth of micro-organism i.e. Clostridium botulinum. However, higher residual level of nitrite is anis an emerging issue as it contributes in the formation of the carcinogenic compound i.e. nitrosamine. Therefore effort should done to reduce the residual levels of the nitrite in the products through processing alterations, which may be an acceptable alternative to reduce exposure of human body to nitrite through processed meats. Considering necessity scientists insisted to exploit citrus fruit for reduction of residual nitrite level. Aleson-Carbonell et al. (2004) and Fernandez-Lopez et al. (2004) observed reduction in the residual nitrite content respectively in dry-cured sausages and bologna sausage on addition of different levels of lemon albedo (2.5 to 10%). Similarly residual nitrite reduction were also noted by Fernandez Gines et al. (2003) for cooked sausages (bolognas) on addition of orange fiber powder (0.5% to 2%), Fernandez Lopez et al. (2007) for dry-cured sausages prepared with addition of orange fiber (0, 5, 10, 15 and 20 g/kg), Fernandez Lopez et al. (2008) for orange fiber (0, 1 and 2%) added "salchichon" and Viuda Martos et al. (2010) for orange fibre treated mortadella, Barıs et al. (2012) for different levels of orange fiber (0, 2 and 4%) added sucuk.

Fat replacer in livestock products

Health consious peoples are demanding for less fat, low calories and less cholesterol containing livestock products. Low-fat and high-fiber foods can help reduce the risk of cardiovascular diseases, obesity, colon cancer, and other disorders (Mansour and Khalil, 1997). Fat is an essential component in meat product which imparts flavour, juiciness and texture, hence it cannot be directly reduced by using less fat or direct replacement with other types of fat (Jimenez-Colmenero, 2000), but addition of suitable ingredient can replace fat without affecting the quality.

Citrus fruits characterize a potential source of dietary fiber, which can be employed as potential fat replacers (Mendoza et al., 2001) without affecting physical, chemical, and sensory properties of the products. Fernandez-Gines et al. (2004) found that the presence of raw and cooked albedo in bolognas decreased (p < 0.05) the fat content, this decrease was found higher in bolognas with raw albedo than cooked albedo. Fat contents of sausages were reduced with increased level of citrus fiber (5-20%). Addition of citrus fiber as a fat replacer increased energy values, but decreased cholesterol contents Emel and Nalan, (2005). However Tainara et al. (2013) added peel, pulp and seeds as well as peel in the ice-cream formulation. They stated that orange fiber reduced approximately 70% fat content of ice cream, which is a good alternative for fat replacement. Similarly, Tainara et al. (2014) observed that the addition of orange juice industry by-products did not change important properties of ice cream, but it reduces approximately 50% mean fat content of light lemon ice cream. However, Sendra et al. (2008) declared that the fiber particles reduced creaminess of fermented milks. Eda et al., (2015) used lemon fiber (LF-2%, 4%, 6%) and carrot fiber (CF-2%, 4%, 6%) to produce low fat beef hamburgers. Irrespective of fiber type (LF/ CF) fat and cholesterol contents of Beef Hamburger decreased significantly.

Promotes growth of beneficial bacteria

Bactria images bad way in everyone's mind but viable bacteria in the fermented milk products have a positive health effect due to the beneficial action. Fermentation of food is as old a custom; purpose of the fermentation is not only to preserve the food, but also to improve its flavor, consistency, texture and nutritive value. Fibers may also interact with the microbial populations of fermented livestock products; either residual essential oil may inhibits growth bad bacteria or fiber components as oligosaccharides may enhance growth of good bacteria known as probiotic bacteria. To exert beneficial effects in the host, it is essential that probiotic bacteria should be alive and abundant in the product at the time of consumption. Usually recommended counts are from 106 to 108 CFU/g, but there is no general agreement regarding achievement of probiotic effects due to unsuccessful efforts for reaching the probiotic concentration (Lourens-Hatting and Viljeon, 2001). Citrus fibers promotes the growth of beneficial bacteria which was also confirmed by Sendra et al. (2008) who stated that the presence of citrus fiber enhanced the growth and survival of yogurt starter bacteria viz, L. acidophilus and L. Casei and B. bifidum. Fernández Lopez et al. (2008) also added orange fibre to "salchichon", a dry fermented sausage, which promoted the growth of micrococcus without affecting flavour of the products. Baris et al. (2012) stated that the growth of ripening bacteria viz, lactic acid bacteria and Micrococcus / Staphylococcus was positively affected on addition of 4% orange fiber in sucuk.

Enhancement of the shelf life of the product

Livestock products are best media for growth of microbes and the presence of air induces fat and protein oxidation. Storage leads to gradual deterioration in the quality and nutritive value of products and production of potentially toxic reaction products such as malonaldehye (MDA) and cholesterol oxidation products (COPs) (Verma and Sahoo, 2000) and to develope rancid flavour of the product (Cottone, 2009). Prevention of microbial growth and oxidative rancidity during storage and retail display is essential to maintain the quality and safety of livestock products. It can be achieved by applying preservation technologies (Aymerich et al., 2008). Generally synthetic preservatives are used to restrict the microbial growth and thereby extending the shelf life of livestock products. However synthetic preservatives are health hazardous, hence now days these are less preferred by consumers over bio-preservatives due the awareness of the consumers regarding health. Plant extracts are main sources of Biopreservatives, containing phenolic compounds as secondary metabolites; these compounds have capacity as an antioxidant by reducing power of free hydroxyl groups and antimicrobial by binding protein. To cater the constantly increasing demand of consumers for the chemical and antibiotic free food and multi-drug resistance of food borne pathogens, recently natural available citrus fruits are commonly used for extending the shelf life of the products.

Citrus products counter acts the oxidation of products and enhances the shelf life. Aleson-Carbonell et al. (2003) observed more slowly increase in TBARS values of sausages containing different levels of lemon albedo than control. Fernandez-Lopez et al. (2004) also reported antioxidant effect of rosemary, orange, and lemon extracts in cooked Swedish-style meatballs. Orange fiber had positive effect with respect to retarding oxidation with enhancement of the shelf-life of sausage (Fernández-Ginés et al., (2003), Fernández-López et al., (2007), (2008) and Viuda-Martos et al., (2010). Hanan et al., (2013) evaluated microbial growth, lipid oxidation and color change of raw ground beef meat stored at 4±1°C by using fruit byproducts such as Grapefruit rind powder (GRP), orange rind powder (ORP) and mandarin rind powder (MRP) with or without y irradiation and observed significantly lower TBARS values for all treated (GRP<ORP<MRP) samples than control throughout storage refrigerated period. However, Inserra et al. (2014) were fed dietary citrus pulp (24% and 35%) feeding to the lamb and found positive effect on reducing lipid oxidation of meat over refrigerated storage.

Antimicrobial activity of oils and vapours of citrus fruits evaluated against wide variety of spoilage and pathogenic microorganisms (Roy et al., 2012). This acitivity of citrus fruit is mainly due to bioactive compounds viz, ferulic acid, hydrocinnamic acid, yaniding glucoside, hisperidin, vitamin C, carotenoid, and naringin, which also imparts important nutritional and flavoring value (Ghafar et al., 2010). Prakash et al. (2013) stated that the fruit peel extracts of Maclura pomifera (Orange), Citrus limetta (Sweet lime or Mousambi) and Mangifera indica (Mango) had moderate to mild inhibiting effect on pathogenic bacteria. Mishra et al. (2012) tested specifically antimicrobial acitivity of citrus fruits against E. coli organism and observed significant effect against the various strains of E. coli. Fisher and Carol, (2008) reported that the essential oils of *citrus* fruits like orange, lemon had good antimicrobial activity against Escherichia coli O157, Campylobacter jejuni, Listeria monocytogens, Bacillus cereus and Staphylococus aureus. Lee and Najiah, (2009) found range of MIC values from 7.8 to 31.3 mg mL-1 for crude extract of Citrus microcarpa against Escherichia coli (ATCC 25922), Citrobacter freundii (ATCC 8090), Aeromonas hydrophila (ATCC 49140), Pseudomonas aeruginosa (ATCC 35032), Streptococcus agalatiae (ATCC 13813), Edwardsiella tarda (ATCC 15947), and Yersinia enterocolitica (ATCC 23715). Fernandez-Lopez et al. (2004) also reported the antibacterial effect of rosemary, orange, and lemon extracts in cooked Swedish-style meatballs. Viuda Martos et al. (2010) revealed that the orange fiber have a positive effect on reducing the growth of unwanted microbes and increasing the shelf-life of the sausage. The counts of artificially inoculated pathogenic bacteria viz, (Escherichia coli, Salmonella typhimurium and Bacillus cereus) into ground beef meat were significantly (p < 0.05) reduced by the addition of Mandarin

rind powder, orange rind powder, grapefruit rind powder (Hanan et al., 2013).

Enhances Technological Parameters during Processing

Researchers found appreciable changes in the technological properties of milk meat products. Citrus fibers plays important role in various functional properties of food products, in addition to the health and nutritional characteristics. Water holding capacity, viscosity, gel-forming ability, and fat binding capacity are major functional properties of dietary fiber. Incorporation of citrus dietary fiber increases water holding capacity as fibers occupies water in the fiber pores, which ultimately increases cooking yields and reduces the caloric contents of livestock products. Viscosity is a resistance to flow, increase in the molecular weight or chain length of the fiber, increases the viscosity of fiber in solution and provides rheological properties in food system. Experiential increase in the viscosity of the orange fiber added yogurt was observed by Sendra et al. (2008). Formulation of the product containing citrus fibers disperses and/or binds fat due to which fat content of the products reduces (Decker and Park, 2010; Weiss et al., 2010). Better texture profile and greater sensory acceptability were observed on addition of 1.5% fruit fiber along with 10% of pork fat in dry-fermented sausages by Garcia et al. (2002). Saricoban et al. (2008) declared that the addition 5% of lemon albedo increased the emulsion capacity (EC) and as well as enhance the functional and technological properties for frankfurter-type meat products. However, addition of lemon fiber resulted in a lighter, redder, and more yellow color (p<0.05) as well as increase in moisture content and cooking yield of low fat burger due to its better water binding properties (Eda et al., 2015).

Limitations

Citrus fruits are good source of vitamin C, but mostly it fade away during heat treatment (processing) like blanching, boiling, cooking, cooking under pressure and sterilization of foods. Citrus fibers can cause acidic or bitter taste because of its low pH. Product texture as well as juiciness gets affected on addition of higher level of citrus fiber. Bioactive compounds of citrus fruit and its byproducts are sensitive to heat treatment which results in losses of it.

Future scope

The development of meat products enriched with citrus fruit & it byproducts is indeed a novel

area. Furthermore, investigations on pretreatment of citrus component to reduce bitterness, stability and interactions of phyto-chemicals with other food ingredients during processing and storage need to be initiated. Research is needed to understand their interactions with meat products citrus constituents to improve physicochemical and textural quality of product. Real challenge actually lies in effective development and marketing of these functional meat products.

Conclusions

- *Citrus* fruit is a good source of vitamin C, phyto-chemical and flavonoids it's available in various forms.
- Milk and meat products are naturally lacking in dietary fiber, vitamin C and phyto-chemicals.
- Incorporation of *citrus* fruit in milk and meat products, in addition to the nutritional properties it can also provide functionality to the product.
- The active ingredients of *citrus* fruits are sensitive to heat treatment so the proper technology for product development and quality assessment is a most important to exploit the beneficial effects of citrus fruit in milk & meat products.

References

- 1. Aleson-Carbonell L, Fernandez-Lopez J, Sendra E, Sayas-Barbera E and Perez-Alvarez JA. Quality characteristics of anon-fermented dry cured sausage formulated with lemon albedo. Journal of the science of food and agricultural. 2004;84:2077-2084.
- Alvarez-Gonzales I, Madrigal-Bujaidar E and Sanchez-Garcia VY. Inhibitory effect of grapefruit juice on the genotoxic damage induced by ifosfamide in mouse. Plant Foods Human Nutrition. 2010;65:369–73.
- Assis CRCL, Hermsdorff HHM. and Bressan, J. Anti-inflammatory properties of orange juice: possible favorable molecular and metabolic effects. Plant Foods Human. Nutrition. 2013;68:1–10.
- Aymerich T, Picouet PA and Monfort JM. Decontamination technologies for meat products. Meat Science. 2008;78:114–29.
- Balasundram N, K Sundram, S Samman. Phenolic compounds in plants and agri-industrial byproducts: antioxidant activity, occurrence and potential uses. Food Chem. 2006;99:191-203.
- 6. Barıs Y, Güzin K and Mükerrem K. The effects

of different levels of orange fiber and fat on microbiological, physical, chemical and sensorial properties of sucuk. Food Microbiology. 2012;29: 255-59.

- Bocco A, Cuvelier ME, Richard H. and Berset C. Antioxidant activity and phenolic composition ofcitrus peel and seed extracts. Journal of Agricultural and Food Chemistry. 1998;46:2123–29.
- Braddock RJ. By-products of citrus fruits. Food Technology. 1995;49:74–77.
- Sarıcoban C, Zalp BO, Yılmaz MT, Zen GO, Karakaya M and Akbulut M. Characteristics of meat emulsion systems as influenced by different levels of lemon albedo. Meat Science. 2008;80:599–606.
- 10. Cha JY, Cho YS, Kim I, Anno T, Rahman SM and Yanagita T. Effect of hesperedin, a citrus flavonoid, on the liver triacylglycerol content and phosphatidate phosphohydrolase activity in oroticacidfed rats. Plant Foods Human Nutrition. 2001;56:349–58.
- Champ M, Langkilde AM, Brouns F, Kettlitz B, Collet Y and Le B. Advances in dietary fiber characterisation. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects. Nutrition Research Revision. 2003;16:71–82.
- Cottone E. Use of natural antioxidants in dairy and meat products: A review of sensory and instrumental analyses. M. Sc. Thesis submitted to KansasState University, Manhattan, Kansas. 2009.
- 13. Decker EA and Park Y. Healthier meat products as functional foods. Meat Science. 2010;86:49-55.
- Eda DS, Nuray K, Neslihan Ç, Görsen SÖ., Ilker T A, Yeliz KA. The Comparative Effect of Carrot and Lemon Fiber as a Fat Replacer on Physico-chemical, Textural, and Organoleptic Quality of Low-fat Beef Hamburger. Korean Journal of Food Science Animal. 2015;35(3):370-81
- 15. Emel C. and Nalan G. Changes in energy and cholesterol contents of frankfurter-type sausages with fat reduction and fat replacer addition. Food Chemistry. 2005;91:443–47.
- Fernandez-Gines JM, Fernandez-Lopez J, Sayas-Barbera E, Sendra E and Perez-Alvarez JA. Lemon albedo as a new source of dietary fiber: Application to bologna sausages. Meat Science. 2004;67(1):7-13.
- Fernández-Ginés JM, Fernández-López J, Sayas-Barberá E, Sendra E and Pérez-Alvarez JA. Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. Journal of Food Science. 2003;68(2):710-14.
- Fernandez-Lopez J, Perez-Alvarez JA, Sayas-Barbera E. and Lo'pez- Santoven F. Effect of Paprika (Capsicum annum) on color of Spanishtype sausages during the resting stage. Journal of Food Science. 2002;67:2410–14.
- 19. Fernandez-Lopez J, Fernandez-Gines JM, Aleson-

Carbonell L, Sendra E, Sayas-Barbera E and Perez-Alvarez JA. Application of functional citrus byproducts to meat products. Trends in Food Science and Technology. 2004;15:176–85.

- Fernández-López J, Viuda-Martos M, Sendra E, Sayas-Barberá E, Navarro C and Pérez-Alvarez JA. Orange fibre as potential functional ingredient for dry-cured sausages. European Food Research and Technology. 2007;226(1–2):1–6.
- Fernandez-Lopez J, Sendra E, Sayas-Barbera E, Navarro C and Perez- Alvarez JA. Physico-chemical and microbiological profiles of "salchichon" (Spanish dry – fermented sausage) enriched with orange fiber. Meat Science, 2008;80: 410-17.
- 22. Fisher K and Carol P. Potential antimicrobial uses of essential oils in food: is citrus the answer. Trends in Food Science and Technology. 2008;19(3):156-64.
- 23. García CG, Suarez MR and Romero HT. Why fiber is recommended in the diet? Magazine Metropolitan Hospital. 2001;1:98-99
- 24. Genovese S, Fiorito S, Locatelli M, Carlucci G. and Epifano F. Analysis of biologically active oxyprenylated ferulic acid derivatives in Citrus fruits. Plant Foods Human Nutrition. 2014;69 (3):255-60.
- Ghafar. MFA, Prasad KN, Weng KK and Ismail A. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. African Journal of Biotechnology. 2010;9(3):326-30.
- Hanan H, Abd El-Khalek, Zahran DA. Utilization of Fruit by-Product in Ground Meat Preservation. Journal of Food Science and Quality Management. 2013;11:49-60.
- 27. Hyun Jung Kim and Hyun-Dong Paik. Functionality and Application of Dietary Fiber in Meat Products. Korean Journal Food Science Animal. 2012;32(6): 695-705.
- Jaime L, Molla E, Fernandez A, Martin-Cabrejas, MA, Lopez Andreu FJ and Esteban RM. Structural carbohydrates differences and potential source of dietary fibre of onion (*Allium cepa* L.) tissues. Journal Agriculture Food Chemistry. 2002;50(1):122–28.
- Inserra L, Priolob A, Biondi L, Lanza M, Bognanno, M, Gravador R and Lucianob G. Dietary citrus pulp reduces lipid oxidation in lamb meat. Meat Science, 2014;96:1489–93.
- Lee S and Najiah M. Antimicrobial property of 2-Hydroxypropane-1,2,3-Tricarboxylic acid isolated from Citrus microcarpa extract. Agricultural Sciences in China. 2009;8(7):880-886.
- Li Y, Ou-Lee TM and Raba R, Amundson RG. and Last RL. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. Plant Cell. 1993;5:171–75.
- 32. Lourens-Hatting A. and Viljeon BC. Yogurt as probiotic carrier food. International Dairy Journal. 2001;11:1–17.

- 33. Magiorkinis E, Beloukas A and Diamantis A. Scurvy: past, present and future. European Journal International Medicine. 2011;22:147–52.
- 34. Mansour EH and Khalil AH. Characteristics of low-fat beefburger as influenced by various types of wheat fibers. Food Research International. 1997;30(3-4):199-205. Retrieved from: http:// www.sciencedirect.com/science/article/pii/ S0963996997000434 http://dx.doi.org/10.1016/ S0963-9969(97)00043-4
- Marín FR, Soler-Rivas C, Benavente-García O, Castillo J and Pérez-Alvarez JA. By-products from different citrus processes as a source of customized functional fibres. Food Chemistry. 2007;100(2):736-741. Retrieved from: http://www.sciencedirect. com/science/article/pii/S0308814605009386 http://dx.doi.org/10.1016/j.foodchem.2005.04.040
- Mendoza E, García ML, Casas C. and Selgas MD. Inulin as fat substitute in low fat, dry fermented sausages. Meat Science. 2001;57(4):387-93. Retrieved from: http://www.sciencedirect.com/science/ article/pii/S0309174000001169 http://dx.doi. org/10.1016/S0309-1740(00)00116-9
- Mishra RP, Yadav S. and Anjali. Study of antimicrobial activities of Citrus limetta. Journal of Pharmaceutical and Biomedical Sciences. 2012;19(15):1-4.
- Pak CY. Medical management of urinary stone disease. Nephron Clinical. Practices. 2004;98:49–53.
- Roy SD, Bania R, Chakraborty J, Goswami R, Laila R and Ahmed SA. Pharmacognostic, phytochemical, physicochemical property and antimicrobial activity studies of lemon peel oil. Journal of National Product and Plant Resources. 2012;2(3):431-35.
- Sanchez E, Garcia S and Heredia N. Extracts of edible and medicinal plant damage membranes of Vibrio cholerae. Applied and Environmental Microbiology. 2010;76(20):6888-6894.

- 41. Schieber A, Stintzing FC and Carle R. Byproducts of plant food processing as a source of functional compounds - recent developments. Trends in Food Science and Technology. 2001;12:401-13.
- Sendra E, Fayos P, Lario Y, Ferna ndez-Lopez, J, Sayas-Barbera E. and Perez-Alvarez JA. Incorporation of citrus fibers in fermented milk containing probiotic bacteria. Food Microbiology, 2008; 25(1):13–21.
- Shahidi F. Natural antioxidants an over view. In: Shahidi F. (Ed.), Natural Antioxidants. AOCS Press, Illinois, 1997.pp.1–11.
- 44. Sica DA. Interaction of grapefruit juice and calcium channel blockers. American Journal of Hypertension. 2006;19:768–73.
- 45. Tainara de. MC, André J, Alessandro de OR, Rosane R and Simone HF. Dietary fiber from orange byproducts as a potential fat replacer LWT - Food Science and Technology. 2013;53:9-14
- Tainara de. MC, Rubilene R. deA., Alessandro de OR. and Simone HF. Orange fiber as a novel fat replacer in lemon ice cream. Food Science and Technology (Campinas). 2014;34(2):332-40 •
- 47. Verma SP and Sahoo J. Improvement in quality of ground chevon during refrigerated storage by tocopherol acetate preblending. Meat Science. 2000;56:403-13.
- Wang L, Wang J, Fang L, Zheng Z, Dexian Z, Wang, S, Li S, Ho CT and Zhao H. Anticancer activities of citrus peel polymethoxyflavones related to angiogenesis and others. Biomedemial Research, 2014, Int. http://dx.doi.org/10.1155/2014/453972.
- Weiss J, Gibis M, Schuh V and Salminen H. Advances in ingredient and processing systems for meat and meat products. Meat Science. 2010;86:196-213.
- 50. Wolfe K, Wu X and Liu RH. Antioxidant activity of apple peels. Journal of Agricultural Food Chemistry. 2003;51:609-14.

Superiority of A2 Milk Over A1 Milk: A Boon for Human Health

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A1 Milk; A2 Milk, Bcm-7; Proline Amino Acid; Histidine Amino Acid.

Abstract

Milk is a complete food as it contains all essential micro-nutrients needed for growth and development of human health as well as for neonate animals. However, milk derivative peptides may cause deleterious effect on human health by elevating risk for type I diabetes (DM-1), coronary heart disease (CHD), schizophrenia and autism. The higher occurrences of these diseases have relationship with consumption of variants A1, B and C beta-casein from cow's milk. The production of BCM-7 is more in A1 milk than A2 milk and the difference is basically at 67th position of the beta casein chain. Due to presence of histidine at amino acid at 67th position, digestion of A1 β -casein milk releases a 7 amino acid bioactive peptide called β -casomorphin 7 (BCM-7) in small intestine, while proline in A2 milk at 67th position prevents splitting at this particular site. So propensity is now towards consumption of A2 milk. It is a matter of great concern for the health of people in India.

Introduction

Milk contains all essential micro-nutrients so it is called a complete food. It contains immunoglobulin's, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and several others bioactive peptides. The lipids in milk are emulsified in globules coated with membranes and proteins are in colloidal dispersions as micelles. The casein micelles are seen and observed as colloidal complexes of protein and salts, especially with calcium. Lactose and most other minerals are present in solution form. Cow's milk contains about 87.7% water, 4.9% lactose (milk sugar), 3.4% fat, 3.3% protein and 0.70% Minerals. Bovine milk contains about 32g protein/liter (USDA, 2007). Bovine milk contains huge array of proteins from antimicrobials to hormones and enzymes to antibodies (Clare et al., 2000). Milk usually has two different types of proteins i.e. caseins and whey proteins. Casein is about 80% (Niki et al., 1994) and rest is whey protein. Milk caseins are assembled into proteins and minerals macromolecules and thereby form casein micelles. The structure of the micelles shows k-CN primarily on to the surface and protects the micelle structure from destabilization. Casein shows phosphorylation as they are basically phoshoproteins. Casein molecular mass is about 18–25 k Da. These are quite heterogenous in nature as it develops through post-

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translational modifications and alternative splicing of the gene product and genetic polymorphisms (Caroli et al., 2009). Milk proteins (e.g. secretory immunoglobulin A, lactoferrin, 1-antitrypsin, β -casein and lactalbumin) in human digestive tract appear as peptides or whole proteins and their functions depending upon their relative resistance towards digestive enzymes. Biological function of caseins is to carry calcium and phosphate and to form a clot in the stomach for efficient digestion. Milk usually has three different types of casein protein i.e. Alpha, Beta and Kappa casein. Whey protein is of two types i.e. alpha-lactalbumins and beta-lactglobulins. Casein contains four components namely as1 (CSN1S1, 39-46%), as2 (CSN1S2, 8–11%), β (CSN2, 25–35%) and κ (CSN3, 8-15%) of total caseins (Eigel et al., 1976; Rijnkels, 2002).

A1 and A2 Milk

Amongst the milk constituents, beta casein has gained importance and popularity amongst the health conscious people due to its recent health related issues. The β -CN constitutes about 30% of total CN in bovine milk and is encoded by the CSN2 gene on chromosome 6 (Rijnkels, 2002). This gene is highly polymorphic and 12 different genetic variants of β -CN based on gene polymorphisms and protein sequences have been identified. Alpha-Casein have 9 variants and Beta-Casein have 12 variants (A1, A2, A3, B, C, D, E, F, G, H1, H2, I) but A1 and A2 variants are reported to be the most common allelic variants of β -case in dairy cattle (Farrell et al., 2004). Kappa-casein has 11 variants. There are two major alleles of the gene i.e. A1 and A2 beta casein alleles. A cow carries two copies of the beta-casein gene; she can carry either of A2A2 (homozygous), A1A2 (heterozygous) or A1A1 (homozygous) alleles. Neither allele is dominant over the other rather they are co-dominant i.e. additive in their effect. Therefore, an A1A2 cow will produce A1 and A2 beta-casein in equal amounts. An A2A2 cow will only produce A2 betacasein and an A1A1 cow will only produce A1 beta-casein. So A2 milk is the milk that contains only the A2 type of beta-casein protein whereas A1 milk contains only A1 beta casein type variant. A1 protein variant is commonly found in milk from crossbred and European breeds of cattle. Foreign breeds like HF and Jersey contains around 60% of A1 protein (Priyadarshini et al., 2018). Indigenous cow (Zebu type), buffalo and exotic cows (taurine type) have shown that A1 allele is frequent in exotic cattle while Indian native dairy cow and buffalo have only A2 allele (Mishra et al., 2009). The Milk of Indian breeds of cows (Red Sindhi, Sahiwal, Tharparkar, Gir and Rathi) and buffaloes have 100% A2 allele gene. A1 β -casein is absent in the milk of pure Asian and African Cattle So, our indigenous cows and buffaloes produce only A2 milk.

Chemistry of A2 into A1 beta-casein

A2 is recognised as being the original or ancestor beta-casein gene in modern cattle. Scientists believe the difference originated as a mutation that occurred between 5000 and 10,000 years ago—as cattle were being taken north into Europe—when the proline at position 67 was replaced by histidine, with the mutation subsequently spreading widely throughout herds in the Western world through breeding. Originally all domesticated cows produced milk containing only A2 type of betacasein. Natural mutation resulting into variant of the A2 protein appeared in a proportion of cows of European breeds producing a casein variant called A1 beta-casein.

Beta casein is a chain of 209 amino acids in length. Cows which produce this protein in their milk with a proline at 67th position are called A2 cows and 67th Position's change from proline to histidine created A1 beta-casein (Greenberg et al,. 1984, Roginski, 2003). A bioactive peptide with seven-amino-acid called β-casomorphin-7 (BCM-7), is released by digestion of A1 β -casein with pepsin, leucine amino peptidase and elastase in the small intestine. But milk with A2 β -casein, which has alternative proline at 67th position, prevents a split at this site. Proline has a strong bond to BCM 7, which does not release from the milk in the gut, so that essentially no BCM 7 is found in the urine, blood or GI tract of indigenous A2 cows. On the other hand, histidine, the mutated protein, only weakly holds on to BCM 7, so it is liberated in the GI tract of animals and humans who drink A1 cow's milk. One amino acid difference allows the formation of beta-casomorphin-7 (BCM7) via digestion. The effects on human health of this tiny protein fragment called beta-casomorphin-7 (BCM7) which is a powerful opioid or narcotic as well as being antioxidant. BCM-7 is an exorphin having potential to elicit opioid activity via its affinity to both mu- and delta receptors on a range of tissues and organs including the digestive tract, respiratory and immune systems.

These peptides yielded by the digestion of β -casein have opioid effects similar to morphine, and so named β -casomorphins (β -CMs). Due to presence of histidine at amino acid 67th position,

digestion of A1 β -casein milk releases a 7 amino acid bioactive peptide called beta-casomorphin 7 (BCM-7) in small intestine, while proline in A2 milk at 67 position prevents the split at this particular site and generates peptide BCM-9 (Roginski, 2003; Kostya et al., 2004). A1-derived BCM-7 has been shown to have a longer half-life (Panksepp et al., 1984). BCM-7 may cross the breast parenchyma-blood barrier into plasma and subsequently penetrate the bloodbrain barrier to reach the central nervous system of the developing foetus (Nyberg et al., 1989). It is believed that generation of BCM-7 is the major causative factor associated with A1 milk related health disorders. However, A2 β-casein not been linked to any of such health issues (Kaminski et al., 2007). A1, B and C β -casein have a histidine residue at position 67 that allows an enzymatic cleavage to occur during digestion, releasing the seven amino acid peptide, β - casomorphin 7 (De Noni and Cattaneo, 2010).

Health problems with A1 milk

Due to ingestion of A1 Milk, Increased incidences of various diseases like Type 1diabetes, Heart diseases, Autism and schizophrenia, Atherosclerosisand Sudden infant death syndrome (SIDS). BCM-7 has been linked as a peptide with the ability to catalyse the oxidation of LDL (Low Density Lipoprotein) in a non cation dependent fashion (Torreilles and Guerin, 1995) and this Oxidised LDL uptake by endothelium-bound macrophages leads to pathogenesis of atherosclerosis (Siow et al., 1999). BCM7 causes human health hazards as it can potentially affect numerous opioid receptors in the nervous, endocrine and immune system. Autism and schizophrenia have associated with consumption of beta casein A1 milk (Laugesen and Elliott, 2003; Tailford et al., 2003).

Elliott (1992) stated that Mice on diets having A1 β -casein got diabetic but no diabetes occurred in the mice fed with A2 β -casein. Approved in infants due to the consumption of bovine milk, of A1 origin, which lead to sudden death in new born (Sun et al., 2003). Protein from some cow's milk (not all the cows) raise risk for type I diabetes (DM-1) and coronary heart disease (CHD), schizophrenia and autism as Well. β -casein A1 proteins may develop and assist in the development of diabetes and cardiac diseases (Birgisdottir, 2002). A correlation between BCM-7 levels with delayed psychomotor function in formula fed human infants has been reported. Recent clinical trials report BCM-7 production to physiologically relevant levels in the gut of healthy adult humans (Boutrou et al., 2013). Neurological problems get potentiated due to the consumption of A1 milk which is being related directly to autistic spectral disorder (ASD) and schizophrenia. BCM-7 and related compounds may be involved in the etiology of a range of chronic diseases, including Type 1 diabetes, ischaemic heart disease, autism and schizophrenia (Elliott et al., 1999; McLachlan, 2001).

Bioactive peptide BCM-7 is an exceptionally powerful opioid and Serum BCM-7 has also been linked to the compromise of breathing in infants fed A1 containing formula. A behavioural study of 70 patients with childhood autism who were put on a gluten free, casein free (GFCF) diet concluded that GFCF diet had helped in reducing autism disorder symptoms (Cade et al., 2000). BCM-7 acts on lymphocytes in the intestinal wall and in some way promotes an auto-immune reaction to insulin-producing β -cells resulting in their damage and so the required amount of insulin cannot then be secreted (Elliott et al., 1997). Cieslinnska et al. (2007) experimentally shown that the level of BCM-7 in β -case A1 hydrolysed milk was four times higher than in A2 milk. BCM-7 has been reported to bind to µ-opioid receptors located on the epithelial cells in the gut and inhibit gastrointestinal (GI) functions, which may lead to decreased GI motility and delayed transit time (Barnett et al., 2014).

Conclusion

Milk for human health ought to be drunk. But, determination of milk quality and standards to A1 and A2 milk is also required. Currently, A2 milk is being marketed as a healthier choice than regular milk. The A1/A2 debate is still up in the air. A few studies indicate that A1 beta-casein may have adverse effects in certain individuals and it is believed that generation of BCM-7 is the major causative factor associated with A1 milk related health disorders. However, the evidence is still too weak for any strong conclusions to be made. That being said, if you feel like you can tolerate A2 milk better than A1 milk, then you should definitely stick to it.

References

 Barnett MPG, McNabb WC, Roy NC, Woodford KB, Clarke AJ. Dietary A1 b-casein affects gastrointestinal transit time, dipeptidyl pepti dase-4 activity, and inflammatory status relative to A2 b-casein in Wistar rats. International Journal of Food Sciences and Nutrition. 2014;65:720-27.

- Birgisdottir BE, Hill JP, Harris DP, Thorsdottir I. Variation in consumption of cow milk proteinsand lower incidence of type 1 diabetes in Iceland vs. Theother 4 Nordic countries. Diabetes, Nutrition and Metabolism. 2002;15:240–45.
- Boutrou R, Gaudichon C, Dupont D, Jardin J, Airinei G, Marsset-Baglieri A. Sequential release of milk protein-derived bioactive peptides in the jejunum in healthy humans. American Journal of Clinical Nutrition. 2013;97:1314-23.
- Cade R, Privette M, Fregly M, Rowland N, Sun Z, Zele V. Autism and Schizophrenia: Intestinal Disorders. Nutritional Neuro science. 2000;3(1): 57–72.
- Caroli AM, Chessa S, Erhardt GJ. Invited review: Milk protein polymorphismsin cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science. 2009;92:5335-52.
- Cieslinska A, Kaminski S, Kostyra E, Sienkiewicz Szlapka E. Betacasomorphin 7 in raw and hydrolyzed milk derived from cows of alternative b-casein genotypes. Milch Wissenschaft. 2007;62: 125-27.
- Clare DA, Swaisgood HE. Bioactive milk peptides: A prospectus. Journal of Dairy Science. 2000;83: 1187-95.
- DeNoniI, Cattaneo S. Occurrence of b-casomorphins 5 and 7 in commercial dairy products and in their digests following in vitro simulated gastrointestinal digestion. Food Chemistry. 2010;119:560-66.
- 9. Eigel WN, Randolph HE. Comparison of calcium sensitivities of aS1-B,b-A2, and g-A2 caseins and their stabilization by k-casein A. Journal of Dairy Science. 1976;59:203-06.
- Elliott RB, Harris DP, Hill JP, Bibby NJ, Wasmuth, HE. Type 1 (insulin-dependent) diabetes mellitus and cow milk: Casein variant consumption. Diabetologia. 1999;42:292–96.
- Elliott RB, Wasmuth HE, Bibby NJ, Hill JP. The role of b-casein variants in the induction of insulindependent diabetes in the non-obese diabetic mouse and humans. In Seminar on Milk Protein Polymorphism. International Dairy Federation. 1997;IDF Special Issue no 9702:445-453.
- Farrell HM Jr., menez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK. Nomenclature of the proteins of cows' milk-sixth revision. Journal of Dairy Science. 2004;87:1641–74.
- Greenberg R, Groves ML, Dower HJ. Human b-casein Amino acid sequence and identification of phosphorylation sites. Journal of Biological Chemistry. 1984;259:5132-38.
- Ivano De N, Richard J, FitzGerald, Hannu JT. Korhonen, Yves Le R, Chris T. Livesey, Inga Thorsdottir, Daniel Tomé, Renger Witkamp. Review of the potential health impact of β-casomorphins and related peptides. EFSA Scientific Report. 2009;

231:1-107.

- Kaminski S, Cieslinska A, Kostyra E. Polymorphism of bovine beta-casein and its potential effect on human health. Journal of Applied Genetics. 2007; 48:189–98.
- Kostyra E, Sienkiewicz-Szapka E, Jarmoowska, B, Krawczuk S, Kostyra H. Opioid peptides derived from milk proteins. Polish Journal of Nutrition Science. 2004;13/54:25–35.
- 17. Laugesen M, Elliott R. Ischaemic heart disease, Type 1 diabetes, and cow milk A1 beta-casein. New Zealand Medical Journal. 2003;116:1168.
- Martin P, Szymanowska M, Zwierzchowski L, Leroux C. The impact of genetic polymorphisms on the protein composition of ruminant milks. Reproduction Nutrition Development. 2002;42:433.
- 19. Mclachlan CNS. Beta-casein A1, ischemic heart diseases, mortality and other illnesses. Med Hypotheses. 2001;56:262-72.
- Mishra BP, Mukesh M, Prakash B, Sodhi M, Kapila R, Kishore A, Kataria RS, Joshi BK, Rasool TJ, Bujarbaruah KM. Status of milk protein, β-casein variants among Indian milch animals. Indian Journal of Animal Sciences. 2009;79(7):722–25.
- 21. Niki R, Kim GY, Kimura T, Takahashi T, Kohyama K, Nishinari K. Physical properties and microstructure of rennet gels from casein micelles of different sizes. Milch Wissenschaft. 1994;49:325-28.
- 22. Nyberg F, Lieberman H, Lindstrom LH, Lyrenas, S, Koch G, Terenius L. Immunoreactive betacasomorphin-8 in cerebrospinal fluid from pregnant and lactating women: correlation with plasma levels. Journal of Clinical Endocrinology and Metabolism. 1989;68(2):283–89.
- Panksepp J, Normansell L, Siviy S, Rossi J, Zolovick, AJ. Casomorphins reduce separation distress in chicks. Peptides. 1984;5(4):829–31.
- 24. Priyadarshini P, Mishra C, Mishra B, Krutanjali S, Mangalika R, Mishra SP. Impact of milk protein on human health: A1 verses A2. International Journal of Chemical Studies. 2018;6(1):531-35.
- 25. Rijnkels M. Multispecies comparison of the casein gene loci and evolution of the casein gene family. Journal of Mammary Gland Biology Neoplasia. 2002;7:327-45.
- 26. Roginski H. Encyclopaedia of dairy sciences. Academic Press, London. 2003.
- Siow, R.C., Richards, J.P., Pedley, K.C., Leake, D.S., Mann, G.E. Vitamin C protects human vascular smooth musclecells against apoptosis induced by moderately oxidized LDL containing high levels of lipid hydroperoxides. Arteriosclerosis Thrombosis and Vascular Biology. 1999;19(10):2387–94.
- Sun Z, Zhang Z, Wang X, Cade R, Elmir Z, Fregly, M. Relation of β-casomorphin to apnea insudden infant death syndrome. Peptides. 2003;24:937–43.

- 29. Tailford Kristy A, Berry Celia L, Thomas Anita C, Campbell Julie H. A casein variant in cow's milk is atherogenic. Atherosclerosis. 2003;170:13-19.
- 30. Torreilles J, Guerin MC. Casein-derived peptides

can promote human LDL oxidation by a peroxidisedependent and metal-independent process, Comptes rendus des séances de la Société de biologie et de ses filiales. 1995;189:933-42. Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

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[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiberreinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online – Trends in suicide by method in England and Wales, 1979-2001. www. statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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