

Training Programme

on

Entrepreneurship Development Programme on Donkey Farming

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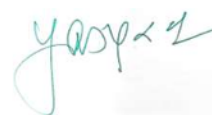
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Director's Forward

Donkeys are known as the beast of burden. These are the most neglected species. These are unclassified and recognized as local and nondescript donkeys. The contribution of donkeys has been enormous in agriculture, herding and carrying materials with migratory herds of cattle, sheep and goats, transportation of agricultural produce, household material and provision as well as material for building construction. Although donkeys support the rural livelihood and low-income poor farmer's families by providing economy at minimal maintenance cost, they are not yet considered as farm animals. These animals have very good draught power, immunity and stress bearing capabilities. The population of donkeys in India is dwindling at a rapid pace and according to the recent livestock census there was 61.23% decline in the donkey population all over India. In this connection, it is utmost important to record, characterize the various donkey breeds present in the country and initiate necessary conservation strategies to minimize the drastic decline in their population.

While the donkey was shunned aside for its 'asinine' values, its milk was venerated as the elixir of life. With increasing numbers of research reports worldwide, donkey milk is now being taken as "gold mine" for future. In western countries, donkey dairies are emerging to produce an alternative milk source for human infants. In our country donkey milk is not gaining wider consumer acceptance, because of a lack of the related food product legislation. There is enhanced interest from consumers for donkey milk in southern states, while there are no official reports or published data with reference to its production, quality and hygiene. Maximum donkeys are found with nomads and labour at brick kilns who don't know how to gain economic benefit from the animal except as pack and load carrying animal. If these donkey owners and marginal donkey farmers are made aware of the benefits and the products of donkey milk, this can be a great financial boon to them. Looking as the upcoming demand for donkey farming and milk pharming, ICAR-NRC on Equines also taking up various projects on characterisation of donkey milk and development of various beauty related products using donkey milk. Many upcoming entrepreneurs in India now looking at opening donkey farms and aim to capture the donkey milk market. The number of requests and queries from the donkey keepers, donkey farm owners are raising day by day with regard to their management of health and production. Keeping this in view, for the first time ICAR-NRCE is organising a three day training programme on Entrepreneurship development programme on donkey farming which will greatly benefit the donkey farm owners. I wish this programme to be successful and benefit the donkey entrepreneurs.



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DONKEY DEVELOPMENT AND CONSERVATION

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Donkeys

The Donkey is a domesticated version of the wild ass. The donkey was domesticated about 5,000 years ago in Egypt, Iraq and Iran and was important, along with horses and camels in the development of trade and mobility. They were an important pack animal for the Roman legions who introduced the donkey to Britain. Genetic fingerprints indicated that wild African asses were the ancestors of domestic donkeys, making donkeys the only important domestic animal known to come from Africa. Donkey DNA analysis showed that there were 2 distinct populations of domestic donkey (*Science* Vol 304, p 1781): one descended from the Nubian wild ass *Equus asinus africanus* and the other being similar to the Somalian wild ass *Equus asinus somaliensis* (<http://www.arthurgrosset.com/mammals/donkey.html>, retrieved August 21, 2010). The Indian donkeys are not much studied animals using molecular genetics tools. There are three registered breeds of donkeys in India viz., Spiti (INDIA_DONKEY_0600_SPITI_05001), Halari (INDIA_DONKEY_0400_HALARI_05002), and Kachchhi (INDIA_DONKEY_0400_KACHCHHI_05003). The total Donkey population in the country has decreased by 61.20% over the previous census and the total donkeys in the country are 0.12 million numbers in 2019. Donkeys are used as pack and carting animal. These are capable of thriving in hot climates (Pal *et al.*, 1997, Pal *et al.*, 2000) and can survive even in adverse conditions such as scarcity of feed (Gupta *et al.*, 1999, Pal and Gupta 2004, Pal and Gupta 2004a). There are following three registered breeds of donkeys in India.

Poitou Donkeys

Breeding tract of Poitou donkeys is in Europe (France). These donkeys were imported from UK in 1990 for superior quality mule production and breed improvement of indigenous donkeys. Good quality donkey jacks and jennies of exotic breed obtained from France and other European countries are being maintained by NRCE, State Animal Husbandry Deptt of Haryana and Uttar Pradesh, and at Equine Breeding Farms of the Army.

Indian Donkeys

Indian donkeys range from white to grey or black in colour. They usually have a dark stripe from the mane to the tail and a crosswise stripe on the shoulders. The mane is short and upright and the tail has long hair only at the end. The very long ears are dark at the base and tip. The different breeds of donkey vary greatly in size and shape but the average donkey stands 40 inches (102 cm) at the shoulder, the Sicilian donkey stands 24 inches (61 cm), the large ass of Majorca stands at about 62 inches (158cm), while the American ass may reach a height of about 66 inches (168cm) (<http://student.britannica.com>, retrieved May 13, 2009).

(a) Spiti Donkey

The donkey (*Equus asinus*) is a sure footed, docile and hard working animal and used broadly for pack purposes especially in difficult terrains. Spiti donkeys are scattered within the trans-Himalyan regions of Spiti and Yangthang in Lahaul Spiti and Kinnour districts of Himachal Pradesh. The climate remains cold and arid, characterized by dry and mild summer without monsoon rains. Heavy snowfall and severe cold is observed in winters. Spiti donkey is smaller in size, having a solid body, straight back and powerful legs. The height at withers varies from 80-95 cm of Spiti donkeys. The body is normally covered with a thick coat of long hairs. The main coat colours were brown, brown with lighter bellies, dark brown/black and light brown with whitish bellies. White markings were observed around muzzle and eyes. These donkeys look like exotic donkeys (Poitou) in appearance and colour. The height at withers of Spiti donkeys varies from 80 -95 cm (Gupta and Pal, 2014). The nasal bone was straight to slightly convex. The ears were comparatively smaller, straight, pointing upwards with slightly lateral orientation. The tail switch was not distinguishable due to presence of thick hair on the tail (Behlet *al.*, 2017). They are a crucial livestock species which adapted to the various environments, hard conditions and harsh climates. Motorized agriculture and transport caused an unfavourable condition for donkeys.

(b) Halari Donkey

These donkeys are native of Saurashtra region of Gujarat state. These donkeys are generally reared by the Rabari shepherds in Halar region in Jamnagar, Devbhoomi Dwarka, Morbi and Thar region of Gujarat. Since Rabari people migrate with their sheep and goats, they follow the donkey to move their goods and households from one place to another. Donkeys of Halari

breed can walk 30-40 km in a day during migration. Halari donkeys are of white colour, muzzle and hooves contain black colour. The average height at withers is 110 cm and weighs about 150 kg. Donkeys of Halari breed are taller than other normally seen donkey breeds. Fore head of Halari donkeys is convex. The numbers of donkeys in India have declined sharply. Halari donkeys can pull loads of up to two times of their body weight without stress whereas with pulling loads of three times of body weight load, fatigue symptoms developed in donkeys (Legha *et al.*, 2020).

(c) Kachchhi Donkey

These donkeys are found in Kachchh District of Gujarat. Coat colour is mainly grey containing dorsal surface grey and ventral surface white followed by white, brown and black. In similar studies, Pal *et al.*, (2013b) observed that donkeys prevalent in Rajasthan are of many coat colours but light and dark gray is more prevalent, accompanied by shoulder crosses, dark ear markings, white muzzles, eye rings, white bellies and inner legs whereas Gupta *et al.*, (2017a) reported small hair on most of the body parts with zebra marking on legs and stripe on back in donkeys of Bihar. Forehead of Kachchhi donkeys is convex and their nasal bone is straight. Height at wither of these donkeys ranges from 77 to 115 cm. Their temperament is docile. In some parts of Gujarat state, donkeys are used for agricultural operations viz., inter-cultivation for weed removal and in transportation as donkey cart. Pastoralists during migration use donkeys as pack to carry their materials. These donkeys can carry approx. 80-100 Kg on back and can pull 200-300 Kg on cart. In contrast to this, Pal *et al.*, (2002) reported that donkeys can carry pack load of up to 50% of their body weight comfortably. Also pack load equivalent to 66% of their body weight causes early onset of fatigue and should be avoided (Legha *et al.*, 2018). Intermittent rest in between work not only reduces stress to the animal but also increases the working efficiency (Pal *et al.*, 2012).

Population of Kachchhi donkeys is approximately 1700 (http://14.139.252.116/Kachchhi_donkey.html retrieved on 10th October, 2020). Although the population size of donkey is decreasing, still there is scope of scientific intervention due to high existing diversity as depicted by the average number of alleles per locus, observed heterozygosity, absence of inbreeding as well as bottleneck (Sharma *et al.*, 2017). Gupta *et al.*, (2017b) reported that Indigenous donkeys can be phenotypically clustered in two groups

namely miniature donkeys (Leh, Spiti and Bihar regions) and small standard donkeys comprising donkeys from Gujarat, Baramati, Rajasthan and Haryana.

UTILITY OF EQUINE PRODUCTS

i) Equine milk: It has been observed that equine milk has tremendous medicinal value, curative agent for metabolic and allergic diseases, use in cosmetics and anti-aging property. In some part of the country, donkey milk is sold for medicinal uses. The donkey milk fetch a good amount of money for donkey milk providers and is in fair demand. Donkey milk based cosmetics and health mixtures can be an attractive agribusiness for equine framers in India. This new possibility of increasing the income of the equine owners is very widespread among general public. Donkey milk is natural hypo-allergenic milk, because it is tolerated by about 90% of infants with food allergies, e.g., breast milk protein allergy, a general food allergy in childhood with a prevalence of approximately 3% during the first 3 years of life (Salimei and Fantuz, 2012). Donkey's milk is similar to human milk for its lactose, proteins, minerals and omega-6, 9 fatty amino acid contents. Donkey milk has high lactose content but low fat content.

Dairy products made with DM are gaining public interest because they are considered as functional foods, rich in major milk components. Dairy products made up of DM include ice cream, cheese, fermented milk products, milk powder for infants' formula. Keeping in view the functional properties of DM, emphasis is given on developing DM-based dairy products that provide human health benefits. Recent studies show that DM and its derivatives are successfully used in the development of food products including cheese, milk powder, yogurt, kefir, ice cream and other fermented products (Perna *et al.*, 2015; Salgado *et al.*, 2021; Tidona *et al.*, 2015).

The donkey milk used as a moisturizer has amazing benefits. In cosmetics, cleaning and hydrating action combined with an antioxidant action of donkey milk stops the aging. The fat content in donkey's milk nourishes the skin and softens it. Also, donkey milk could be used therapeutically in numerous cases, like liver problems, infectious diseases, fevers, asthma, etc. (Hippocrates 1843, Pliny the Elder 1893).

ii) Vermicomposting: Earlier disposal of equine dung was havoc. The technology of use of equine dung for converting into vermicompost has been standardized. But, now the same is being used for production of vermicomposting at some research and private farms. The

vermicompost is quite beneficial in agricultural fields also. This can also be exploited as a source of livelihood.

iii) Mushroom production: To dispose the equine dung and fodder waste, it was decomposed and utilized for the production of mushroom. Now, the standardized technology of button mushroom production using equine dung and waste is available at NRCE.

Conservation of equine genetic resource

In the view of global liberalization, competitiveness and coming of General Agreement on Tariffs and Trade (GATT) agreement, the conservation and preservation of local breeds of horses and donkeys in their home tract needs main concern. The management of genetic diversity is a major factor of any program of preserving species and breeds to protect genetic resources of animals (Gupta *et al.*, 2015). It is therefore important that breeds of horse and donkey need preservation and conservation both *in-situ* and *ex-situ*.

***In-situ* Conservation**

In-situ Conservation implies the maintenance of entire breeds in their natural habitat with no interference in the form of management, breeding and other ecological conditions. The breeds which are under the threat of extinction must be preserved under *in-situ* conservation schemes. This type of conservation is possible through two approaches. The active *in-situ* conservation leads to breed development through well designed breeding programmes, whereas, passive *in situ* conservation is concerned with the maintenance of live animals under an adaptive environment.

In-situ conservation may be planned in two ways. Firstly, *in-situ* conservation can be done at institutional or Government farms where the animals are kept for breeding to increase the number of true to breed animals of same genetic makeup and subsequent conservation. Secondly, *in-situ* conservation can be attempted at farmers' doors using their own stock. For institutional herds, conservation may be undertaken considering population size, nucleus herd, multiple ovulations embryo transfer (MOET), single ovulation embryo transfer (SOET), artificial insemination (AI) and improved management tools. *In-situ* conservation in farmers' herds needs governmental help in the form of incentives, the participation of research institutes in the form of advisory services to the farmers, self-help groups and cooperatives etc.

Ex-situ Conservation

It may be undertaken for endangered as well as thickly populated breeds. *Ex-situ* preservation involves the conservation of animals in a situation removed from their usual habitat. It is used to refer to the collection and freezing in liquid nitrogen of animal genetic resources in the form of living semen, ova or embryos (Tischner, 1979). Pal *et al.*, (2011a) concluded that semen of all the stallions do not freeze equally and they may be classified as poor, moderate and good freezers. Vapor freezing technique for stallion and jack semen was standardized by Pal *et al.*, (2011b) and Legha and Pal (2012). Arangasamy *et al.*, (2013) accomplished that dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) can be used as an alternative cryoprotectant, wherever the stallion semen facing problems with glycerol in freezing. Ravi *et al.*, (2013) cryopreserved semen of Kathiawari stallions at farmers' door using vapour freezing technique for encouraging artificial insemination and their conservation. Legha *et al.*, (2012) observed individual variation in fertility rate of jacks and found that double AI per cycle has higher conception rate as compared to single AI in mares.

PHYSICO-CHEMICAL PROPERTIES AND PROTEIN PROFILING OF DONKEY MILK

Anuradha Bhardwaj, Varij Nayan, Harish Kumar, TR Talluri, RK Dedar, Ram Avatar Legha, J Singh, SC Mehta, Yash Pal, Bhupendra Nath Tripathi

INTRODUCTION

Donkey (*Equus asinus*), considered as an animal with versatile customs and is mainly known as a pack animal but also provides milk, meat and also uses in breeding as well as in transportation too. The total Livestock population is 536.76 million in the country showing an increase of 4.8% over Livestock Census-2012. Contribution of livestock in total agriculture and allied sector GVA (at Constant Prices) has increased from 24.32 per cent (2014-15) to 28.63 per cent (2018-19) (National Accounts Statistics, 2020). Total population of Horses, ponies, Mules & Donkeys is 0.55 million during 2019. Total population of Horses, Ponies, Mules & Donkeys has decreased by 51.7% over previous livestock census (2012) (20th Livestock Census-2019) (Table 1-2; Figure 1-2).

Indian Equines	Population (Million) (2007)	Population (Million) (2012)	Population (Million) (2019)	% Decrease (2012-2019)
Horse & Ponies	0.61	0.62	0.34	45.2%
Mules	0.14	0.20	0.08	57.1%
Donkeys	0.44	0.32	0.12	61.2%

Table 1: Indian Equine Population

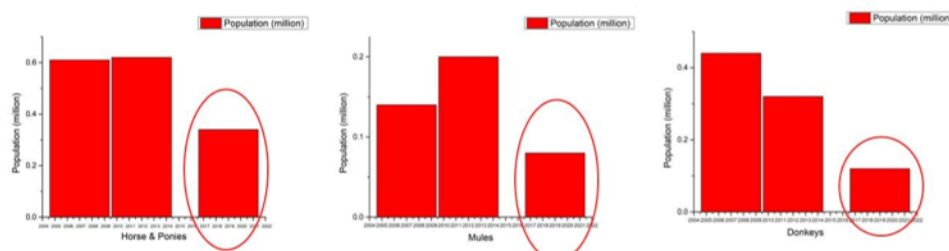


Figure 1: Indian Equine Population changes

States	Population (in lakhs) 2012	Population (in lakhs) 2019	% Change
Rajasthan	0.81	0.23	-71.31
Maharashtra	0.29	0.18	-39.69
Uttar Pradesh	0.57	0.16	-71.72
Gujarat	0.39	0.11	-70.94
Bihar	0.21	0.11	-47.31
Jammu & Kashmir	0.17	0.10	-44.55
Karnataka	0.16	0.09	-46.11
Madhya Pradesh	0.15	0.08	-45.46
Himachal Pradesh	0.07	0.05	-34.73
Andhra Pradesh	0.13	0.05	-65.16

Table 2: the Major States with Donkey population (in Lakhs)

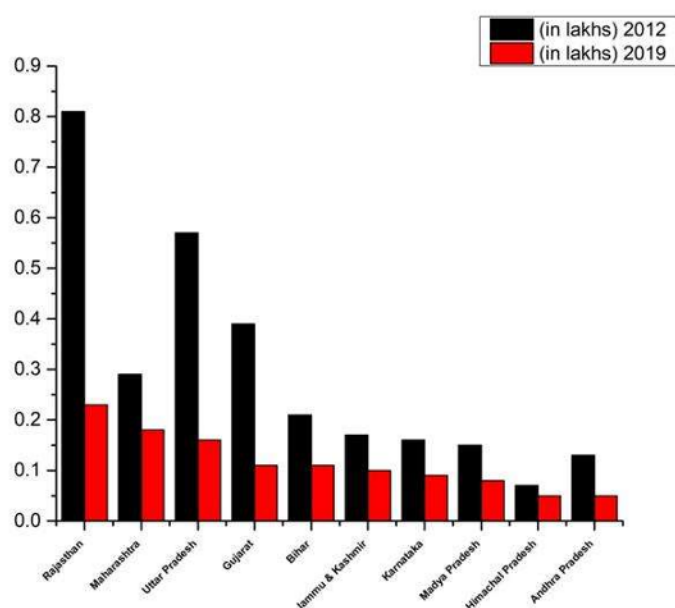


Figure 2: State-wise (Major) Donkey population (in Lakhs)

The traditional dairy-cattle-based industry is becoming increasingly diversified with milk and milk products from non-cattle dairy species. The interest in non-cattle kinds of milk has increased because there have been several anecdotal reports about the nutritional benefits

of these kinds of milk and reports of individuals tolerating and digesting some non-cattle kinds of milk better than cattle milk and of certain characteristics that non-cattle milk is thought to share in common with human milk. Thus, non-cattle kinds of milk are considered to have potential applications in infant, children, and elderly nutrition for the development of specialized products with better nutritional profiles. However, there is very little scientific information and understanding about the physicochemical and digestion behavior of non-cattle kinds of milk. Donkey milk is still undiscovered and unexplored research area in India and is the need of the hour. Milk is a nutritive drink obtained from various animals and consumed by humans. Generally, milk is obtained from dairy cows and buffaloes; although milk from Goats, Camel and Donkey is also used in various parts of the world. It is well known that the best nutritional option for newborns is their mother's milk: however, some infants may not be exclusively breast fed during the first months of life. Therefore, there is a need for another substitute of close composition and properties as human milk. In such cases, when breast feeding is not possible, a cow's milk free diet often resolves symptoms, although some infants can present intolerance to the foods used as alternatives. The nutritive content of donkey's milk is almost similar to breast's milk hence beneficial for babies that could not feed on mother milk due to diseases or improper milk formation etc.

The milk of cow, goat and sheep contributes the majority of the global milk production and buffalo milk production is in second place worldwide (Tozzi *et al.*, 2016). Bovine milk represents over 80% of the world's milk production and is a major source essential nutrient for human health (Wochner *et al.*, 2018). Medhammar *et al.*, (2012) reported that donkeys, Bactrian camel, reindeer, musk ox, llama, moose, yak and alpaca have been underutilized milk producing animals and are defined as “species with underexploited potential for contributing to food security, health and nutrition”. **Donkey milk** is being used as substitute of breast milk in several countries. Now-a-days, the consumers' interest in donkey milk is escalating in European countries due to its compositional similarity to human milk, with good tolerability and palatability. Hence, in western countries, donkey dairies are emerging to produce an alternative milk source for human infants. Also, there is enhanced interest of consumers for donkey milk in southern states of India, while there are no official reports or published data with reference to its production, quality and hygiene. Consumer acceptance for donkey milk will further increase in India after its approval as a food item by FSSAI.

Equine (donkey) milk production differs greatly from that of conventional dairy species, especially in terms of milk supply which is much more limited. The equid mammary

gland has the low capacity (maximum 2.5L) and a part of the milk production should be left to foal and that milking may be carried out two or three hours after separation from the foal (Doreau 1991). Donkeys produce milk from 20 to 90 d after foaling for three times a day. A female gives between 0.5 and 1.3 liters of milk a day for about 6-7 months. Among the functional proteins detected in donkey milk, there are molecules active in antimicrobial protection such as lysozyme and lactoferrin. The lactoferrin content of donkey milk is intermediate between the lower values of cow milk and the higher values of human milk. Lysozyme in donkey milk is present in large amounts, indeed ranges from 1.0mg/ml milk to 4 mg/ml, depending on the analytical method used (chemical or microbiological): this substance is present also in human (012 mg/ml) but only in trace amounts in cow and goat protease and may play a significant role in the intestinal immune response (Tidona *et al.*, 2011). Donkey mammary secretions contain human-like leptin at levels close to human milk (3.35 e 5.32 ng/ml). The bioactive peptides insulin like growth factor 1, ghrelin and triiodothyronine were also found in frozen donkey milk. These molecules, and many others present in human milk, are increasingly receiving attention from a nutraceutical point of view because of their potential direct role in regulating food intake, metabolism, and infant body condition.

Donkey milk is used as natural hypo-allergenic milk, because it is tolerated by about 90% of infants with food allergies, e.g., breast milk protein allergy, a common food allergy in childhood with a prevalence of approximately 3% during the first 3 years of life (Salimei and fantuz, 2012). Donkey's milk is similar to human milk for its lactose, proteins, minerals and omega-6, 9 fatty amino acid content. In terms of energy despite the high lactose content of donkey milk the average fat content is lower.

The Donkey is a domesticated version of the wild ass. The donkey was domesticated about 5,000 years ago in Egypt, Iraq and Iran and was important, along with horses and camels in the development of trade and mobility. They were an important pack animal for the Roman legions who introduced the donkey to Britain. Genetic fingerprints indicated that wild African asses were the ancestors of domestic donkeys, making donkeys the only important domestic animal known to come from Africa. Donkey DNA analysis showed that there were 2 distinct populations of domestic donkey (Science Vol 304, p 1781): one descended from the Nubian wild ass (*Equus asinus africanus*) and the other being similar to the Somalian wild ass (*Equus asinus somaliensis*) (<http://www.arthurgrosset.com/mammals/donkey.html>, retrieved August 21, 2010). The Indian donkeys are not much studied animals using molecular genetics tools as well donkey milk was not recognized in eligible milk list also. However, based on the

phenotype, the Indian donkeys have been grouped under “Large White” or “Small Grey” categories. There are only three breeds assigned to donkeys (<http://www.nbagr.res.in/regdnky.html>) in India i.e. Halari donkeys which are large white donkeys of Gujarat, Spiti donkeys and recently Kachchhi donkeys have been registered. The total donkey population in the country has decreased by 61.0 % over the previous census and the total donkeys in the country are 0.12 million numbers in 2019. India is bestowed with precious equine germplasm and equines have contributed significantly in the development of human race from the time immemorial. India possesses about 0.54 million equines as per 2019 livestock census. Major population of equids comprising of donkey, mule, horse and ponies provide livelihood to the rural and semi-urban societies. There are seven registered horse breeds i.e. Marwari, Kathiawari, Kachchhi-Sindhi, Spiti, Zanskari, Bhutia and Manipuri and three registered breeds of donkeys, namely Spiti, Halari and Kachchhi. The management of genetic diversity is a major factor of any program of preserving species and breeds to protect genetic resources of animals. Therefore, it is important that breeds of horse and donkey are preserved and conserved both in-situ and ex-situ especially in view of their reducing populations in India (Pal *et al.*, 2020). The donkey milk profiling in India needs to be done and it has been observed that equine milk has tremendous medicinal value, curative agent for metabolic and allergic diseases, use in cosmetics and anti-aging property. In some part of the country, donkey milk is sold for medicinal uses. The donkey milk fetch a good amount of money for donkey milk providers and is in fair demand. Donkey milk based cosmetics and health mixtures can be an attractive agribusiness for equine framers in India. This new possibility of increasing the income of the equine owners is very widespread among general public. ICAR-NRCE is establishing a Jenny Dairy Unit at Hisar campus with Jennies of Halari breed. It will serve as a demonstration farm for developing donkey milk based research and also for entrepreneurship accordingly.

Donkey breeds of India

1	<i>Spiti</i>	<i>Himachal Pradesh</i>	<i>INDIA_DONKEY_0600_SPITI_05001</i>
2	<i>Halari</i>	<i>Gujarat</i>	<i>INDIA_DONKEY_0400_HALARI_05002</i>
3	<i>Kachchhi</i>	<i>Gujarat</i>	<i>INDIA_DONKEY_0400_KACHCHHI_05003</i>

Milk yield

In terms of milk supply, donkey milk production differs greatly from that of conventional dairy species. Recent studies on donkey lactation curves showed that individual milk yield ranged between 1.54 and 1.73 kg/day on specialized farms (Bordonaro *et al.*, 2013), which generally raise animals in semi extensive conditions and care about their wellness. Equid mammary gland has a low average capacity (maximum 2.5 L); therefore, to increase milk supply, dairy equids may need to be milked many times a day (Salimei and Fantuz 2012). Alabiso *et al.*, (2009) showed that the highest milk yield can be obtained with three milkings per day compared to two per day, with an increase in milk fat content, too. The donkeys that gave birth in an autumn–winter period yielded more milk than donkeys foaling in spring–summer period since seasonal thermal stress can have detrimental effect on the quantity and quality of milk.

Milk composition

The composition of donkey's milk differs considerably from the milk of cow, buffalo, goat, camel and sheep. In comparison with bovine milk, donkey's milk contains less fat, protein and inorganic salts but more lactose, with a concentration close to that human milk. Lactose is a source of fast energy. It makes this milk sweet, palatable and well accepted by children. On the other hand, the majority of adults and certain ethnic groups exhibit intolerance to milk sugar (Guo *et al.*, 2007). The high lactose content also stimulates the intestinal absorption of calcium, which is essential for bone mineralization and for nervous system development in infants (Schaafsma 2003). Moreover, the high lactose content suggests use of donkey milk for probiotic purpose (Coppola *et al.*, 2002) because it is an ideal substrate for a correct development of intestinal lactobacilli and makes donkey's milk an ideal matrix for the preparation of probiotic drinks following the incubation with *Lactobacillus rhamnosus* strains (Coppola *et al.*, 2001).

The gross composition of milk is affected by genetic and environmental factors, including the breed, individuality of animals, stage of lactation, frequency and completeness of milking, maternal age, health and type of feed. Many times fat content in donkey milk was estimated negligible in our studies (Pal *et al.*, 2016) and hence, donkey milk is termed as natural defatted milk. Hence forth, it is encouraging for studies on the possible use of donkey milk in dietotherapy.

The pH of donkey milk, as well as human milk, is neutral or slightly alkaline, likely due to low content of caseins and phosphates, in comparison to cow milk (Pal *et al.*, 2018). The average size of donkey milk fat globules may be important for milk digestibility. Although there are no explanatory results in the literature, it seems that the diameter of the milk fat globules has a different effect on the way in which fat is digested and metabolized (Michalski *et al.*, 2005a). In fact, some authors have speculated that smaller native milk fat globules may have the best digestive parameters due to the larger surface available for the lipase action (Raynal-Ljutovac *et al.*, 2008). Differences between donkey and other dairy species have also been found regarding the number of milk fat globules/mL of milk. In fact, the number of donkey milk fat globules has been found to be lower than that found in cows, goats and sheep (Martini *et al.*, 2013a). Donkey milk fat globules showed an average diameter of 2 μm for 70% of total globules, resulting similar to the horse ones but smaller than both human (4 μm) and cow (2.8–4.6 μm) fat globules (Claeys *et al.*, 2014, Martini *et al.*, 2013). Moreover, equine milk does not cream due to the lack of cryoglobulin, a protein that adsorbs onto the fat globules as the temperature is reduced, and thus the agglutination of fat globules occurs very slowly (O'Mahony and Fox 2014).

Milk contains numerous nutrients. The content of most minerals is higher in donkey milk than in human milk, but significantly lower than in ruminant milk (Fantuz *et al.*, 2012). The vitamin content of donkey milk is generally comparable or slightly lower than human milk, and, on average, lower than the vitamin content of ruminant milk, except for the vitamin C level (Claeys *et al.*, 2014).

The principal salts in donkey milk are calcium, phosphorus, potassium, sodium, and magnesium. The concentrations of these elements, except potassium, are higher in donkey milk than in human milk but all are considerably lower compared with species such as cows, buffaloes, goats or sheep. The calcium, phosphorus concentrations were observed to be about 2-3 times higher than in human milk (Salimei and Fantuz 2012). Casein micelles are primarily a source of amino acids, calcium, phosphate and bioactive peptides for neonates (Shekar *et al.*, 2006). Although the mechanism of tolerance of donkey's milk has not yet been fully clarified, it is reasonable to hypothesize that the reduced allergenic properties of this milk can be related to structural differences of its protein component with respect to cow's milk. The lower casein concentration in donkey's milk (about 50% of total protein) compared with bovine milk (80% of total protein) (Zicker & Lonnerdal 1994) and the relevant percentage of essential amino-acids make this milk a new dietetic food and a promising breast milk substitute (Guo *et al.*,

2007). A more recent study on DM has revealed the percentage of four caseins (α s1, α s2, β and κ -CN) in decreasing order: β (54.28) > α s1 (35.59) > α s2 (7.19) > κ -CN (2.79) as evaluated by Urea-PAGE analysis at pH 8.6, followed by immuno-detection with polyclonal antibodies, coupled to densitometric analysis (Cosenza *et al.*, 2019).

Table 1: Chemical composition of milk from different species (Guo et al, 2007)

Components g/100g	Donkey	Bovine	Human
Total solids	8.8-11.7	12.5-13.0	11.7-12.9
Fat	0.3-1.8	3.5-3.9	3.5-4.0
Lactose	5.8-7.4	4.4-4.9	6.3-7.0
Ash	0.3-0.5	0.7-0.8	0.2-0.3
Total protein	1.5-1.8	3.1-3.8	0.9-1.7
Casein	0.64-1.03	2.46-2.80	0.32-0.42
Whey protein	0.49-0.80	0.55-0.70	0.68-0.83
pH	7.0-7.2	6.6-6.8	7.0-7.5

Milk proteins have been studied in depth for well over 50 years and lot of studies were performed in order to analyse the various milk protein components, in various milk from different mammals. However, many questions concerning milk protein expression, structure and protein modifications remain still not completely covered such as some details of protein modifications due to disease and processing. The milk proteome is extremely complex because of the presence of post-translational modifications, alternative splicing and different genetic variants. The post-translational modifications are: glycosylation, phosphorylation, disulphide bonds formation and proteolysis, they create a large number of different protein variants from a single gene product. In milk the molecular composition of proteins is very important since it influences the functional properties of milk proteins such as solubility, clotting aptitude, thermal denaturation and the nutritional properties of the milk. Usually, the analytical procedures used for structural analysis of milk proteins are based on chromatographic techniques (ionic exchange, reversed phase chromatography, size-exclusion chromatography) followed by one-dimensional electrophoretic techniques (PAGE, Urea-PAGE and SDS-PAGE), or more efficiently on bi-dimensional electrophoresis technique (2-DE). Recent clinical evidence has renewed the interest in donkey milk because of high tolerability in infants

with cows' milk protein allergy. To be successful as a substitute for human milk in infant nutrition, donkey milk must be capable of performing many biological functions associated with human milk. The specific protein fraction in donkey milk can be good indicators of its potential role. From the proteomic map the presence of two isoforms of α -lactalbumin, three isoforms of β -lactoglobulin, lysozyme, albumin and lactoferrin was revealed. The high lysozyme and α -lactalbumin content found in donkey milk may be responsible for the low bacterial count reported in literature. Lysozyme, lactoperoxidase and lactoferrin have been recognized as antimicrobial and bacteriostatic agents. Their action may extend the conservation of fresh donkey milk and the relative potential commercial supply. In the past it has been widely used to replace human milk because its chemical composition and particularly protein content are close to that of human. Great attention must be obviously given to the hygienic characteristics of donkey milk production.

Therapeutic properties of donkey milk

Cows are the primary dairy animal species throughout the world because of the abundance of their lacteal secretion, needed to satisfy the demand of milk and dairy products for human nutrition. However, cow milk is not suitable for the infants affected by cow milk protein allergies (CMPA), the prevalence for which was estimated between 5% and 15%, including infants who show symptoms related to adverse reactions to cow milk protein (Vandenplas *et al.* 2007). Donkey milk has been successfully used in clinical studies, in children with cow's milk protein allergy (CMPA), and has good palatability (Monti *et al.*, 2007; DelloIacono and Limongelli, 2010). Its composition is more similar to human milk than ruminant milk, however it is poor in lipids, and adequate lipid integration is needed for a toddler's diet (D'Auria *et al.*, 2011). Recently, the potential role of donkey milk has also been of increasing interest in the prevention of atherosclerosis and cardiovascular diseases (Tafaro *et al.*, 2007).

The good tolerance of donkey milk in children suffering from CMPA (Monti *et al.*, 2007) could thus be due to the levels of its major allergenic milk components, in fact its low casein content and casein:whey protein ratio play an important role in the sensitization capacity of the milk (Lara-Villoslada *et al.*, 2005). Other factors may also help to explain the good tolerability of donkey milk, for example the number of casein fractions, the primary structure of the milk proteins, and the differences in digestibility of potential milk allergens, factors

which have not yet been analyzed in depth (Salimei and Fantuz 2012). In addition, the lactose content represents a substrate for the development of intestinal microbiota with health-promoting properties (Coppola *et al.*, 2002).

In fact, milk contains a variety of bioactive compounds with special properties associated with the development, growth and survival of infants beyond those provided by nutrition alone (Schanbacher *et al.*, 1998). The major antimicrobial proteins in milk are immunoglobulins, lactoferrin, lactoperoxidase and lysozyme (Tanaka 2007). The protein, α -lactalbumin has a property of antiviral, antitumor and anti-stress. Cow's milk represents the most common feeding during the infant weaning and widely used as a substitute for human milk, but the cow's milk is one of the most common food allergies in children, it can lead to an abnormal immunological response (Criscione *et al.*, 2009). Cow milk protein allergy (CMPA) is the most common food allergy in early child hood; affecting 2 to 5% of the child population with less than three years of age (Huang and Kim 2012).

Cosmetic properties of donkey milk

The use of donkey milk as a moisturizer in cosmetics is more fabulous. In cosmetics, donkey's milk is used for its cleaning and hydrating action combined with an antioxidant action that prevents the aging. In fact, the fat content in donkey's milk nourishes the skin and gives it softness. Cleopatra and other privileged ladies of ancient times were taking their bath in donkey milk in order to maintain their skin fresh, lenitive and shiny. Nowadays, so-called therapeutic and cosmetic properties of donkey milk seem to be validated by many trials (Brumini *et al.*, 2016). Donkey milk is rich in vitamins and polyunsaturated fatty acids (Aspri *et al.*, 2016) and contains anti-ageing, anti-oxidant and regenerating compounds, which are described as naturally active in skin hydration and skin ageing prevention. The lipid fraction is characterized by high levels of essential fatty acids and low saturated fatty acids (Gastaldi *et al.*, 2010). Compared with ruminants' milk, the considerable presence of unsaturated fatty acids found in donkey milk makes it very useful in the prevention of the cardiovascular, auto-immune and inflammatory diseases (Martemucci and D'Alessandro 2012, Martini *et al.*, 2014). In addition, donkey milk could act therapeutically in numerous cases, such as liver problems, infectious diseases, fevers, asthma, etc. (Hippocrates 1843, Pliny the Elder 1893).

Agri-entrepreneurship through donkey dairy farming

It has been observed that donkey milk has tremendous medicinal value as curative agent for metabolic and allergic diseases along with its use in cosmetics. In some part of the country especially the southern regions, donkey milk is sold for medicinal uses. The donkey milk fetch a good amount of money for donkey milk providers and is in fair demand. Donkey milk based cosmetics and health mixtures can be an attractive agribusiness for equine framers in India. This new possibility of increasing the income of the equine owners is very widespread among general public.

Since time immemorial the donkey milk was known for its unique therapeutic values. However, as cow's milk became a more popular choice for the masses due to high production and breeding policies, the values of donkey's milk was forgotten with time. But, economic gains from donkey milk are still on air, it is the costliest milk around the world fetching around 2000 to 5000/- per liter according to the demand. Not only this now- a-days the cosmetic properties of donkey milk are gaining momentum. One Agri- entrepreneur from Kerala, Mr Aby Baby decided to embark on a journey to bring back the lost glory of donkey's milk. Mr Aby Baby is now a man with a donkey farm. Aby Baby's one-of- its-kind donkey farm at Ramamangalam is the source of donkey milk used in a range of natural wellness and skincare products manufactured by him and getting favorable economic gain. Mr Aby Baby is finally finding joy in his venture after receiving positive feedback from consumers. Similary, Ms Pooja Kaul a young entrepreneur and founder of "Organiko" is leading her firm Organiko for making Donkey milk Soaps in India.

Future perspectives

While the donkey was shunned aside for its 'asinine' values, its milk was venerated as the elixir of life. With increasing numbers of research reports worldwide, donkey milk is now being taken as "gold mine" for future. In contrast to the affluent Western societies in India donkey serves for the livelihood of the landless, small and marginal farmers. Maximum donkeys are found with nomads and labor at brick kilns who don't know how to gain economic benefit from the animal except as pack and load carrying animal. If these donkey owners and marginal donkey farmers made aware of the benefits and the products of donkey milk, this can be a great financial boon to them. The future prospects are really high by proper breeding, milking and product marketing.

REFERENCES

From NRCE

- Bhardwaj, A., Pal, Y., Legha, R. A., Sharma, P., Nayan, V., Kumar, S., ... & Tripathi, B. Donkey milk composition and its therapeutic applications. *Indian Journal of Animal Sciences*, 90, 6.
- Bhardwaj, A., Kumari, P., Nayan, V., Legha, R. A., Gautam, U., Pal, Y., ... & Tripathi, B. N. (2019). Estimation of antioxidant potential of indigenous Halari and French Poitu Donkey milk by using the total antioxidant capacity and ferric reducing antioxidant power assay. *Asian Journal of Dairy and Food Research*, (4), 307-310.
- Garhwal R, Mehra R, Sangwan K, Bhardwaj A, Pal Y, Kumar H. A systematic review of the bioactive components, nutritional qualities, and potential therapeutic applications of donkey milk . Accepted in *Journal of Equine Veterinary Science* 2022
- Pal Y, Kumar S, Mohanty A K and Bhardwaj A. 2016. *Annual Report 2015-2016*, ICAR-National Research Centre on Equines, Hisar: 31-32
- Pal Y, Legha RA, Bhardwaj A, Tripathi BN. Status and conservation of equine biodiversity in India *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*. 2020, Volume: 41, Issue: 2 pp- 174-184.
- Pal Y, Legha R A, Kumar S, Bhardwaj A and Tripathi BN. 2018. Composition of Equine milk in comparison to different milk species. XV Annual Convention of Society for Conservation of Domestic Animal Biodiversity (SOCDAB) and National Symposium on “Sustainable Management of Livestock and Poultry Diversity for enhancing the Farmers’ Income” February 8-10, 2018, RAJUVAS, Bikaner, Rajasthan: 234.
- Gupta, A. K., Kumar, S., Pal, Y., Bhardwaj, A., Chauhan, M., & Kumar, B. 2018. Genetic Diversity and Structure Analysis of Donkey Population Clusters in Different Indian Agro-climatic Regions.

Bibliography

- Alabiso A, Giosuè C, Alicata M L, Mazza F and Iannolino G. 2009. The effects of different milking intervals and milking times per d'ay in jennet milk production. *Animal* 3: 543-47.
- Akan, E. (2021). An evaluation of the in vitro antioxidant and antidiabetic potentials of camel and donkey milk peptides released from casein and whey proteins. *Journal of Food Science and Technology*, 58(10), 3743–3751.

- Aspri M, Economou N and Papademas P. 2016. Donkey milk: An overview on functionality, technology, and future prospects. *Food Reviews International* 33(3), 316-333.
- Bordonaro S, Dimauro C, Criscione A, Marletta D and Macciotta NPP. 2013. The mathematical modeling of the lactation curve for dairy traits of the donkey (*Equus asinus*). *Journal of Dairy Science* 96: 4005-14.
- Brumini D, Criscione A, Bordonaro S, Vegarud GE and Marletta D. 2016. Whey proteins and their antimicrobial properties in donkey milk: a brief review. *Dairy Science and Technology* 96:1–14.
- Carminati D, Tidona F, Fornasari ME, Rossetti L, Meucci A and Giraffa G. 2014. Biotyping of cultivable lactic acid bacteria isolated from donkey milk. [*Letters in Applied Microbiology*](#), 59: 299-05.
- Cavallarin L, Giribaldi M, Soto-Del Rio, MD, Valle E, Barbarino G, Gennero M S and Civera T. 2015. A survey on the milk chemical and microbiological quality in dairy donkey farms located in North Western Italy. *Food Control* 50: 230-35.
- Claeys WL, Verraes C, Cardoen S, De Block J, Huyghebaert A, Raes K, Dewettinck K and Herman L. 2014. Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. *Food Control* 42: 188-201.
- Coppola R, Salimei E, Sorrentino E, Nanni M, Succi M, Belli Blanes R and Grazia L. 2001. Latte d'asina: unsuitable substrate for the preparation of probiotic beverages. In Proc. 36th Simp. *Internazionale Zootecnica*, Ancona, Italy, 57-61.
- Coppola R, Salimei E, Succi M, Sorrentino E, Nanni M, Ranieri P, Belli Blanes R and Grazia L. 2002. Behaviour of *Lactobacillus rhamnosus* strains in ass's milk. *Annals of Microbiology* 52:55-60.
- Cosenza G, Mauriello R, Garro G, Auzino B, Iannaccone M, Costanzo A, Chianese L and Pauciullo A. 2019. Casein composition and differential translational efficiency of casein transcripts in donkey's milk. *Journal of Dairy Research*, 1-7.
- Criscione A, Cunsolo V, Bordonaro S, Guastella AM, Saletti R, Zuccaro A, D'Urso G and Marletta D. 2009. Donkey milk protein fraction investigated by electrophoretic methods and mass spectrometry analysis. *International Dairy Journal* 19: 190-97.
- D'Auria E, Mandelli M, Ballista P, Di Dio F and Giovannini M. 2011. Growth impairment and nutritional deficiencies in a cow's milk-allergic infant fed by unmodified donkey's milk. *Case Reports in Pediatrics*, 1-4.

- DelloIacono I and Limongelli M G. 2010. Impiego del latte di asina nel bambino con allergia alle proteine del latte vaccino: nuovi contributi. *Rivista di Immunologia e Allergologia Pediatrica* 4:10-15.
- Fantuz F, Ferraro S, Todini L, Piloni R, Mariani P and Salimei E. 2012. Donkey milk concentration of calcium, phosphorus, potassium, sodium and magnesium. *International Dairy Journal* 24(2): 143-145.
- Gastaldi D, Bertino E, Monti G, Baro C, Fabris C, Lezo A and Conti A. 2010. Donkey's milk detailed lipid composition. *Frontiers in Bioscience*, E2: 537–546.
- Guo HY, Pang K, Zhang XY, Zhao L, Chen S W, Dong ML and Ren FZ. 2007. Composition, physiochemical properties, nitrogen fraction distribution and amino acid profile of donkey milk. *Journal of Dairy Science* 90:1635-1643.
- Hippocrates, Francis Adams (trans.). *The Genuine Work of Hippocrates*, Vol. 1. Sydenham Society; 1843.
- Huang F and Kim JS. 2012. IgE-mediated cow's milk allergy in children. *Pediatric Allergy and Immunology* 12(6):630-640.
- Lara-Villoslada F, Olivares M and Xaus J. 2005. The balance between caseins and whey proteins in cow's milk determines its allergenicity. *Journal of Dairy Science* 88:1654-1660.
- Li, L., Liu, X., & Guo, H. (2017). The nutritional ingredients and antioxidant activity of donkey milk and donkey milk powder. *Food Science and Biotechnology*. <https://doi.org/10.1007/s10068-017-0264-2>
- Martemucci G and D'Alessandro AG. 2012. Fat content, energy value and fatty acid profile of donkey milk during lactation and implications for human nutrition. *Lipids in Health and Disease* 11: 113-127.
- Martini M, Altomonte I and Salari F. 2014. Amiata Donkeys: Fat Globule Characteristics, Milk Gross Composition and Fatty Acids. *Italian Journal of Animal Science* 13(1): 3118.
- Martini M, Altomonte I and Salari F. 2013a. Evaluation of the fatty acid profile from the core and membrane of fat globules in ewe's milk during lactation. *Lebensmittel-Wissenschaft Technologie* 50:253-258.
- Medhammar, E., Wijesinha-Bettoni, R., Stadlmayr, B., Nilsson, E., Charrondiere, U. R. and Burlingame, B. (2012). Composition of milk from minor dairy animals and buffalo breeds: A biodiversity Perspective. *Journal of the Science of Food and Agriculture*, vol. 92, no. 3, pp. 445–474.

- Michalski MC, Briard V, Desage M and Geloën A. 2005a. The dispersion state of milk fat influences triglyceride metabolism in the rat - a ^{13}C breath test study. *European Journal of Nutrition* 44:436-444.
- Monti G, Bertino E, Muratore MC, Coscia A, Cresi F, Silvestro L, Fabris C, Fortunato D, Giuffrida M G and Conti A. 2007. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an in vivo and in vitro study. *Pediatric Allergy and Immunology* 18:258-264.
- O'Mahony J A and Fox PF. 2014. Milk: an overview (Chapter 2). In: Boland M., Sing, H and Thompson A. (Eds.), *Milk Proteins: From Expression to Food*, second ed. Elsevier, Oxford, UK, 19-73.
- Pliny the Elder, *Naturalis Historia*. Second volume, book XXVIII. Bostock J and Riley HT (trans.); Ed: H.G. Bohn; 1893.
- Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I and Chilliard Y. 2008. Composition of goat and sheep milk products: an update. *Small Ruminant Research* 79:57-72.
- Saarela T, Kokkonen J and Koivisto M. 2005. Macronutrient and energy contents of human milk fractions during the first six months of lactation. *Acta Paediatrica* 94:1176-1181.
- Salerno M, Paterlini F, Martino PA. 2011. Microbiologia e attività battericida del latte di asina. In: Milonis, E., Polidori, P. (Eds.), *Latte di asina: produzione, caratteristiche e gestione dell'azienda asinina. Fondazione Iniziative Zooprofilattiche e Zootecniche*, Brescia, Italy. ISBN: 978-88-904416-6-0: 193-205.
- Salimei E and Fantuz F. 2012. Equid milk for human consumption. *International Dairy Journal* 24:130-142.
- Šarić L, Šarić BM, Mandić AI, Torbica AM, Tomić JM, Cvetković DD and Okanović DG. 2012. Antibacterial properties of domestic Balkan donkeys' milk. *International Dairy Journal* 25:142-146.
- Schaafsma G. 2003. Nutritional significance of lactose and lactose derivatives. In *Encyclopaedia of Dairy Science*, Roginski, Fuquay & Fox, eds., London 3:1529-1533.
- Schanbacher FL, Talhouk RS, Murray FA, Gherman L I and Willett LB. (1998). Milk-borne bioactive peptides. *International Dairy Journal* 5(6):341-598.
- Shekar CP, Goel S, Rani SDS, Sarathi DP, Alex JL, Singh S. 2006. k-Casein-deficient mice fail to lactate. In *Proceedings of the National Academy of Sciences*, USA 103:8000-8005.

- Tafaro A, Magrone T, Jirillo F, Martemucci G, D'Alessandro AG, Amati L and Jirillo E. 2007. Immunological properties of donkey's milk: its potential use in the prevention of atherosclerosis. *Current Pharmaceutical Design* 13:3711-3717.
- Tanaka T. 2007. Antimicrobial activity of lactoferrin and lactoperoxidase in milk. *Dietary proteins research trends*. Nova Science Publishers. New York: 101-115.
- Tozzi, B., Liponi, G.B., Meucci, V. et al., (2016). Aflatoxins M1 and M2 in the milk of donkeys fed with naturally contaminated diet. *Dairy Science and Technology*, vol. 96, no. 4, pp. 513–523.
- Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje AP, Brueton M, Staiano A and Dupont C. 2007. Guidelines for the diagnosis and management of cow's milk protein allergy in infants. *Archives of Disease in Childhood*, 92: 902-8.
- Wochner, K. F., Becker-Algeri, T.A., Colla, E., Badiale-Furlong, E., and Drunkler, D. A. (2018). The action of probiotic microorganisms on chemical contaminants in milk. *Critical Reviews in Microbiology*, vol. 44, no. 1, pp. 112–123.
- Zhang X, Zhao L, Jiang L, Dong M and Ren F. 2008. The antimicrobial activity of donkey milk and its microflora changes during storage. *Food Control* 19:1191–1195.
- Zicker SC and Lönnerdal B. 1994. Protein and nitrogen composition of equine (*Equus caballus*) milk during early lactation. *Comparative Biochemistry and Physiology* 108A:411-421.

STATUS AND CONSERVATION OF DONKEY GENETIC RESOURCES

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Status of Equine Genetic Resources: World

There are 59.99 m horses in the world, which are found in nearly 134 countries. Most horses are found in the United States, followed by Mexico, China, Brazil, Mongolia, Argentina etc. India has 0.34 m horses and is ranked 28th in the world in holding the horse population. There are 52.96 m donkeys in the world, which are found in around 104 countries. Most asses are found in Africa. India has 0.25 m donkeys and is 25th in the world from the view point of population of the donkeys, while there are 7.91 m mules in the world, which are found in about 65 countries. Most mules are found in Mexico, followed by China, Brazil, Ethiopia and Morocco. India has 0.08 m mules and is 12th in the world from the perspective of mule population. (FAOSTAT data 2020)

The World donkey population is 52.96 m and the trend over previous years shows that it is increasing. The major share in the population of donkeys is of Africa, where 62.59% of the donkeys are found. This is followed by Asia, America, Europe and Oceania. The population trend analysis shows that the population of donkeys is decreasing in all continents except Africa. The details have been presented in Table 1.

Table 1. Distribution of donkeys: Continents

Regions	Population	Share	Trend
World	52961925	-	Increasing
Africa	33148206	62.59%	Increasing
Americas	6665687	12.59%	Decreasing
Asia	13028473	24.60%	Decreasing
Europe	110665	0.21%	Decreasing

Oceania	8894	0.02%	Decreasing
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The country-wise distribution of donkey population shows that the Ethiopia alone has 20.4% of the world's donkey population. Next is Sudan with 14.4 % population. Thus, these two African countries constitute about 34,8% of the donkeys' population of the world. Further details have been presented in Table 2.

Table 2. Distribution of donkeys: Countries

Rank	Country	Population	Share in World Population
1	Ethiopia	10791896	20.4%
2	Sudan	7631699	14.4%
3	Pakistan	5517000	10.4%
4	Chad	3860282	7.3%
5	Mexico	3292267	6.2%
6	China, mainland	2324000	4.4%
25	India	253500	0.5%

Evolution and domestication

If we look at the evolution of the species, it dates back to about 52 million years, when there was little to no ice at the poles. During that time a species appeared, called the *Eohippus*. It is believed that this is the first genus that later developed into the *equus* genus, which contains horses and donkeys. The origin of the present day asses is purely African and the domestication took place in the Neel Valley about ~5000 to ~6000 years ago. It is believed that today's donkey breeds are based off Nubian and Somali donkeys. Even today, the wild and feral population of the donkeys exists in the world. The details are as under: -

Wild Donkeys: -

- Kiang (*Equus asinus kiang*): India and Nepal.
- Onager (*Equus onager*): Iran, Syria and North Saudi Arabia.
- Kulan (*Equus hemionus*): Central Asia
- Indian Wild Ass (*Equus hemionus khur*) : Gujarat

Feral Donkeys: -

- Burro (*Equus africanus asinus*) : North America
- Asinara donkey: Italy,
- Sahara Ass (*Equus asinus atlanticus*): It is already extinct.
- Somali Ass (*Equus asinus somaliensis*): Somalia, Ethiopia and parts of Kenya.
Closely related to domestic donkeys.
- Nubian Ass (*Equus asinus africanus*): Egypt, Sudan, Eritrea and part of Ethiopia.
Equal contributors to gene pool of the domesticated donkey

It is believed that today's donkey breeds are based off Nubian and Somali donkeys, but with the passage of time and requirement, several breeds of donkeys have evolved. As per Domestic Animal Diversity Information System (DAD-IS) of Food and Agriculture Organisation (FAO), 2006, there are 185 breeds of donkeys in the world. Africa has 26, Asia and Pacific region has 32, Europe and Caucasus region has 51, Latin America and the Caribbean has 24, Near and Middle east region has 47 and US and Canada have 5 breeds

Status of Equine Genetic Resources of India

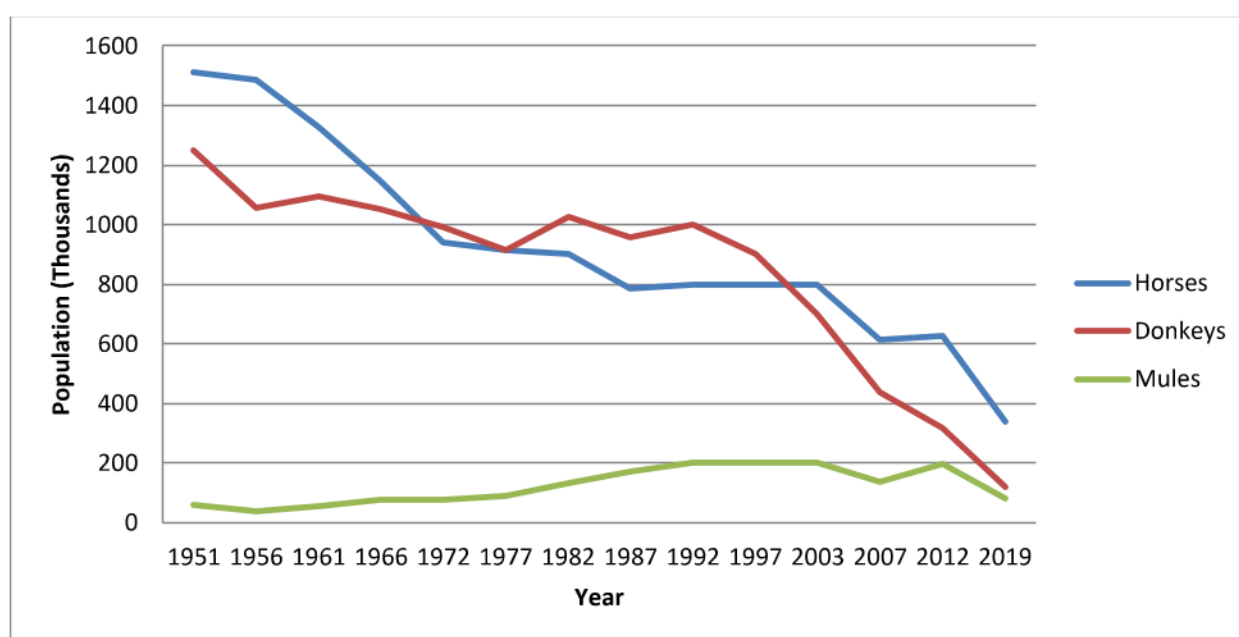
The equine population of the country is dwindling at a very fast rate. The 19th and 20th Livestock Census (2012-2019) data reveals that the population of horses, donkeys and Mules in the country has gone down by 45.58%, 61.23% and 57.09% respectively. Collectively this amounts to 52.71% reduction in the population of equines (Table 3, Fig. 1).

Table 3 : Population of Equines in the Country (in thousands)

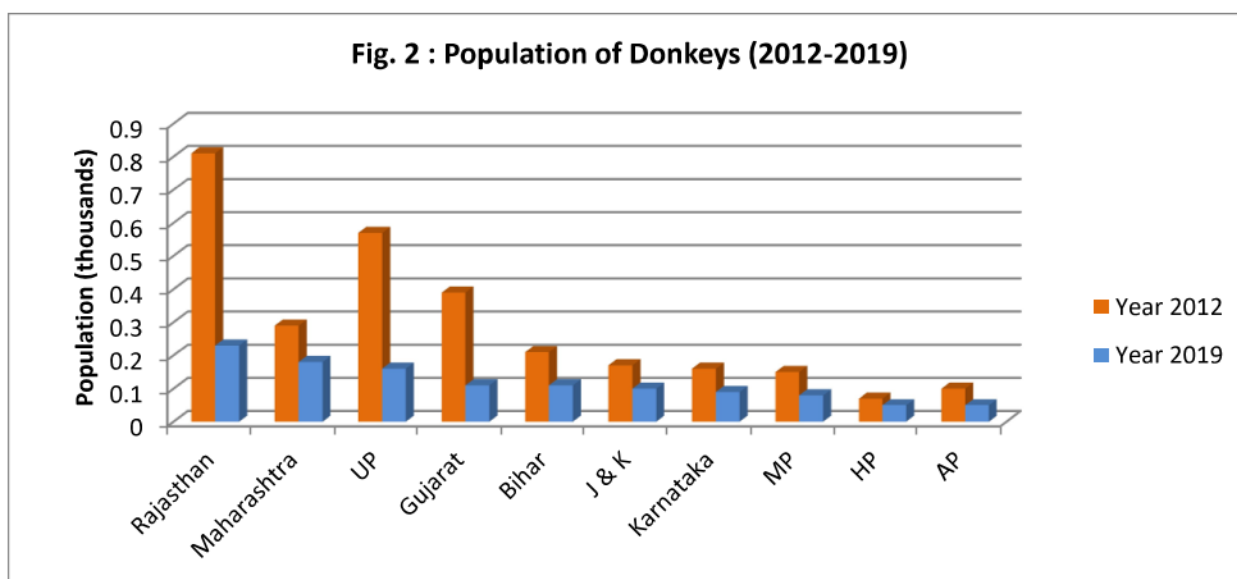
Year	Horses and ponies	Donkeys	Mules
1951	1514	1249	61
1956	1484	1057	39
1961	1327	1096	53
1966	1148	1054	75
1972	942	994	75
1977	916	917	89

1982	900	1024	131
1987	784	960	169
1992	800	1000	200
1997	800	900	200
2003	800	700	200
2007	612	438	137
2012	625	319	198
2019	340 (-45.58%)	120 (-61.23%)	80 (-57.09%)

Fig 1. Equine Population Trend in India (Livestock Census 1951 -2019)



The states like Uttar Pradesh, Jammu & Kashmir, Rajasthan, Bihar, Gujarat, Maharashtra and Punjab are among top states having sizable number of horses and ponies. The donkeys are most numerous in Rajasthan, Maharashtra, UP, Gujarat and Bihar states. The Mules are most numerous in the states like Uttarakhand, Himachal Pradesh, J & K, Uttar Pradesh and Madhya Pradesh. The 20th livestock census data shows that the population of donkeys have reduced significantly in Rajasthan, UP and Gujarat (Fig.2)



Indian Donkey Breeds

The origin of the asses is purely African and it is domesticated in the Neel Valley. Today's asses are considered related to Nubian species. According to the World Food Organization, Indian asses, Indian wild asses and kiang asses are found in India. Indian asses are found throughout the country while Indian wild asses are found in Kutch region of Gujarat and Kiang asses are found in Sikkim and Ladakh. More recently, the Indian Agricultural Research Council and other institutions have worked on donkey breeds and hope that extended information on the species of Indian donkeys will soon be available. Recently, Halari and Kachchhi donkey of Gujarat have been recognized as a separate breed of donkeys. However, as per Domestic Animal Diversity Information System (DAD-IS) of FAO, the listed breeds are Halari, Indian Donkey, Indian Wild Ass, Kachchhi, Kiang and Spiti.

SPITI DONKEY

Habitat: Trans-Himalayan regions of Spiti and Yangthang in Lahaul- Spiti and Kinnour districts of Himachal Pradesh.

Characteristics: Spiti donkeys are comparatively smaller in size, strongly built with compact bodies, straight backs and strong legs. The body is normally covered with a thick coat of long hairs. The coat colour is predominantly brown but it varies from dark brown to light brown with lighter or whitish bellies. White markings were observed around muzzle (68%) and eyes (12%). The dorsal cross was visible only in lighter shades. Head is comparatively shorter and broader. The face length is around 32 cm. The nasal bone is straight to slightly convex. The

ears are comparatively smaller, straight, pointing upwards with slightly lateral orientation. The head is supported by strong neck having mean lengths of 31.1 ± 2.21 (male) and 30.15 ± 1.52 cm (female). Height at withers of Spiti donkeys is around 89 cm. The tail extends up to hocks and the tail switch is not distinguishable due to presence of thick hairs on rest of the tail. The adult body weight of Spiti donkeys is around 75 Kg.

Uses: They are mainly used as pack animals for transportation of material. They are able to carry up to 100 kg of load in this rugged terrain with hypoxic conditions.

HALARI DONKEY

Habitat: Jamnagar, Dwarika and Rajkot districts of Gujarat.

Characteristics: The coat colour is predominantly white. The Halari donkeys are very docile. The ears are larger than any other breed of donkeys found in the country. The face is characterized by the presence of roman nose. The body size is large to massive. It is the largest breed of indigenous donkeys. The height at wither in adults is around 110 cm. The adult body weight of Halari donkeys is around 280 Kg.

Uses: They are mainly used as pack animals for transportation of material.

POITOU DONKEY

Habitat: Poitou regions of France.

Characteristics: The Poitou donkeys are one among the largest donkeys of the world and are known for production of large working mules. They have a shaggy coat of long and soft hairs called "*Cadanette*". The coat colour is always dark brown or black. They have white underbelly, nose and rings around its eyes. They have large ears, long heads, strong necks, long backs, short croups and round haunches. The limb joints and feet are large, and the legs are strong. The minimum height of Jacks is 140 cm and that of Jennies is 135 cm.

Use: They are used for production of large working mules for agricultural work, driving and riding.

Present Status

Before the development of ammunition weapons (fire arms) in the world, the horse was the most important from view point of the war, even the donkeys were used in wars for carrying the wounded soldiers and riding. The donkeys were used as pack animal in day-to-day life of the human beings. Even today, they are still being used in hills and other places for the transport

of man and material. However, invention of steam engine by James Watt in 1763 changed the entire scenario of transportation in the world. The animal driven carts were replaced by mechanized vehicles. Due to this, all draught species, e.g., horses, donkeys, bullocks, camels, mule etc. lost their utility. Still, the people in the world are versatile and they use most of the livestock species for everything that is possible, i.e., draught power, sport, meat, milk, skin, bone, urine, dung and other by-products. This is the main reason that the population of draught species is increasing globally, e.g., donkey, camel etc. Even now a days, in approximately 55 countries around the world, 7,72,829 tonnes of horse meat is produced. In eight countries 1,19,699 tonnes of donkey meat is produced (Table 4) and in two countries 12,087 tonnes of mule meat is produced (FAOSTAT data 2020). In the world, asses and mules are being used exclusively for working in difficult situations and traveling to inaccessible places.

Table 4 : Donkey meat production (tonnes per annum)

Rank	World-Country	Meat (tonnes)
	World	119699
1	China, mainland	97282
2	Niger	11567
3	Mali	3617
4	Senegal	3331
5	Mauritania	2604
6	Burkina Faso	1132
7	Argentina	162
8	Kazakhstan	4

Present Day Uses of Donkey

Looking at the distribution of donkeys in the world and the countries, it is very much evident that about 80% of the donkey population exists in underdeveloped countries. It is still being used as pack animals for day-to-day transportation of man and material. In difficult terrains, even today there is no alternate transport system available to poor populations. It is a zero-input animal. People use it and let it loose for eating grasses. The nomads in the country (India also) still use it to carry house hold material, ladies, kids, poultry, lambs etc. from one place to the other. In few pockets it is being used in agricultural operations, especially when the land

holdings are very small and the farmer is very poor. In Europe, it is also being reared as pet animal. Meat and milk is also being produced and consumed or sold.

Traditional Chinese medicine - Ejiao

The traditional Chinese medicine Ejiao (donkey-hide gelatin) is being produced by boiling the donkey's skin and refining the product into a tonic. It is claimed that Ejiao has the following biological activities: -

- Anti-fatigue
- Immunity improvement
- Tumor suppression
- Anti-anemia effect etc.

It is also being used to treat cases of bleeding, dizziness, insomnia and dry cough. The manufacture of Ejiao Bar by adding ejiao, nuts, sesame, Chinese dates and cooking wine is also being done.

Donkey Milk: Cosmetic and Therapeutic Uses

Looking at the use of donkey milk by the queen of ancient Egypt, Cleopatra to take care of her skin and preserve the beauty, the cosmetic properties of the donkey milk are being explored. Several products are being made and marketing is being done. It is said that the donkey milk is nutritious, it has anti-ageing and healing effect due to the kinds of essential Fatty acids it has, it is rich in antioxidants. It is also rich in Vitamins A, B1, B6, C, D, E; Amino acids, Omega 3, and 6 fatty acids. It is also being used as moisturizer and softener. Several milk products for day-to-day use are also being tried.

The Ragusano breed of donkey, which is found in Italy has been evaluated for its' milk production potential and it has been found that the milk produced during 6 am to 3 pm by two times milking was about 1.64 kg/day. The foals were allowed to suckle the dams after 3 pm and were allowed to be with dam till 6 am in the morning.

Conservation of Donkey Genetic Resources

As per Breed-wise Report of Livestock and Poultry (based on 20th Livestock Census) published by the Department of Animal Husbandry and Dairying, Spiti is the only breed of donkey, which has been counted for the purpose and the population of Spiti donkey in the country has been reported as 10235 heads. It appears that the figures need to be rechecked. The

sources indicates that the population of Halari donkeys is about 400 to 500 in the country. Looking at the reduction in the donkey population at the country level (-61.23%), most of the breeds of donkeys, irrespective of the fact that they have been described or not; may be expected to have declined at the same rate. Hence, it is perhaps the appropriate time, if not late, to have proper *In Situ* and *Ex Situ* programmes for the conservation and preservation of the breeds of donkeys. Under *Ex Situ* programmes, attempts should be made to characterize the breeds and populations at the earliest to reduce the proportion of non-descript animals to bare minimum. All recognized breeds should be preserved by establishing the Institutional Herds. Appropriate, improvement and propagation programmes be implemented. The cryopreservation of semen, somatic cells, DNA and embryos be done for future use.

In Situ conservation should also be focused upon. Breeders' Association to safe guard the interest of the breeders be formed. The social taboos, associated with donkey farming be addressed. The associations or FPOs or the Societies should address the issues of product development, marketing, skill development and should organize the events to promote the donkey farming.

Breeding Strategies

Whenever the number of heads of a particular breed or species is going down at such an alarming rate (-61.23% in 5 years), it is essential to maintain the pedigree information as well as all other records pertaining to that farm. Planned mating under supervision of qualified breeder should be initiated, selection criteria for rearing and improvement of the animals be well thought and implemented. Whenever, there is sudden reduction in the population, the bottleneck issues should be looked in to. The inbreeding should be avoided and the easiest way of sire rotation should be implemented. Further strategies can be prepared by consulting the respective scientist.

Conservation Avenues

Looking at the present-day trend of eco-tourism, the donkey farming can well be connected with the concept of eco-village. Some traditional games maybe suitably modified and introduced in such concept. The donkey fairs be organised with full zeal and enthusiasm. Several competition and handsome prizes be kept. The discovery of some biological compound or active principal for betterment of human life, from the in the milk or blood of the species may further support the conservation efforts of the biologists.

References

- Breed-wise report of livestock and Poultry (based on 20th Livestock Census, 2019). Published by Department of Animal Husbandry and Dairying, GoI, New Delhi.
- Livestock Census (2019). 20th Livestock Census. Published by Department of Animal Husbandry and Dairying, GoI, New Delhi.
- FAOSTAT data (2020). Food and Agriculture Organization of United Nations. Rome, Italy.
- D. Karatosidi, G. Marsico and S. Tarricone (2013). Modern Use of Donkeys. Iranian Journal of Applied Animal Science (2013) 3(1), 13-17
- Orhan Yılmaz, Saim Boztepe, Mehmet Ertugrul (2012). The Domesticated Donkey: II -types and breeds. Canadian Journal of Applied Sciences. 2(2): 260-266.
- Orhan Yılmaz, Saim Boztepe, Mehmet Ertugrul (2012). The Domesticated Donkey: I -Species Characteristics. Canadian Journal of Applied Sciences. 4(2): 339-353.
- Albano Beja-Pereira, Phillip R. England, Nuno Ferrand, Steve Jordan, Amel O. Bakhiet, Mohammed A. Abdalla, Marjan Mashkour, Jordi Jordana, Pierre Taberlet, Gordon Luikart (2004). African Origins of the Domestic Donkey. Science (304) 1781.
- Birgitta Kimura, Fiona B. Marshall, Shanyuan Chen, Sonia Rosenbom, Patricia D. Moehlman, Noreen Tuross, Richard C. Sabin, Joris Peters, Barbara Barich, Hagos Yohannes, Fanuel Kebede, Redae Teclai, Albano Beja-Pereira and Connie J. Mulligan (2010). Ancient DNA from Nubian and Somali wild ass provides insights into donkey ancestry and domestication. Proc. R. Soc. B (2011) 278, 50–57. doi:10.1098/rspb.2010.0708.
- Paolo Polidori, Daniela Beghelli, Pierluigi Mariani, Silvia Vincenzetti (2009). Donkey milk production: state of the art. Ital. J. Anim. Sci. vol. 8 (Suppl. 2), 677-683.
- Stine Rossel, Fiona Marshall, Joris Peters, Tom Pilgram, Matthew D. Adams and David O'Connor (2008). Domestication of the donkey: Timing, processes, and indicators. PNAS (105) 10 : 3715–3720.

COMMON HEALTH PROBLEMS IN DONKEYS

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Introduction

Traditionally donkey is reared by the very poor economic class of farmers in India. Even it is taken rarely to the hospitals for treatment or diagnosis of health problems. So exposure to the donkey diseases is very less to the veterinarians in our country. Even sufficient literature regarding donkey health problems in India is also lacking. Recent interests of new entrepreneurs on donkey milk is changing the pattern of donkey farming and now donkeys are being kept as a dairy animal for utilization of donkey milk. So for the new entrants in the donkey farming it is important to be introduced with the some common health problems of the donkeys which are described below.

1.0 Stunted growth, foot problems and lameness: Donkeys are being used as pack animals in urban areas especially by the contractors in infrastructure building sector where they start the use of donkeys at very early age. Donkey farmers do not use deworming, so donkeys have very heavy worm load. Female donkeys with foal at foot are given no extra care and nutrition. Due to malnutrition in mother donkeys, agalactia is very common. Donkeys have to take very heavy load through stairs upto top floors of very high buildings. Donkeys are kept in very small house in unhygienic conditions. Donkeys are not given proper ration especially there protein and mineral requirements are not fulfilled.

Prevention: There should be legal provision of lower age limit about donkeys to use in such type heavy work and these activities should be monitored by welfare agencies or related government departments. Contractors should be educated to provide at least maintenance ration to there donkeys balanced with protein and minerals. Donkeys should be kept in properly cleaned house.

2.0 Wounds and injuries: It is the most common problems in all the donkeys. Most of injuries occur due to fighting with each other and biting each others during this fighting. Dog bites wounds are also common. Due to ill fitting or inappropriate harness repeated injury is very

common finding. Donkeys are good healers if proper management is applied but in most of the cases donkeys are not rested, hospitalized or treated. Over work, old age and malnutrition often complicate and retard the healing.

3.0 Laminitis: Donkey owners often neglects mild, acute episodes of frequently occurring laminitis, it causes serious structural damage to the hoof. Like horses providing carbohydrates rich dietary source to the donkeys is the main cause of laminitis. Overlong hooves, uneven weight bearing (Long standing lameness) and generalised or systemic infections may also cause laminitis in donkeys.

Symptoms includes reluctance to move, preference for soft standing, alternate lifting of the forefeet, weight bearing on the heels. Pain may cause increased pulse and respiration rate. Increased volume and pressure to the pulse in digital arteries can also be felt.

4.0 Pedal Sepsis: Most common cause of lameness, donkey with severe non- weight bearing lameness, abscess track proximally from the white line at the bearing surface, eventually rupturing at the coronary band.

5.0 Puritis: It is characterised by severe itching and is often complicated by self trauma. Chronic mild puritis can cause scaling, hypertrophy and even lichenification of the skin. Parasitic infestation of the skin and hypersensitivity due to insect bite are the most common reasons. Puritis may also occur due to hepatic problems. Sometimes puritis is also a sign of rabies in donkeys. Infestation of the lice is also common in groups of closely managed donkeys. Both biting (*Damalina equi*) and sucking lice (*Haematopinus asini*) occur in donkeys. In both cases mild or intense pruritis may be seen or donkey may develop moth eaten appearance without pruritis. Where pruritis is present it is usually worse in warm weather. Opportunistic pathogens may develop on traumatised skin. Scaling can develop if the infestation is prolonged. Clinically significant anaemia may develop if severe infestations occur when *Haematopinus asini* is present. Infestation of mange also causes severe pruritis and alopecia in donkeys.

6.0 Habronemiasis: Habronemiasis is a parasitic disease of equids (horses, donkeys, mules and zebras) caused by the nematodes *Habronema musca*, *H. majus* and *Draschia microstoma*. The adult worms live on the wall of the stomach of the host without internal migration.

Embryonated eggs are excreted in the feces to the environment where they are ingested by the larvae of intermediate hosts, such as houseflies and stable flies. Most cases of gastric habronemiasis do not show clinical signs, however heavy worm infestation can result in gastric perforation. Development of the parasite is synchronous with the development of the intermediate host, and infectious larvae (L3) are deposited on the host when the flies are feeding. When a fly lands near the horse's lips, the L3 is liberated and is swallowed by the horse, resulting in completion of its life cycle. Larvae which are deposited on mucous membranes or on injured tissue will not complete their life cycle but will induce a local inflammatory reaction causing cutaneous ("summer sores") and/or ophthalmic habronemiasis. In donkeys the facial form and conjunctival form are most common forms of habronemiasis. Extensive, raw, superficial, ulcerated and eroded facial dermatitis can be found extending from the medial canthus of the eye to down face.

7.0 Hyperlipaemia: It is a life threatening disease of the donkeys, occurs when animals mobilise triglyceride from body fat reserves in response to a negative energy balance. The end result is malty organ failure as lipid is deposited in the liver and kidneys. Risk factors involves obesity, stress, older age, late pregnancy or early lactation, laminitis and concurrent diseases such as dental diseases, parasitism, colitis, laceration, impaction and endotoxemia. Absence of glucose in circulations leads to mobilization of body fat stores by hormone sensitive lipase. Failure of insulin to block the action of hormone sensitive lipase once the energy demand is met leads to increased levels of lipids in blood. Free fatty acids (FFA) released into the blood stream in quantity that exceeds the liver's pathways of oxidation, glucogenesis, and ketogenesis. The FFAs are re-estrified by the liver to form triglycerides and released back into the plasma as very low density lipoproteins (VLDLs). The circle will continue if the energy demand and insulin resistance persists, leading to impaired liver function. Clinical Signs includes dullness, anorexia, gut stasis, mucus stained dry fecal balls, halitosis, congested mucus membranes, ataxia, tachycardia, ventral oedema, hepatic encephalopathy, abdominal pain.

8.0 Colic: Dullness, lack of appetite, rolling and pawing at the ground, fast breathing and excessive sweating are the common signs of colic in the donkeys. Appearance of brick red colour of mucus membranes is a sign of poor prognosis. Sudden change in the diet, poor quality feed, too much rich grass and too much grain can cause colic in the donkeys. In some circumstances, housed donkeys eat their bedding, which can be a cause of colic. Eating strange

things such as polythene bags or pieces of rope they may get colic. Like horses, donkeys' teeth continue to grow throughout their lives. Sometimes teeth problems reduce the ability to properly chew food. Donkeys may eat sand especially when grazing on sandy soil with little grass. Lack of access to water can make donkeys more vulnerable to colic. In cold weather, when water becomes cold donkeys' water intake may be reduced. Migration in the body of larval stages of parasites or large numbers of adult worms in the guts can cause colic. Eating poisonous plants can also cause colic. Some donkeys do not deal with certain stressful situations as well as others and may show signs of colic. Impactions or blockages with partially digested food may also cause colic, most frequently in parts of the large intestine. Spasmodic colic appears to be a more common type of colic in horses, but it is rarer in the donkeys.

8.0 Umbilical infection: Umbilical infections are common in foals and can lead to septicaemia, Patent urachus and joint ill in foals.

9.0 *T. Evansi* infection: It is caused by *Trypanosoma evansi* a blood protozoon. It is transmitted by the flies. Intermittent fever up to 107°F, weakness and progressive thinness, tired easily and may stop eating, anaemia/ pale mucus membranes (gums, conjunctiva), swollen lymph nodes, limbs and belly with edema, sensitivity to light, dullness, dragging hind limbs, loss of balance, seizures, head pressing are the most common signs. It is important to realize that drugs alone will not cure trypanosomosis. Trypanosomes overwhelm the immune system of the host; they are immunosuppressive. Chemotherapy, by stopping the multiplication of the trypanosomes, helps the immune system to overcome the infection. Treatment will be more effective in a well-fed and rested animal, in which the immune system is not adversely affected by stress and lack of food. The drugs used must be used with a lot of caution and be injected deep intramuscularly due to tissue necrosis and toxicity. Quinapyramine sulfate: prior to its use the donkey must be well rested; fatigue can cause curare like reaction- 10%, 5 mg/kg body weight.

10.0 Theileriosis: Theileriosis is caused by an intra-erythrocytic protozoan parasites. This is a tick transmitted disease, whose vectors include Hyalomma, Dermacentor and Rhipicephalus ticks; intrauterine infection, particularly with *T. equi*, is also relatively common. The incubation period ranges from 5 - 21 days. The signs include fever, inappetence, increased respiratory rate, muscle tremors, anaemia, jaundice, depression, marked thirst, lacrimation, swelling of the

eyelids, weight loss; hemoglobinemia and hemoglobinuria occur in the final stages. Affected donkeys are constipated, passing small, hard ball of faeces covered with yellow mucus, may lose the health condition. Sometimes donkeys can show colic symptoms viz. looking at flank, pawing, kicking at belly region, lying down and rolling, due to sluggish intestinal peristaltic movements and constipation. The most characteristic sign is the development of icterus, mucus membranes vary from pale pink to pale yellow to bright yellow in color. Petechiae or ecchymosed haemorrhages are seen on the mucus membranes of nasal passages, vagina or third eyelids. Extremely large spleen in the affected animals is the common symptom. Untreated or neglected cases become severely anemic, malaise, reluctant to move, down neck, in appetite, disinterest in surroundings and show signs of general weakness. Development of progressive anemia and haemoglobinuria in the last clinical phase of the disease is pathognomonic sign in *T. equi* infection in horses and also in donkeys. Chronic cases are more common in donkeys and clinical signs are usually non specific including mild in appetite, poor work performance or poor body weight gain. Mixed infections are not uncommon therefore making diagnosis difficult. Clinically, theileriosis can be confused with other conditions that cause fever, anemia, hemolysis, jaundice, or red urine. Therefore, confirmation of a diagnosis by microscopic examination of Giemsa-stained blood or organ smears is essential. From the live animal, thick and thin blood smears should be prepared, preferably from capillaries in the ear or tail tip. If equine *T. equi* is diagnosed and treated early, there is an excellent chance of recovery. Imidocarb (Imizol®) is administered @ 4mg /kg is administered 4 times at 72-hour intervals.

11.0 Strangles: It is a bacterial disease caused by the *Streptococcus equi*. Early clinical signs include rise of temperature, watery discharge from the nose and coughing later phase cough disappears, difficulty of swallowing, thick creamy nasal discharge appears. Animal can be treated by providing rest and offering soft, moist food, and antibiotics .

12.0 Tetanus or lock jaw: It is caused by *Clostridium tetani*; it is associated with soil contamination of sores and wounds from ill-fitting harnesses and poor hygiene during surgery. Clinical signs include history of deep wound(s) which can already be healed completely. Due to muscle stiffness and locked jaw the donkey become unable to eat and swallow food, Pupils become dilated. Donkeys have frightened expression and the ears stickup. The disease can be prevented by complete treatment of wounds and cleanliness/ hygiene in surgical procedures. Donkeys should be vaccinated with Tetanus toxoid.

13.0 Glanders: This is a bacterial disease of zoonotic importance caused by *Burkholderia mallei* a gram negative aerobic . The disease affects the skin and lungs. Mules and donkeys die within two weeks. Fever, watery nasal discharge initial that becomes thicker in later stage. Lumps appear in the nostril and break open. After healing the lump leaves a star-shaped scar in the nostril. The burst lump also releases a sickly honey-like discharge.

14.0 Rabies: It is also an important viral disease of donkeys. Specially the donkeys which are being kept open usually get exposed to dog bite without the knowledge of owner. Depression, agitation, occasional colic, foamy salivation, jerking, teeth grinding, laboured breathing, sternal recumbency and self-mutilation are common signs of rabies. There is no treatment of rabies. So there is need of annual vaccination to control the incidences of rabies in donkeys.

VALUE ADDED DONKEY MILK BASED PRODUCTS

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1.0 Introduction

Milk and dairy products are integral part of our daily diet and important items for all occasions. Cow milk and products based on it like curd, buttermilk, *ghee* and *makhhan* are considered as sacred items in old Vedic literatures. In our country livestock farming has contributed immensely in ensuring the livelihood, food and nutritional security. Milk alone contributes more than wheat and rice in value terms in agrarian economy. Milk and milk products are third largest component in Indian food basket and their share has increased to about 22.5 percent in recent years. Livestock are also deployed for draught purpose especially in areas where farmers have small land holdings and mechanization is not possible due to undulated terrains. With the emergence of functional foods and nutraceuticals in global and domestic market, the attention is shifted to milk-based products because of the great diversity of nutrients present in it. Milk production in India is growing at the rate of about 5.6% per annum which is much higher than the global milk production growth rate. Besides being the largest milk producers and consumer, our country is also blessed with excellent biodiversity mainly in livestock. As per FAO, there are 61 breeds of cattle, 19 of buffalo, 59 of sheep, 34 of goat, 3 of pig, 6 of horse, 3 of donkey, 8 of camel and poultry breeds in India. Although, the share of minor milch species i.e., goat, sheep, camel etc. is only about 4 percent in national milk pool; their chemical make-up and richness in certain bioactive have attracted the attention of stakeholders in exploring the nutritional and therapeutic properties of non-bovine milks.

According to 19th livestock census survey, there are 1.12 lakhs donkeys in India that showed nearly 70 percent decline in donkey population decreased since last census (DAHDF, 2019). As per Food and Agriculture Organization (FAO), India has three types of donkeys: Indian, Indian wild, and Kiang. The Rann of Kutch located in Gujarat is home to two big breeds of Indian wild donkeys, while Sikkim and Ladakh are home to Kiangs. In recent past there is growing interest in understanding the nutritional, health promoting properties of donkey milk and products based on it.

2.0 Nutrition and Health Properties of Donkey Milk

Donkey milk is the lacteal secretion obtained from the milking and healthy mammary gland of a domesticated donkey (jenny milk). Milk used in human nutrition, for the preparation cosmetics items and meat production is examples of food applications which is limited certain ethnic populations in some countries. Donkey milk is gaining popularity because of the emergence of literatures related to its health-promoting properties. Great diversity of nutrients, richness of certain nutraceutical components and functional properties; donkey milk is now referred as "pharma food." Donkey milk's high lactose content which impart it a pleasant sweet taste and promotes calcium absorption in the intestine. Higher calcium bioavailability is essential for infant bone mineralization. Donkey milk is ideal choice for infants allergenic to cow milk because its protein profile similarity to that of human milk. The three major whey proteins in donkey milk are: α -lactalbumin, β -lactoglobulin, and lysozyme. It has recently been discovered that α - lactalbumin has antiviral, anticancer, and anti-stress effects. Donkey milk has a higher degree of resemblance to human milk especially long chain polyunsaturated fatty acids which is about 15-20 percent of the total fatty acids. PUFA containing a high proportion of linoleic acid and a lower ω -6 to ω -3 ratios (LA/ALA ratio) because of above-mentioned properties, it was found that donkey milk can lower the risk of coronary heart disease, hypertension, thrombosis and lower the cholesterol level. Moreover, richness in Long Chain Polyunsaturated Fatty Acids (LCPUFA) further amplify the nutrition properties by providing it as ideal base for eicosanoid biosynthesis, which play important role in enhancing the immune inflammatory system.

Mineral content in donkey milk vary in the range of 0.3 to 0.9 g/100 g which is equivalent to human milk and greater than cow milk. The mineral composition of milk varies according to the breed, feed, stage and period of lactation. Major minerals present in higher concentration in milk include calcium, phosphorus, sodium, potassium, magnesium, iron, zinc, and copper. Although the amount of various vitamins in donkey milk is yet to be determined but it contains substantially more vitamin C than cow milk. Except for niacin (vitamin B₃), donkey milk has more thiamine (vitamin B₁), riboflavin (vitamin B₂), cobalamin (B₁₂), and vitamin C than human milk. Low bacterial counts in donkey milk are due to the higher lysozyme (1.00 g/L) content, which plays an important role in combating various infections. Lysozyme level in donkey milk is lower than the human milk but several folds higher than bovine milk. This antimicrobial protein is quite effective against gram positive bacteria. Being a protein lysozyme is heat sensitive and thermal processing may reduce both its level and anti-microbial efficacy

against various microbial groups. Comparative evaluation of donkey milk with other mammalian species indicated that donkey milk has superior nutrient profile, greater diversity of bioactives, more digestible and similarity with human milk. There is need to harness the nutritional and health promoting virtues of donkey milk for human being. The presence of milk constituents in significant amounts such as immunoglobulins, lysozyme, lactoferrin, 3-fatty acids, bioactive peptides, and favourable casein: whey protein ratio is mainly attributed to (a) antimicrobial activities, (b) immunomodulation activities and (c) hypoallergenic. Donkey milk's high lysozyme content may be responsible for the low bacterial count recorded almost universally in the literature, as well as making it ideal for preventing or reducing gastrointestinal infections in infants. In cheese making, donkey milk has been used as an alternative to egg lysozyme to prevent spore-forming clostridia strains that cause late blowing in hard Italian cheeses.

3.0 Value Added Donkey Milk Based Products

Traditionally donkey milk is utilized for the preparation of cosmetics, drinking purpose and sporadically for the manufacture of certain artisanal cheeses. Raw (unprocessed) and heat-treated (pasteurized and ultrahigh temperature (UHT)) donkey milk is currently available. Donkey milk has been frozen, freeze-dried (lyophilized), and powdered, among other methods of processing.

There is increasing attention towards donkey milk because of its nutritional, nutraceutical, and functional properties so that it is considered as a functional food and raising interest in the development of donkey milk products. Cheese, ice cream, milk powder, fermented milk products, and other dairy products have all been made from donkey milk. The thermal stability of donkey milk is low. Even at low temperatures, such as 75°C for 10 minutes, sedimentation was observed. Donkey casein micelles are more vulnerable to heat treatment than whey protein due to their low colloidal stability and presence of a high calcium environment.

Cheese

Since donkey milk has low rennet clotting activity, cheese manufacturing using donkey milk is difficult. Moreover, low total solid content and lower proportions of caseins, especially α -S1-casein, resulted in a very weak gel and prevent the formation of a firm curd after renneting. The rennet curd has been reported to fragile. Donkey milk with a low-fat content may affect the texture and flavour of the cheese produced. Recent study carried out by using pure camel

chymosin as a clotting agent for donkey milk cheese production resulted in 3.32 percent yield of fresh cheese. After 12 hours, the fresh cheese had a total solids content of 35.65%. Niro *et al.* (2017) investigated the efficacy of donkey milk as a dietary supplement. In Italian Grana cheeses, a replacement for hen's egg white lysozyme in preventing cheese blowing. The microbiological and sensory quality of cow-donkey mixed milk was fine. The authors proposed using donkey's milk in hard cheese production as a new technical solution to prevent late-blowing defects.

Ice cream

Donkey milk was used to make strawberry flavoured ice cream, which was then mixed with two strains of lactic acid bacteria that were found to have probiotic properties. *Bifidobacterium adolescentis* ATCC15703 and *Lactobacillus plantarum* 998 with a fat and overrun of about 24.7 and 1.2 percent, the ice cream had a very low-fat content. There was a reduction in vitamin C content after 4 months of storage at -20°C. The total phenols and antioxidant potential of the ice cream, however, did not alter. Throughout the storage time, the viable probiotic bacteria count was over 9 log CFU/g.

Donkey milk powder

Donkey milk and donkey milk powder are good substitutes for cow milk and infant milk powder since they contain lower levels of fat and cholesterol, a good calcium-phosphorus ratio, and are resistant to oxidation. Among the milk of two breeds of donkeys, i.e. dwarf grey and largely white, it was noticed that dwarf grey breed donkey milk was better for powder processing. It was related to the higher particle size, protein and better nutrient composition i.e., lysozyme, Ca, B₃, cysteine, omega-3. Heat treatment of donkey milk at 85°C resulted in a greater loss of lysozyme and β -lactoglobulin. The levels of lysozyme and beta-lactoglobulin drop by 60% and 87 %, respectively. Heat treatments above 65°C resulted in a 100 percent loss of lactoferrin.

At temperatures less than 85°C, lysozyme was found in raw and heat-treated foods in equivalent amounts. The spray drying procedure considerably reduced lysozyme activity (58 percent residual activity) and lactoglobulin content (6.43 mg/mL in fresh milk vs 5.51 mg/mL in spray-dried milk). The high temperature used to process donkey milk reduced lysozyme activity and beta-lactoglobulin levels.

Fermented milk products

Carminati *et al.* (2014) isolated and characterized culturable and cultivable acidifying and thermotolerant LAB microbiota in donkey milk. *Lactobacillus plantarum* strains have been suggested as possible candidates for producing novel fermented milk from donkey milk, not only because of their probiotic potential but also because of their ability to improve the growth of *Streptococcus thermophilus*. The pH values of the fermented donkey kinds of milk were 2.91 ± 0.16 and 1.78 ± 0.66 . Lysozyme resistance was strong in both *S. thermophilus* and *L. plantarum*. Coppola *et al.* (2002), studied donkey milk as an excellent option for making probiotic beverages. They looked at how heat treatment affected the microbiological and biochemical consistency of ass's milk (Martina Franca breed) as well as the growth of four distinct *Lactobacillus rhamnosus* strains. *Lactobacillus* bacteria are commonly utilized to make probiotic beverages from equid milk, such as donkey and mare milk. *Koumiss* is fermented equid milk that has been utilized in Central Asia since antiquity. The lower protein content mainly casein and lack of adequate nutrient from microbial proliferation could be the reason for poor fermentation characteristics of donkey milk. Further experimentation are required in terms of increasing the total solids in donkey milk by supplementing goat or bovine milk, ultrafiltration, use of hydrocolloids and utilization of cross-linking enzymes.

4.0 Conclusion

Donkey milk has unique physico-chemical, sensory and microbial properties. The composition and characteristics of donkey milk vary with the stage of lactation, feed and also seasons. Similarity with human milk offers emerging opportunities for its utilization to develop specialized infant foods. Among the thermal processing, HTST pasteurization can be employed successfully for delivering microbiologically stable product with better functional properties. However, a systematic investigation is required for preparing acceptable quality fermented dairy products using donkey milk by employing novel processing and formulation interventions.

PACKAGING AND LABELLING OF MILK AND DAIRY PRODUCTS

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1.0 Introduction

Dairy products contain essential and non-essential nutrients required for the growth and development of human beings. They are highly perishable commodities and serves as a very good media for the growth of many spoilage causing microorganisms. Hence, there is an obvious need to preserve our precious food sources. Food packaging is an external means of preservation of food during storage, transportation and distribution. Hence, it forms an integral part of the product manufacturing process. Food packaging performs four major disparate functions such as containment, protection, convenience and communication. In pursuit of achieving these goals, many materials have been discovered by man for use as food packaging materials. They include wood and paper, glass, metals (such as tin, aluminium), plastics, composites, etc. The inherent properties of these packaging materials make them either highly suitable or unsuitable for a particular food product. Hence, a packaging material has to be judiciously chosen depending upon the nature of the product, availability, machinability, cost, etc. Further, the labelling is important in the current era of increased consumer awareness. In India, the FSSAI, New Delhi has released the FSSR (2020) labelling requirements for pre-packaged foods. In the present article, an overview of the materials suitable for dairy products packaging and the mandatory FSSR (2020) regulations have been presented.

2.0 Paper and Paper-Based Materials

Paper and packaging is a natural pair. Paper is the most important packaging material and packaging is an important use of paper. Paper is a generic term encompassing a broad spectrum of materials derived from cellulosic fibers. It is the oldest packaging material and the most versatile. Pulp is the fibrous raw material used for the production of paper and paper-based products such as paperboard, corrugated board, etc. The distinction between paper and paperboard is not always very clear, but paperboard is heavier and more rigid than paper. Usually, all paper sheets which are greater than 12 divisions thick when measured by a micrometer (300 μm or 1200 gauge or 0.012 inch) are classified as paperboard. Some important

paper and paper-based packaging materials used in dairy and food industry are described below:

2.1 Kraft Paper

Strength is one of the outstanding characteristics of Kraft paper and accounts for its wide use throughout the industry. Four of the more important grades of Kraft paper are: (a) grocer's bag or sack paper, (b) shipping sack paper, (c) wrapping paper, and (d) gumming and asphaltting paper. Kraft paper is used for bulk packaging of skimmed milk powder, whole milk powder, whey protein concentrates, whey protein isolates, etc. in dairy industry, usually with a plastic inner lining. Unbleached Kraft retains much of the natural colour of the wood pulp from which it was produced.

2.2 Greaseproof Paper

Greaseproof paper is a translucent machine-finished paper that has been hydrated to give oil and grease resistance. Prolonged beating or mechanical refining is used to fibrillate and break the cellulose fibers, which absorb so much water that they become superficially gelatinized and sticky. This physical phenomenon is called as hydration. Although, they are strictly not "greaseproof" since oils and fats will penetrate them after a certain interval of time, they are often used for packaging butter and similar fatty foods.

2.3 Vegetable Parchment

Vegetable parchment takes its name from its physical similarity to animal parchment, which is made from animal skins. It is free of lint, odour and taste, and resistant to grease and oils. Because of its grease resistance and wet strength, it strips away easily from food material without defibering, thus finding use as an interleaver between slices of food such as meat or pastry. It can be treated with mold inhibitors and used to wrap foods such as cheese.

2.4 Waxed Paper

Waxed papers provide a barrier against penetration of liquids and vapours. Many base papers are suitable for waxing, including greaseproof and glassine papers. The primary purpose of the wax is to provide a moisture barrier and a heat sealable laminant. Frequently, special resins or plastic polymers are added to the wax to improve adhesion and low temperature performance, and to prevent cracking as a result of folding and bending of the paper.

2.5 Folding cartons

Paper is generally termed board when its grammage exceeds 224 gm-2. Folding cartons are containers made from sheets of paperboard (typically with thickness between 300 and 1100 μm), which have been cut and scored for bending into desired shapes; they are delivered in a collapsed state for assembly at the packaging point. A number of steps are involved in converting paperboard into cartons. Where special barrier properties are required, coating and laminating are carried out. Folding cartons, for example, are used for packing bulk/family-sized ice cream packs. The most common packaging material used for UHT milk is the paperboard laminate carton.

2.6 Corrugated fibreboard

Corrugated board is manufactured from three basic sheets – two liner boards and a central corrugated sheet or medium (flute). These materials can be varied as to weight, type and number and/or height of the corrugations in the fluting medium. The properties of a corrugated board depend largely on the type, number and position of the corrugations. Mostly corrugated fibreboards are used as secondary or tertiary packaging in the distribution and marketing of dairy products (Fig. 1). Different types of corrugated fibreboards viz. (a) single face, (b) single wall, (c) double wall and (d) triple wall are shown in Fig.2.

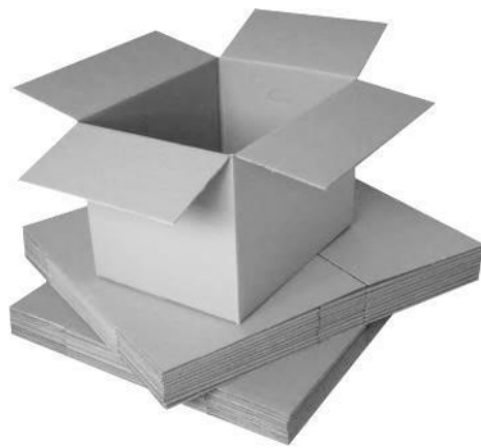


Fig.1. Corrugated Fibreboard

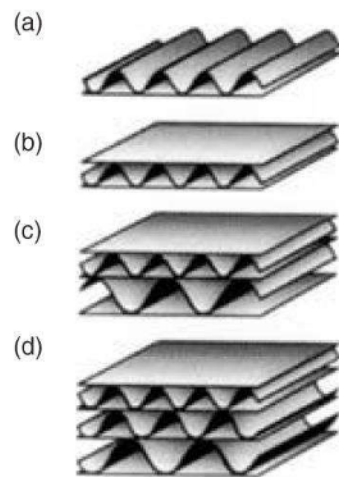


Fig.2 Types of corrugated fibre boards

3.0 Glass Packaging Material

Glass is substance that is hard, brittle and usually transparent. Soda-lime glass is made from limestone (about 10%), soda (about 15%) and silica (about 75%). Lesser percentages of aluminium, potassium and magnesium oxides may be included. When the components are melted together they fuse into a clear glass which can be Readily shaped while in a semi-molten

state. The melting of glass is usually accomplished at a temperature of about 1540°C in a very large furnace. For obtaining different coloured glasses various chemicals are added to the melt such as iron oxide for amber colour, cadmium compounds for yellow colour, etc. Glass containers such as jars, bottles, etc. are made by various methods such as (a) extrusion, cutting and shaping; (b) press and blow or blow and blow; and (c) pressing. Different types of closures are used for closing the containers viz. Simple wrapping without sealing, twist wraps, heat seals, threaded screw closures, etc. Milk for retail sale was traditionally packaged in refillable glass bottles. However, today, single-serve paperboard cartons and plastic containers of various compositions and constructions dominate the market. Presently, sterilized flavoured dairy drinks are marketed in glass bottles by some reputed dairy industries.

4.0 Metal Packaging Materials

Four metals are commonly used for the packaging of foods viz. steel, aluminium, tin and chromium. Tin and steel, and chromium and steel are used as composite materials in the form of tinplate and electrolytically chromium-coated steel, sometimes referred to as tin-free steel. Aluminium is used in the form of purified alloys containing small and carefully controlled amounts of magnesium and manganese.

4.1 Tin plate

The term tin plate refers to low ca material that has good strength, combined with excellent fabrication qualities such as ductility and drawability as well as good solderability, weldability, nontoxicity, lubricity, lacquerability and a corrosion-resistant surface of bright appearance. Tin plate is used for the manufacture of two-piece and three-piece cans (Fig.4). The traditional method for packaging milk powders for consumer's uses three-piece tinplate cans where the atmospheric air is withdrawn from the powder and replaced with an inert gas such as nitrogen prior to seaming the base onto the can.

4.2 Aluminium

Aluminium is the earth's most abundant metallic constituent, comprising 8.8% of the earth's crust, with only the non-metals oxygen and silicon being more abundant. Aluminium is used in the form of foil, tubes or retort pouches in the food industry. Aluminium foil is a thin-rolled sheet of alloyed aluminium varying in thickness from about 4 to 150 µm. It is essentially impermeable to gases and water vapour when it is thicker than 25.4 µm. it can be converted

into a wide range of shapes and products including semi-rigid containers with formed foil lids, caps and cap liners, composite cans, laminates containing plastic and sometimes paper or paperboard, etc. Aluminium foil has an important role as light and gas barrier in the 5 or 7 layered laminate used for packaging of UHT milk.

5.0 Plastic Packaging Materials

Plastic is derived from the Greek *plastikos*, meaning easily shaped or deformed. Food industry is a major user of plastic packaging materials. Plastics are usually divided into two broad categories: thermosetting and thermoplastic. Polyethylene, polystyrene, polypropylene, polyvinyl chloride, etc. are thermoplastic type of plastics. Mostly thermoplastics are being used for packaging of dairy products.

5.1 Polyolefins

Polyolefins form an important class of thermoplastics and include low, linear and high density polyethylenes (PE) and polypropylene (PP). Industry commonly divides polyethylenes into two broad categories based on their densities: high density polyethylene (HDPE) and low-density polyethylene (LDPE). The properties that make polyethylenefilm a popular packaging medium in dairy industry are its low price, nontoxic, excellent heat-sealing property, flexibility, pleasing appearance and softness, chemical inertness. Low-density polyethylene (LDPE) accounts for the biggest proportion of the plastics used in food packaging due to its versatility. LDPE film accounts for a little over 50% of total PE in this sector because it provides strength, gloss, flexibility, and the good clarity. It can be extruded into film, blown into bottles; injection molded into closures, extruded as a coating on paper, Al foil or cellulose film, and made into large tanks and other containers. It is easily heat sealable. LDPE film is used for packaging of milk, oil, salt etc. For an equal wall thickness, HDPE gives greater rigidity to bottles than LDPE. HDPE films have a cloudy appearance, occasionally utilized as a liner for the bulk packaging of skim milk powder due to their high strength and barrier properties. Furthermore, HDPE is the most commonly used film as carton liner. However, polyethylene is not suitable for packing foods with strong aromas or the products, which have to be packed under vacuum. Chemically, the polypropylene (PP) is similar to LDPE and HDPE. It is harder than either and has a less waxy feel. It can be converted into film and sheet or can be thermoformed to give thin – walled trays of excellent stiffness. PP has an excellent grease resistance. It has better mechanical strength and less prone to stress cracking than PE. It has poor low temperature performance

and impact resistance. The properties of PP can be altered by orienting the film. The oriented PP (OPP) has better tensile strength, low permeability to water vapour, and oxygen gas. Polypropylene is non-toxic, and extensively used as packaging material for various food products. The advantages of PP are: low cost, good impact strength, process able by all thermoplastic equipment, low coefficient of friction, and good moisture and chemical resistance. The oxygen permeabilities of commonly used polyolefin-based and other plastic films in dairy and food industry are given in Table-1.

Table-1. Permeability of some selected packaging materials

Film Type	Permeability ^a			Water vapour transmission rate (WVTR) ^b
	Oxygen	Nitrogen	Carbon dioxide	
LDPE	7800	2800	42000	18
HDPE	2600	650	7600	7 – 10
Polypropylene, cast	3700	680	10000	10 – 12
Polypropylene, oriented	2000	400	8000	6 – 7
Polypropylene, oriented and PVdC coated	10 – 20	8 – 13	35 – 50	4 – 5
Polystyrene, oriented	5000	800	18000	100-125
Ethylene vinyl alcohol	3 – 5	---	---	16 – 18
Plasticized PVC	500 - 30000	300 - 10000	1500 - 46000	15 – 40
PVdC-PVC copolymer	8 – 25	2 – 2.6	50 – 150	1.5 – 5.0
Nylon-6	40	14	150 – 190	84 – 3100

^a Gas transmission rates are expressed as mL/m²/day/atm for 25 μ m thick film at 25°C

^b WVTR expressed as g/m²/day/atm (38°C and 90% RH)

Source: Fellows (2009)

5.2 Polystyrene

Polystyrene (PS) is amorphous and in pure form, it is very brittle but when blended with chemicals like butadiene and copolymerized with acrylonitrile, it becomes tough. It is relatively cheap and has high water vapour and gas transmission rate, hence also called as “breathing

film". PS is crystal clear, sparkling and is also used for thermoformed cups, trays and glasses for yoghurt, ice cream, meat, soft drinks, etc. However, it is unsuitable for heat sterilization, and deteriorates on exposure to sunlight.

5.3 Polyvinyl chloride

Polyvinyl chloride (PVC) has several overall balanced properties required by the food packaging systems: glass like clarity, good mechanical strength, and resistance to water vapour, gases and chemicals, retention of flavour, excellent printability, and lower weight / volume ratio. Due to these properties, PVC is commonly used for food packaging in various forms like films and sheets, bags/liners, shrinkable tubes/films, skin/blister packs, film laminates, bottles and sachets.

5.4 Polyvinylidene chloride

Polyvinylidene chloride (PVDC) is a polymer based on the vinylidene chloride monomer. The film has excellent water vapour and gas barrier properties, besides outstanding characteristics like retention of odours and flavours, resistance to oils and fats, heat sealing characteristics, non-toxicity, abrasion, and chemical resistance. The laminates comprising of PVDC are widely used for the packaging of baby foods, snacks, chips, powdered soups and sauces, powdered coffee, coffee beans, sweets, biscuits and crackers, and powdered sugar. Saran, a copolymer of vinyl chloride and vinylidene chloride, is extensively used for food packaging. Saran is suitable for the packaging of several traditional dairy products such as sweet meats.

5.5 Nylon film

The unique properties of nylon film are that it has high mechanical strength, high elongation capability, excellent resistance to cutting, perforation, abrasion and bursting, high chemical resistance to oils and fats, outstanding impermeability to gases and vapours, easy printability, easy metallizing. The film can be biaxially oriented, and its properties remain unchanged between -30° to 120° C. The film is used in food packaging, especially where aroma retention is required.

5.6 Polyester

Polyethylene terephthalate (PET) is important polyester used in food industry. The main advantages of polyester films are that they are tough, sterilizable, very clear, chemical resistant,

has low water absorption, low moisture vapour permeability, low gas permeability and are used as a laminate base. Its main applications include in metallized films, vacuum and gas packaging, shrink packaging, cured meat, and in boil-in-the-bag applications. This film is of great interest to the food packagers as it contains no plasticizers and is non-toxic.

7.0 Characteristics of Dairy Products and their Packaging Requirements

Liquid milk and dairy products differ in their composition and hence, their packaging requirements differ. In general, dairy products are prone to spoilage due to sun light, air / oxygen, water vapour and microorganisms. Typical packaging requirements of dairy products are given in Table-4. Hence, while selecting the packaging materials and packaging technique, these things should be kept in mind.

Table-4. Characteristics of Dairy Products and their Packaging Requirements

Classification	Products	Significance	Packaging Requirement
Fluid Milk Products	Toned milk, Double toned milk, Full cream milk, Flavoured milks, etc.	Perishable due to high moisture, ideal for microbes and presence of photosensitive components	Protection against light and oxygen
Dried Milk Products	WMP, SMP, dried mixes, WPC, etc.	Low moisture, high fat and photosensitive	Protection against water vapour, light and oxygen
Fat rich products	Butter, spreads, etc.	High fat with fat soluble vitamins and pleasant aroma	Protection against oxidative and hydrolytic rancidity, aroma protection
Fermented dairy products	Cheese, Kefir, dahi, yoghurt, lassi, labneh, etc.	Beneficial bacteria with characteristic	Protection against light; oxygen, moisture and gas

		flavours and B-vitamins	barrier; aroma protection
Traditional Indian Dairy Products	Burfi, peda, paneer, gulabjamun, rasogolla, ghee, etc.	High fat, low moisture products with characteristic flavours,	Protection against moisture, light, preservation of aroma
Long-life products	UHT milk, Sterilized milk, retort processed products (like paneer curry), canned products (rasogolla, gulabjamun, condensed milks), etc	Sterile, medium to high fat, characteristic flavours with minimum head space in the pack	Protection against microorganisms, light, oxygen, moisture and container surface and preservation of aroma

8.0 Packaging of liquid milk

Milk has been packaged in different types of containers throughout the world. Although from protection point of view, milk could be packaged under rigid packs such as glass bottles and plastic bottles, but for economical and convenience reasons, their use is discouraged. Alternate flexible materials such as plastic films with more or less the same desired functional properties have been evolved. Of the total milk packed, it is estimated that flexible pouches dominate (92%), followed by bottles (5%) and aseptic packaging cartons (3%). The unique advantages offered by the plastic packages include good barrier properties, visibility of the contents, light in weight, can be used for single-service, are easy to carry home, more economical and attractive due to multi-colour printing and ergonomic designs. Also, the use of plastic containers eliminates noise of the milk bottling plants and during delivery, and also reduces water pollution caused by milk residues and detergents used in the bottle washing process.

Plastic pouches have fast replaced glass bottles for packaging of milk. Flexible pouches have proved to be a safe, quick and cost-effective packaging method with a wide distribution network, providing ease of packaging and handling. Presently, milk is sold in various package

forms like pouches, cartons, bags, plastic bottles and jars, etc. The recommended thickness of PE film for one litre pouches shall be not less than 75 microns and for half litre pouches not less than 60 microns. Presently, pasteurized and homogenized market milks such as toned, double toned, standardized, full cream milks, etc. are all packaged in TiO₂ pigmented LDPE or LLDPE pouches. Recently, the dairy industry started marketing liquid milks for institutional or catering sales using LDPE or LLDPE pouches with a capacity of 6litres. Although there are no guidelines for the thickness of such packs, the industry uses films of minimum 110 microns. In a form-fill-seal (FFS) machine, the plastic film is formed into a tube, sealed along its length, sealed at the bottom to form a pouch, filled with milk and then sealed at the top. Liquid milk is also packaged in various capacities (500 mL to 4 L) of bottles made from TiO₂ pigmented HDPE or polyethylene terephthalate (PET).

9.0 Packaging of fat-rich dairy products

Cream contains a high percentage of butterfat and moisture which make it susceptible to spoilage. In addition, it must be protected from water loss. The shelf life of refrigerated creams ranges between 1-2 days without proper protection. Packaging helps in extending the shelf life of cream. Both retail and bulk packaging of cream are provided by the industry. Bulk packaging of cream is done for catering or institutional use. Normally polythene bags contained in plastic crates or cardboard cartons are used for bulk packaging. The package size for bulk uses ranges from 5 to 25 liters. Pasteurized cream is packaged in cartons and bottles for retail sale with package size usually being in the range of 100-1000 mL. To improve the barrier properties of plastics packaging for fresh cream, multilayer materials may incorporate an ethylene vinyl alcohol (EVOH) layer. Once filled, the containers are closed with a heat-sealed polyethylene or aluminium foil laminate and often a clear plastic lid is provided for consumers to reseal the container once opened. In case of retort sterilized cream, tin cans and glass bottles are commonly used packaging formats. The can and glass bottle have been the traditional containers for retort processing, but retortable multilayered laminates containing polyamides (nylon), aluminum foil and polypropylene materials are now available for packaging. The current retail market is dominated by UHT cream (250 mL) packaged aseptically using multilayered laminates similar to those used for UHT milk. Whipped creams and synthetic formulations are sold in aerosol cans and polyethylene tubs with snap-on lid.

Packaging of butter is done in bulk packs of more than 5 kg and in retail packs from 10 g to 5 kg. Various types of machines are used, depending on the type of packaging. The machines are usually fully automatic, and both portioning and packaging machines can often be reset for different sizes such as 250 and 500 g or 10 and 15 g. The wrapping or packaging material must be greaseproof and impervious to light, flavouring and aromatic substances. It should also be impermeable to moisture, otherwise the surface of the butter will dry out and the outer layers become more yellow than the rest of the butter. Butter is usually wrapped in aluminum foil for retail marketing. Parchment paper was once the most common wrapping material (still being used) which has now been largely replaced by the less permeable aluminum foil. After wrapping, the pat or bar packets continue to a cartoning machine for packing in cardboard boxes. Bulk packaging of butter is done in boxes, tubs or casks. Mostly, HDPE liners are used for wrapping the butter and corrugated fiberboard boxes of multiple plies (3, 5 or 7 ply) are used as secondary packaging material. Tubs made of HDPE or PET with press-on lids are also commonly used for packaging of butter.

Ghee has a long keeping quality and can be stored for 6 to 12 months under ambient temperature. Minimum or no head space should be provided while filling of *ghee*. It is better to fill the product upto the brim of the container to attain a long shelf life. Glass bottles, food grade plastic containers such as high density polyethylene pouches, laminates with metallic layer support (aluminum) and tin cans are in use for packaging of *ghee*. Majority of dairies in public as well as private sector are using lacquered or even unlacquered tin cans of different sizes (250 g, 500 g, 1 kg and 15 kg) for packaging of *ghee*. The advantages of using tin cans are manifold. The only drawback of tin cans is their high cost. Regular tin pack sizes available in the market are 15, 5 and 1 L and 500 mL. Glass bottles also provide excellent protection, as they do not react with the food material and can be used for high-speed operations; but are not in much use for packaging of ghee because of their fragility and high weight. Since *ghee* is an expensive commodity and all consumers cannot afford to buy large size packs, some of the producers have started packaging *ghee* in glass bottles for retailers in sizes of 100 to 500g. Semi-rigid plastic containers are replacing tin plate containers and all have good scope for packaging of *ghee* and butter oil. These are mainly made from HDPE or PET. The advantages of using these containers are that they provide a moderately long shelf life (not as long as tin cans), are lightweight, economical and transport-worthy. Blow moulded bottles made up of HDPE, PET or PVC, lined cartons and multi-layered laminates are available for packaging of

ghee. Blow moulded HDPE is available in the form of bottles (200, 400 g), jars (1 kg and 2 kg.), and jerry cans (2 kg, 5 kg, and 15 kg). PET bottles have excellent clarity, are odour free and have gas barrier properties. Limited quantities of *ghee* are also packed in flexible pouches (less than 1 kg pack size) and bag-in-box containers (1 kg). The most attractive feature of packaging *ghee* in flexible pouches is that they are most economical than any other packaging system. The selection of a laminate or a multilayer film is governed primarily by the compatibility of the contact layer, heat sealability, heat-seal strength and shelf life required apart from aroma, grease, water vapour, oxygen and light barrier properties. Laminated pouches made of PVDC-Al foil-PP are suitable for long term storage of *ghee*. Self-standing laminates are used for 250 mL and 500 mL packs which are barrier to moisture, air and light.

10.0 Packaging of fermented dairy products

Dahi is a popular set-type fermented dairy product in India, whereas yoghurt is the best known of all cultured-milk products and the most popular worldwide. *Dahi* and yoghurt are highly perishable products and packaging protects them during handling and helps to maintain their physicochemical, nutritional and sensory characteristics. Since, these products are soft in nature with firm texture especially in set-type products, they require rigid or semi-rigid containers such as glass or plastic containers, while for stirred, pourable products or beverages flexible packaging materials in the form of paperboard cartons and plastic pouches could be used. Plastic materials such as high-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC) provide desired strength to the packaging materials for yoghurt and *dahi*. The PS containers, especially the high-impact PS (HIPS) which are now extensively used by dairy industry for packaging of *dahi* and yoghurt are very clean in appearance, gives good shining look, are light in weight and unbreakable. But the problem associated with PS and PP cups is wheying-off in set-type products such as *dahi* during storage. Preformed and cut aluminum foil and PE or PS laminates of about 100 mm diameter with a pull-tab for easy opening is usually used as lids to seal *dahi* and yoghurt cups or tubs. Currently, for institutional sales or bulk packaging, *dahi* is also being sold in HDPE buckets or pots of size 1 kg, 5 kg and 10 kg.

Cheese packaging can be broadly grouped as (a) wrapping of cheese for storage and ripening and (b) retail packaging for consumers. Bulk cheeses are either paraffin wax coated or vacuum

packed in flexible film. For retail consumers, blocks of cheese are cut into small random weight size, vacuum packed in barrier bags, hot water shrunk and then weighed, labeled and reassembled as a large block and put into the carton for distribution. Also, about 8-10 pieces of rectangular or triangular chiplets of cheese or processed cheese of convenient sizes (25 g) are individually wrapped with aluminum foil and placed in an outer plastic or paperboard container. Cheese spreads may also be packed in plastic tubs (usually polypropylene) and sealed with aluminum foil laminates and closed with a press on plastic lid, or plastic laminated or co-extruded squeezable tubes or bottles. Cheese is also available in slices individually wrapped in plastic films. About ten such slices are placed and sealed in an outer high barrier plastic pouch.

11.0 Packaging of traditional Indian dairy products

Traditionally the major portion of *khoa* is manufactured in the countryside, from where it is collected by middlemen in bamboo baskets lined with green leaves or used newspaper sheets fitted with loose fitting bamboo strip covers. Of late, *khoa* is being wrapped in plastic films such as PET, placed in a bamboo basket and packed using jute or hessian fiber cloth (gunny bags). A huge quantity of *khoa* contained in PE bags and tied with gunny bags is also stored for future use. Use of different packaging materials and packaging conditions resulted in different shelf lives. Hot filling of *khoa* at such a high temperature though helps in extending the shelf life, it imparts undesirable browning in *khoa* making it unsuitable for sweets making. High barrier structures or laminates based on PET-EVOH-PE are increasingly being used. Such laminates can be used for bulk packaging of *khoa* in cold stores for longer duration. Tin cans and rigid plastic containers of 15 kg capacity can also be used for bulk packaging of *khoa*.

Amongst the several *khoa*-based sweets, *burfi* and *peda* occupy most dominating place in terms of popularity and market demand. Currently, these sweets are largely prepared by sweetmeat makers (*halwai*) on small scale and mostly packaged in paper cartons or duplex board boxes with or without butter paper lining. The traditional packages do not provide sufficient protection to these sweets from atmospheric contamination and unhygienic handling, thereby rendering them susceptible to become dry, hard and mouldy, and develop off flavours. Currently, *burfi* is packaged in HDPE or polypropylene (PP) boxes and cartons of 500g and 1 kg size with an optional inserts or cavities. *Peda* is also similarly packaged in paperboard cartons with a parchment paper liner or greaseproof paper liner. Hot filling of *burfi* in pre-

sterilized polyester (PET) tubs of 250 g followed by vacuum packaging in multilayer co-extruded nylon-based pouches could extend the shelf life to 52 days at 30°C as against 16 days when packaged in ambient air. *Gulabjaman*, another important *khoa*-based sweet is largely packaged without syrup like *burfi* and *peda* in paper cartons or plastic boxes made up of HDPE or PP or PET. Although lacquered tin can is the most suitable packaging material for *gulabjaman*, it is expensive and hence, used for customers living at far-flung areas or Indian diaspora living abroad. Currently, high barrier multilayered stand-alone or gusseted pouches made from nylon and EVOH are being used for packaging of *gulabjamun*. With growing interest and commercial success of modified atmosphere packaging (MAP) technique, several Indian traditional dairy products have been successfully packaged under MAP conditions.

Commercial production of *paneer* has witnessed gradual increase in the country which has encourages development of better packaging solutions for it. Flexible packaging films like polypropylene, retort pouches (PET-Al foil-PP) and co-extruded laminates like nylon-nylon-nylon or PET-EVOH-PE or nylon-EVOH-PE or similar materials are promising materials for long-term storage of *paneer*. Flexible pouches made of high-density polyethylene bags could be used for vacuum packaging of *paneer* blocks to achieve a shelf life of about 30 days at refrigeration temperatures ($6\pm1^{\circ}\text{C}$). Vacuum packaging of *paneer* in a specially developed film (EVA/EVA/PVDC/EVA) followed by heat treatment at 90°C for one minute could help extending the shelf life up to 90 days at refrigeration temperature. Commercially *paneer* has also been successfully packaged under MAP conditions. Several large dairy industries such as AMUL, Mother Dairy, Punjab Milkfed, Heritage, Creamline Jersey, etc. have started selling vacuum packaged *paneer*.

Since the body and texture of *rasogolla* is very delicate and it has to be preserved in sugar syrup, it is invariably packaged in lacquered tin cans of 500 g and 1 kg capacity. When the proportion of *rasogolla* (10-14 pieces) and syrup is kept 40:60, and hygienic conditions are maintained during processing and handling, the product stays in good condition for about 6 months at ambient conditions, since hot filling (at about 90°C) technique is adopted. PP-Al foil laminate could be used for hot filling of syrup and *rasogolla*. High barrier multilayered stand-alone or gusseted pouches made from nylon and EVOH could also be used for packaging of *rasogolla*.

12.0 Packaging of condensed and dried milk products

Usual pack sizes of condensed milk for retail sale are 200 g, 400 g and 1 kg. However, 200 g and 400 g packs are popular. According to the Bureau of Indian Standards (IS: 9991, 1981), the condensed milk containers shall be round cans made from tinplate of a nominal thickness of about 0.22 mm and shall have a nominal internal diameter of about 74 mm with a gross lidded capacity of about 300 mL. The body of the can shall be single seamed, but the ends shall be double seamed. The side seam may be unsoldered, soldered, welded or cemented. When the side seam is cemented, the type of cement used shall not impart any off-flavour to the condensed milk and shall be non-toxic. Internally can surface may be plain or lacquered while externally it could be plain or printed. Bulk packaging may be done in barrels of various sizes, drums with polyethylene liners or tin containers.

For retailing to consumers, milk powder is packed into metal cans (open top sanitary (OTS) cans) with aluminum foil tagger, multilayered refill-pouches, bag-in-box type of packs or shrink sleeved glass or plastic jars. Milk powder packed in pouches is commercially available in a capacity range of 250 g to 2.5 kg. For institutional sales such as for railways and airways, the pack size could be as small as 10 g. Metal cans have been highly popular for a long time for packaging of milk powders with varying capacities (200 g, 400 g, 500 g, 1 kg, 2 kg and 2.5 kg) due to their excellent physical strength, durability, absolute barrier to moisture, oxygen and light, absence of flavour or odour and rigidity. The metal cans are usually coated with a thin layer of tin and an additional layer of organic lacquers such as epoxy-phenolic lacquer to prevent corrosion and avoid metal-powder contact. Powdered milk including infant formula may have hormonally active contaminants such as bisphenol A (BPA) introduced in the manufacturing process or leached from the lacquers of the containers. In recent times, several packaging materials (laminates and co-extruded films) have emerged as alternative to metal cans such as LDPE/nylon/LDPE, Kraft paper/aluminum foil/HDPE, LDPE/HDPE, metallized PET, PET/aluminum foil/BOPP, PET/aluminum foil/HDPE, PET/aluminum foil/LDPE, etc. Alternatively, with pouches for which shorter shelf life is acceptable, the aluminum layer may be replaced with a high-barrier plastic layer such as copolymer of EVOH or PVdC, possibly with the addition of a thin layer of metal or silica oxide deposition to enhance its oxygen barrier characteristics. A study has reported that the shelf life of whole milk powder packaged in either PET/aluminum foil/HDPE or metallized PET (400 microns) was about 12 months. Bulk packaging of milk powders is usually done in 25 kg multiwall Kraft paper sacks or jute (hessian

fabric) bags laminated with woven or non-woven plastic and having a liner made of plastic film.

13.0 Food Package Labelling Requirements

A label is a piece of material affixed to a container or product on which is written or printed information about the product. Labels have many uses including providing information on a product's origin, use, shelf life and mode of disposal, etc., some or all may be governed by the law of land (legislation). Food package labelling is any written, electronic or graphic communication on the package of a food product or on a separate label, but are integral part of the package. These may carry price, directions for use, addresses of manufacturer, brand, bar code, etc.

13.1 General Requirements

In India, the food package labelling is governed by the Food Safety and Standards (Labelling and Display) Regulations (2020) which has recently replaced the previous Food Safety and Standards (Packaging and Labelling) Regulations (2011). The general requirements are as follows:

- a) Every pre-packaged food shall carry a label.
- b) When a food product is sold through e-commerce or any other direct selling means, the mandatory requirements of the label as given in the regulations shall be provided to the consumer through appropriate means before sale except batch number/lot number, best before, use-by-date, expiry date, date of manufacturing / packing.
- c) Pre-packaged food shall not be described or presented on any label or in any labelling in a manner that is false, misleading or deceptive or is likely to create an erroneous impression regarding its character in any respect.
- d) Any information or pictorial device written, printed or graphic matter may be displayed on the label provided that it is not in conflict with the requirements of the regulations.
- e) The particulars of declaration required under these regulations are to be specified on the label either in English or Hindi in Devnagri script. However, nothing prevents the use of any other language in addition to English or Hindi.
- f) The label on the pre-packaged foods shall be applied in such a manner that they will not become separated from the container.

- g) The contents on the label shall be clear, prominent, indelible (cannot be removed) and readily legible by the consumer under normal conditions of purchase and use.
- h) Where a package is provided with an outside container or wrapper and such container or wrapper is displayed for retail sale, it shall also contain all the declarations which are required to appear on the package except where such container or wrapper itself is transparent and the declarations on the package(s) are easily readable through such outside container or wrapper.

13.2 Labelling Requirements

In addition to the general labelling requirements mentioned above, every package of food shall carry the pre-packaged food product information on the label. The details are as follows:

- a) **The name of the food:** The name of the food shall carry name of the food which indicate the true nature of the food contained in the package, on the front of the pack: where a food is specified by certain essential composition under FSSR made under the Act, that establishes its identity the name provided therein shall be used; in the absence of such name, either a common or usual name or an accompanying description of true nature of food shall be used; it may additionally have a “coined”, “fanciful”, “brand” or “trade name” subject to compliance of Food Safety and Standards (Advertising and Claims) Regulation (2018).
- b) **List of ingredients:** Except for single ingredient food, a list of ingredients shall be declared on the label. Declaration of the list of ingredients shall contain an appropriate term or title such as “Ingredients”. Further, the names of the ingredients shall be listed in descending order of their composition by weight or volume, as may be the case, at the time of its manufacture. Also, a specific name shall be used for ingredients in the list of ingredients that fall into a particular class e.g. all types of cheese under “*Cheese*”; cocoa bean, cocoa nib, cocoa mass, cocoa powder under “*Cocoa solids*”. If the ingredient itself is the product of two or more ingredients, such a compound ingredients shall be declared in the list of ingredients and shall be accompanied by a list, in brackets, of its ingredients in descending order of weight or volume. If water is added as an ingredient it shall be declared in the list except in cases where it forms a part of an ingredient such as brine, syrup or both. In case of dehydrated or condensed foods, which

are intended to be reconstituted by addition of water, the ingredients in such reconstituted food shall be declared in descending order of weight or volume as the case may be and shall contain a statement such as “*Ingredients of the product when prepared in accordance with the directions on the label*”.

c) **Nutritional Information:** Every pre-packaged food label shall contain nutritional information or nutritional facts per 100 g or 100 mL or per serving of the product as per the following:

- Energy value shall be in “kcal”
- The amounts of protein, carbohydrate (with quantity of cane sugar specified) and fat in “g” or “mL”.
- The amount of any other nutrient for which a nutrition or health claim is made. If the claim is made regarding the amount or type of fatty acids or the amount of cholesterol, the amount of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in gram (g) and cholesterol in milligram (mg) shall be declared. Further, the amount of *trans* fatty acid content in gram (g) shall be declared. A health claim of “*trans fat free*” may be made where the *trans* fat is less than 0.2 g per serving of food and the claim “*saturated fat free*” may be made in cases where the saturated fat does not exceed 0.1 g per 100 g or 100 mL of food.
- The information on vitamins and minerals shall be expressed in metric units.
- Where the nutritional information is made per serving, the amount in gram (g) or millilitre (mL) shall be declared.
- The following foods are exempted from mandatory nutritional labelling: (a) unprocessed products that comprise a single ingredient; (b) processed products in which the only processing they have been subjected to is maturing and that comprise a single ingredient; (c) waters intent for human consumption, including those where the only added ingredients are CO₂; (d) a herb, a spice or mixtures thereof (masalas) meant for direct consumption; (e) salt and salt substitute; (f) table top sweeteners; (g) coffee extracts and chicory extracts, whole or milled coffee beans and whole and milled decaffeinated coffee, soluble coffee powder, coffee-chicory mixture; (h) herbal and fruit infusions, tea, decaffeinated tea, instant or soluble tea or tea extract; (i) fermented vinegars and

substitutes for vinegar; (j) flavourings, food additives, processing aids, food enzymes, gelatine, yeast; (k) chewing-gums; (l) alcoholic beverages; and (m) Foods for Special Dietary Uses (FSDU), Foods for Special Medical Purposes (FSMP) subject to compliance of requirements specified in the Food Safety and Standards (HS, N, FSDU, FSMP, FF and NF) Regulations (2016).

- d) **Declaration regarding veg or non-veg:** Every package of “non-vegetarian” food shall bear a declaration to this effect on the principal display panel made by a symbol and colour code as shown below (Fig.1) to indicate that the product is non-vegetarian food. The symbol shall consist of a brown colour filled triangle inside a square with brown outline, having the sides not less than the minimum size specified. Where any article of food contains egg only as non-vegetarian ingredient, the manufacturer, packer or seller may give declaration to this effect in addition to the above said symbol. Every package of “vegetarian” food shall also bear a declaration to this effect by a symbol and colour code. The symbol shall consist of a green colour filled circle, having a diameter not less than the minimum size specified, inside the square with green outline, length of each side being double the diameter of the circle (Table-1).

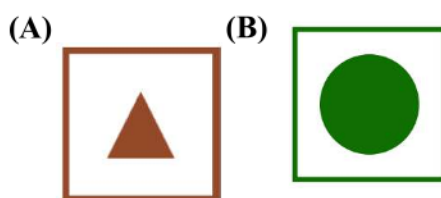


Fig. 1 An illustration of (A) “non-veg” and (B) “veg” logos on a packaged food label

Table-1. Size of the Veg or Non-Vege Food Logo as per FSS (L&D) Regulations (2020)

Area of the principal display panel	Minimum size of diameter of circle (in mm)	Minimum size of each side of Triangle (in mm)	Minimum size of each side of square (in mm)
Up to 100 cm ²	3	2.5	6
Above 100 cm ² upto 500 cm ²	4	3.5	8

Above 500 cm ² upto 2500 cm ²	6	5	12
Above 2500 cm ²	8	7	16

Principal display panel is the part of the package or container which is intended or likely to be displayed or presented or shown or examined by the customer under normal selling conditions. Further, the symbol shall be prominently displayed on the package having contrast background on the principal display panel, just close to the name or brand of the product and also on the labels, containers, pamphlets, leaflets, advertisements, brochures, etc.

- e) **Declaration regarding food additives:** All the food additives that have been used in the manufacture of a packaged food product shall be declared on the label as specified in Food Safety and Standards (Food Product Standards and Food Additives) Regulations (2011). For food additives falling in the respective classes and appearing in lists of food additives generally permitted for use in foods, the title class given in Table-2 below shall be used together with the specific names or recognized international numerical identifications (INS numbers).

Table-2 List of food classes listed under permitted food additives

Food Class	Example	Identification Number
Acidity regulator	Citric acid	INS 330
Anticaking agent	Calcium carbonate	INS 170
Antifoaming agent	Methylphenylpolysiloxane	INS 900 b
Antioxidant	Ascorbic acid	INS 300
Bulking agent	Polydextroses A and N	INS 1200
Colour	Sunset Yellow FCF	INS 110
Colour retention agent	Ferrous gluconate	INS 579
Emulsifier	Sorbitan monostearate	INS 491
Emulsifying salt	Trisodium citrate	INS 331 (ii)
Firming agent	Qulillaia extracts	INS 999
Flour treatment agent	Potassium bromate	INS 924 a
Flavour enhancer	Monosodium glutamate	INS 621
Gelling agent	Carrageenan and its salts	INS 407
Glazing agent	Shellac	INS 904

Humectant	Sorbitol and sorbitol syrup	INS 420
Preservative	Sodium benzoate	INS 211
Propellant	Propane	INS 944
Raising agent	Sodium hydrogen carbonate	INS 500 (ii)
Stabilizer	Sodium alginate	INS 401
Sweetener	Sucralose	INS 955
Thickener	Xanthan gum	INS 415

Where extraneously colouring matter has been added, it has to be mentioned with one of the following statements in capital letters, just beneath the list of ingredients on the label of the pack.

CONTAINS PERMITTED NATURAL COLOUR(S)

OR

CONTAINS PERMITTED SYNTHETIC FOOD COLOUR(S)

OR

CONTAINS PERMITTED NATURAL AND SYNTHETIC FOOD COLOUR(S)

Where extraneously flavouring agent has been added, it has to be mentioned with one of the following statements in capital letters, just beneath the list of ingredients on the label of the pack.

CONTAINS ADDED FLAVOUR

If both colour and flavour are used in the product, one of the following combined statements in capital letters shall be displayed, just beneath the list of ingredients on the label of the pack.

CONTAINS PERMITTED NATURAL COLOUR(S) AND ADDED FLAVOUR(S)

OR

CONTAINS PERMITTED SYNTHETIC FOOD COLOUR(S)

AND ADDED FLAVOUR(S)

OR

CONTAINS PERMITTED NATURAL AND SYNTHETIC FOOD COLOUR(S)

- f) **Name and complete address of the manufacturer:** The name and complete address of the manufacturer and the manufacturing unit if these are located at different places and in case of the manufacturer is not the packer or bottler, the name and complete address of the packing or bottling unit shall be declared on every package of food. If a product is manufactured or packed or bottled by a person or company under the written authority of some other manufacturer, the name and complete address of the manufacturer, for and on whose behalf it is manufactured, are to be mentioned. Further, if the food is imported into India, the package of food shall also carry the name and address of the importer in India.
- g) **FSSAI Logo and License Number:** The FSSAI logo and license number under the Act shall be displayed on the label of the food package in contrast colour to the background as given below:



The FSSAI logo and license number of the brand owner shall be displayed on the label. IN addition, the license number of manufacturer or marketer or packer or bottler, as the case may be, if different from the brand owner, shall be displayed on the label. In case of imported food products, the importer shall display FSSAI logo and license number along with name and address of the importer. Further, fortified food and organic food shall be marked with logo as specified in the regulations (given below).



Fortified with....
SAMPOORNA POSHAN
SWASTHA JEEVAN



- h) **Net quantity, Retail Sale Price and Consumer Care Details:** Every package label shall carry the net weight or volume or number of the product, as the case may be. In addition to the declaration of net quantity, a food packed in a liquid medium shall carry a declaration of the drained weight of the food. Liquid medium includes water, aqueous solutions of sugar and salt, fruit and vegetable juices or vinegar, either singly or in combination. Further, the declarations shall be as provided in Legal Metrology Act (2009) and Rules made there under.
- i) **Lot/ Code/ Batch identification:** A batch number or code number or lot number which is a mark of identification by which the food can be traced in the manufacture schedule and identified in the distribution system, shall be given on the label. However, in case of packages containing bread and milk including sterilized milk, particulars under this clause shall not be required to be given on the label.
- j) **Date Marking:** “Date of manufacture or packing”, and “Expiry / Use by” shall be declared on the label. However, expression “Best before” may also be used as optional or additional information. The manner of declaration of date of manufacture or packing / Expiry / Use-by / Best Before shall be as the date, month and year using the DD/MM/YY format for products with a short shelf life of up to 3 months; the month and the year for products with a shelf life of more than three months, shall be declared in un-coded numerical sequence except that the month shall be indicated by capital letters and abbreviations (at least first three letters of the month) may be used.

In addition to the expiry or Use by, any special conditions for the storage of the food shall be declared on the label if the validity of the date depends thereon. If required, storage conditions after opening the pack may also be specified.

Notwithstanding anything contained in this regulation, an indication of the “Expiry” shall not be required for:

- ✓ Fresh fruits and vegetables, including potatoes which have not been peeled, cut or similarly treated.
- ✓ All types of wine
- ✓ Alcoholic beverages containing 10% or more by volume of alcohol
- ✓ Vinegar

- ✓ Sugar boiled confectionery
- ✓ Food grade salt for industrial use
- ✓ Solid sugars
- ✓ Chewing gum and bubble gum

“Date of manufacture or packaging” and “Expiry / Use by” shall be grouped and given at one place.

“Date and time of manufacture” shall be declared on packed meals served in airlines / railways / mobile catering units.

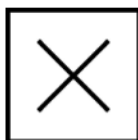
- k) **Labelling of Imported Foods:** In addition to the requirement mentioned in these regulations, labelling requirements of imported foods shall be governed by the Food Safety and Standards (Import) Regulations (2017).
- l) **Country of origin for imported foods:** The country of origin of the food shall be declared on the label of the food imported into India. Further, when a food undergoes processing in a second country which changes its nature, the country in which the processing is performed resulting in change in HS Code (Harmonized System Code, a multipurpose international product nomenclature developed by the World Customs Organization) at the 6 digit level shall be considered to be the country of origin for the purpose of labelling.
- m) **Instructions for use:** Instructions for use, including reconstitution, where applicable, shall be included on the label, to ensure correct utilization of the food, or where such food requires directions for reasons for health and safety (e.g. “Refrigerate after opening”).
- n) **Declaration regarding Food Allergen:** The following foods and food ingredients which are known to cause allergy shall be declared separately as **Contains _____** (Name of allergy causing ingredients).
 - ✓ Cereals containing gluten (To be declared as name of the cereal)
 - ✓ Crustacean and their products (To be declared as Crustacean)
 - ✓ Milk and milk products (To be declared as Milk)
 - ✓ Egg and egg products (To be declared as Egg)

- ✓ Fish and fish products (To be declared as Fish)
- ✓ Peanuts, tree nuts (e.g. almonds, walnuts, pistachio, cashew nuts) and their products (To be declared as Nut)
- ✓ Soybeans and their products (To be declared as Soy)
- ✓ Sulphite in concentrations of 10 mg/kg or more (To be declared as Sulphite)

Provided that in case presence of ingredients due to cross contamination which are known to cause allergy may be declared separately as “**May Contain _____**” (Name of allergy causing ingredients).

Raw agricultural commodities are exempted from the allergen labelling requirements.

- o) Every package of food material sold in retail but which is not meant for human consumption example Pooja water, Ghee for diya, Oil for Pooja, etc. shall bear a declaration to this effect by a symbol as stipulated below. The symbol shall consist of a black colour cross inside a square with black outline having sides of square not less than the minimum size specified in the Table mentioned in the regulation (Table-1).



1.0 Exemptions from certain labelling requirements

The following are the exemptions given in the FSS (L&D) Regulations (2020) regarding labelling of pre-packaged foods:

- a) Where the surface area of the package is not more than 100 cm², the label of such package shall be exempted from the requirements of list of ingredients; lot number or batch number or code number; nutritional information; labelling of irradiated food, declaration of food additives; license number and logo and complete address of the importer and instructions for use, but this information shall be given on the multi-unit packages.
- b) In case of liquid products marketed in bottles, if such bottle is intended to be reused for refilling, the requirement of list of ingredients shall be exempted, but the nutritional information shall be given.

- c) In case of food products with shelf life of not more than seven days, the “date of manufacture” may not be required to be mentioned on the label of packaged food articles, but the “Expiry/Use by” shall be mentioned on the label by the manufacturer or packer.
- d) In case of prepared food served for immediate consumption such as in hotels or by food service vendors or caterers or halwais or hospitals or at religious gathering or food served in airline/railway/passenger vehicle or any mobile unit shall accompany or display the minimum information as mentioned in the regulations at the point of sale/serve of the food.
- e) The following labelling requirements are exempted if they are provided in a Barcode / Global Trade Identification Number (GTIN):
 - ✓ Address of the brand owner whether or not, he himself is the manufacturer, marketer, packer or bottler, as the case may be.
 - ✓ The license number of the manufacturer or marketer or packer or bottler, as the case may be, if different from the brand owner.
- f) For assorted packs, shelf life declared on assorted pack should be that of the product having the earliest shelf life declared amongst the different pre-packaged food packed inside.

References

- Deshwal G.K. and Panjagari N.R. (2020). Review on metal packaging: materials, forms, food applications, safety and recyclability. *J Food Sci Technol* **57**, 2377–2392 (2020).
- Deshwal G.K., Panjagari N.R. and Alam T. (2019). An overview of paper and paper based food packaging materials: health safety and environmental concerns. *J Food Sci Technol* **56**, 4391–4403.
- Panjagari N.R., Singh R.R.B., Singh A.K. (2016) Indian Traditional Fermented Dairy Products. In: Kristbergsson K., Oliveira J. (eds) *Traditional Foods. Integrating Food Science and Engineering Knowledge Into the Food Chain*, vol 10. Springer, Boston, MA.
- Raju P.N. and Singh A.K. (2016). Packaging of Fermented Milks and Dairy Products. In Puniya AK (ed) *Fermented Milks and Dairy Products*, CRC Press, Boca Raton, FL, USA. Pp: 637-672.
- Sabikhi L, Khetra Y. and Raju P.N. (2018). Processing and packaging of dairy-based products. In Mohan CO, Carvajal-Millan E, Ravishankar CN and Haghi AK (eds) *Food Process Engineering and Quality Assurance*, Apple Academic Press Inc. Oakville, Canada.

INNOVATIVE PACKAGING SOLUTIONS FOR DAIRY PRODUCTS

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1.0 Important packaging techniques

1.1 Aseptic packaging

Aseptic packaging can be defined as the filling of a commercially sterile product into sterile containers under aseptic conditions and hermetically seal the containers so that re-infection or contamination is prevented. The prime necessity of an aseptic packaging unit is to prevent recontamination of the sterilized product. Thus, the principal consideration in this regard includes sterilization of the filling machine and packaging material by suitable physical and/or chemical means and maintaining aseptic barriers during filling and sealing. A schematic representation of aseptic packaging process is shown in Fig. 3.

The basic operations in aseptic packaging mainly consists of the following:

- Heating the product to sterilization temperatures (140-150°C for no hold to few seconds).
- Maintaining the sterility of the products till they are cooled/packed.
- Filling into sterile containers and sealing aseptically.

The major reasons for using aseptic packaging are:

- a) To enable the containers to be used which are otherwise unsuitable for in-package sterilization.
- b) To take advantage of HTST sterilization processes, which are thermally efficient and generally give superior quality compared to those processes involving lower temperatures and longer times.
- c) To extend the sterilization of products at normal temperatures by packaging them aseptically.

The aseptic packaging system must be capable of filling the sterile product in an aseptic manner and of sealing the container hermetically to maintain the sterility of product throughout the

storage and distribution process. The major requirement of an aseptic packaging unit is to prevent recontamination of the sterilized product. The aseptic packaging system should be capable of meeting the following four criteria:

- a. Able to be connected to the processing system in a manner that enables aseptic transfer of the product to take place.
- b. Able to be effectively sterilized before use.
- c. Able to carry out the filling, sealing and critical transfer operations in a sterile environment.
- d. Able to be cleaned properly after use.

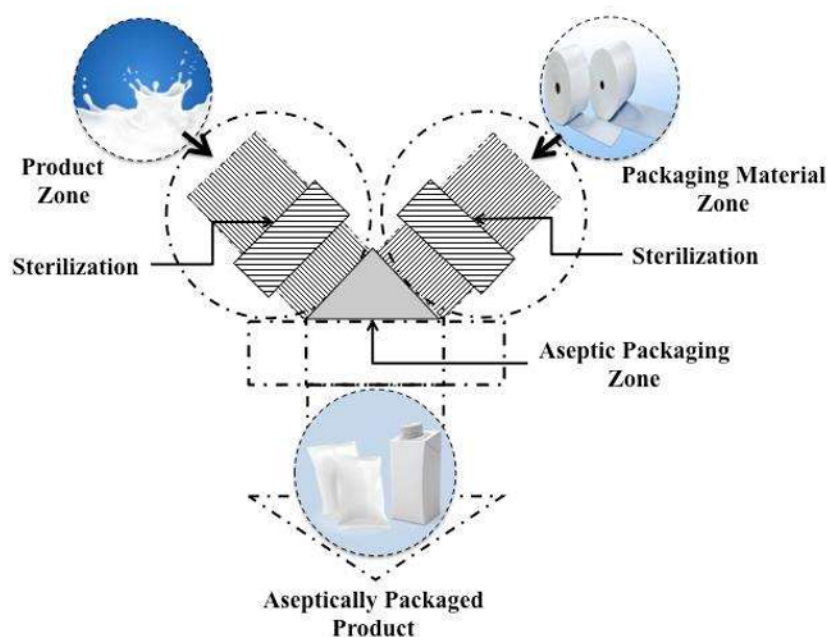


Fig. 3. Schematic representation of aseptic packaging process

The type of packaging material used is influenced by the nature of the product, the cost of both the product and the package, and the preferences of the consumers. Although the most widespread aseptic package for aseptic products is the paperboard laminate carton, five major categories of aseptic packaging forms are available namely (a) carton system, (b) can system, (c) bottle system, (d) sachet and pouch system, and (e) cup system

The carton material consists of layers of unbleached or bleached paperboard coated internally and externally with polyethylene, resulting in a carton that is impermeable to liquids and in which the internal and external surface may be heat-sealed. Such packaging materials are

commonly used in aseptic filling systems for milk, cream, fruit juices, soups etc. The filling systems could be either of the following two types: those in which the carton is formed within the filler from a continuous reel of material; and those in which the cartons are supplied as preformed blanks, folded flat, which are assembled into cartons in the filler. The packaging material is mainly composed of printed-paper coated with aluminium foil and several plastic layers (Polyethylene-paper board-polyethylene-aluminium foil-polyethylene). The inner material side of the finished package is coated with a special layer facilitating the sealing process. The structure of a typical paperboard carton is given in Fig 4. Each layer has a specific function. The outer polyethylene layer (15 g/m^2) protects the ink and enables the sealing process of the package flaps. The bleached paperboard (186 g/m^2) serves as a carrier of the décor and gives the package the required mechanical strength. The laminated polyethylene (25 g/m^2) binds the aluminium to the paperboard. The aluminium foil ($6.3 \mu\text{m}$) acts as a gas and light barrier. The two inner polyethylene layers (15 g/m^2) provides liquid barrier.

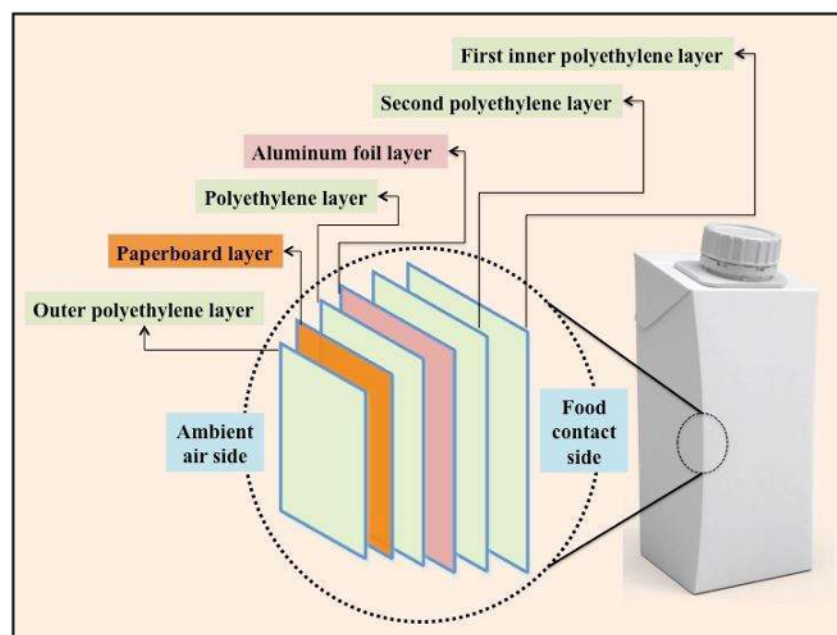


Fig. 4. The structure of a typical laminated carton for aseptic packaging

For a form-fill-seal carton, the packaging material is supplied in rolls that have been printed and creased, the latter is necessary to ease the forming process. The plastic strip is sealed to one edge and the packaging material is sterilized using a wetting system or a deep bath system. In the wetting system, a thin H_2O_2 film (15 to 35% concentration) containing a wetting agent is applied to the inner packaging material surface. The material then passes through a pair of rollers to remove excess liquid and under a tubular electric heater, which heats the inside surface to about 120°C and evaporates the H_2O_2 . In deep bath system, the packaging material

is fed through a deep bath containing H_2O_2 (35% concentration) at a minimum temperature of 70°C , the residence time is 6 sec. After the squeezer rollers have removed much of the peroxide, both sides of the material are heated with air at a temperature of 125°C to evaporate the peroxide.

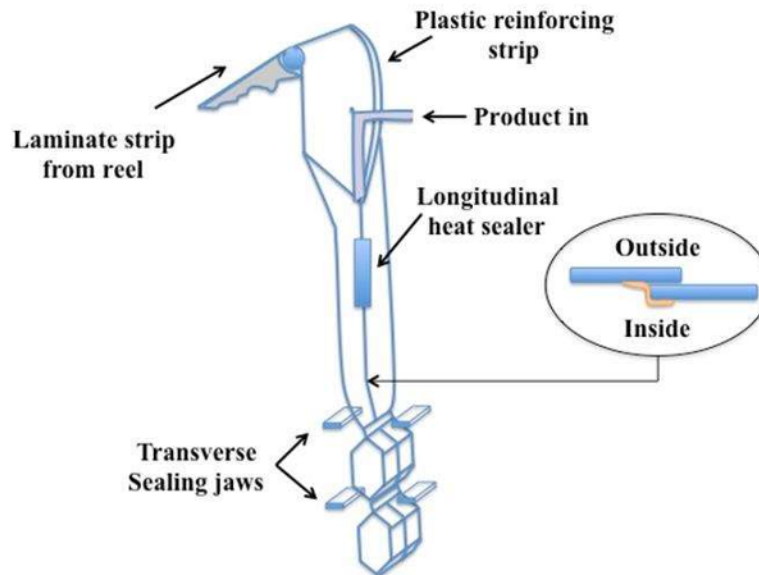


Fig. 5 An illustration of aseptic carton formation from a continuous web

(Redrawn from Robertson (2013))

The sterilized packaging material is fed into a machine where it is formed into a tube and closed at the longitudinal seal by a heat-sealing element. In the process, the strip that was added prior to sterilization is heat sealed across the inner surface of the longitudinal seal to prevent contact between the outside and the inside of the carton. It also provides protection to the aluminium and paperboard layers from the product, which could corrode or swell the layers if such a strip were absent. The tube is then filled with the product and a transverse seal made below the level of the product, thus ensuring that the package is completely filled. Alternatively, the packages may be produced with a headspace of upto 30% of total filling volume by injection of either sterile air or other inert gases. The sterilization, filling and sealing process are all performed inside a chamber maintained at an overpressure of 0.5 atm with sterile air. The system has been illustrated in Fig 5.

6.2 Modified atmosphere packaging

The normal composition of air is 78 % nitrogen (N_2), 21 % oxygen (O_2), 0.03 % carbon dioxide (CO_2) and traces of the noble gases. The modification of the atmosphere within the package by reducing the oxygen content while increasing the levels of CO_2 and / or N_2 has been shown to significantly extend the shelf life of perishable foods at low temperature. The modified atmosphere packaging (MAP) is the technology of producing a gas atmosphere around a product that deviates from ambient air composition by either passive or active methods in order to achieve improved quality and prolonged shelf life. Four packaging techniques are included within this definition namely vacuum packaging, passive MAP, gas-flush MAP and MAP by active packaging. MAP has also been referred to as “*protective atmosphere packaging*” or when used in labeling as “*packaged in a protective atmosphere*”. The diagrammatic representation of a dairy product in MAP is shown in Fig. 6.

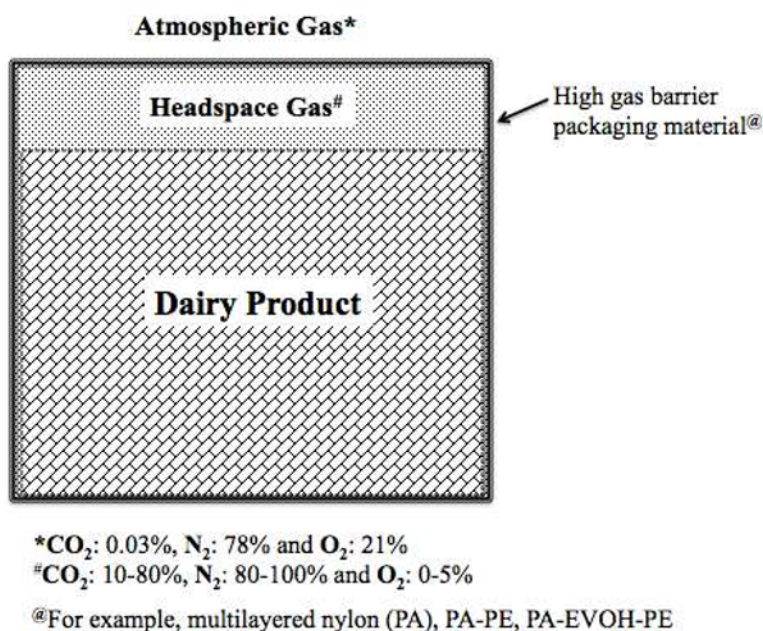


Fig 6. Diagrammatic representation of modified atmosphere packaged dairy products.

The principle of MAP involves the removal of air from the pack and its replacement with a single gas or a mixture of gases by either passive or active methods. The gas mixture used is dependent on the type of product. Passive modification is a slow process. It requires reactions between the food and its surrounding gases to take place, and the package to play the role of a regulator. On the contrary, active modification is faster and can be achieved by gas flushing, vacuum application or by using gas scavengers / emitters. Passive MAP relies on the selective permeability of the packaging materials to different gases and on product respiration, and is traditionally used with fresh and minimally processed fruits and vegetables and only with some

selected cheese varieties. Gas-flush MAP involves the establishment of a specific gas composition within the package in a single stage during the packaging operation, by flushing with the selected gas mixture before sealing. Depending on the desired residual O₂, a vacuum operation may be needed prior to gas flushing. The instant modification of the package atmosphere is the main advantage of gas-flush MAP over the relatively slow passive process. Vacuum packaging, which involves the removal of air from a package without replacement by another gas could also be considered as a type of MAP because the removal of air from the environment itself is a modification of the atmosphere.

One of the important considerations in MAP is defining the optimal gas atmosphere for a food product in a specific packaging design. This optimal atmosphere depends on the intrinsic parameters of the food product (pH, water activity, fat content, type of fat) and the gas / product volume ratio in the chosen package type. The intrinsic parameters will determine the sensitivity of the product for specific microbial, chemical and enzymatic degradation reactions. Products that are susceptible to microbial spoilage due to the development of Gram-negative bacteria and yeasts (e.g. cheese, yoghurt) will be packed in a CO₂ enriched atmosphere as the growth of those microorganisms is significantly retarded by CO₂. In general, oxygen is excluded from the gas mixture. For prolonging the shelf life of products, which are spoiled due to mold growth (e.g. hard cheeses) or due to oxidation, it is essential to package in oxygen-free atmospheres. The use of CO₂ is however limited to its solubility in water and fat. High solubility can cause collapsing of the package when too high CO₂ concentrations are applied. This will especially be the case with food products containing high amounts of unsaturated fat.

Moreover, too high CO₂ concentrations in the atmosphere can lead to an increased drip loss during storage. This can be explained by the pH drop induced by CO₂ dissolving in the water phase of the product, causing a decrease in the water binding capacity of the proteins. Also, when a respiring food is sealed in an impermeable film and in-pack O₂ levels fall to very low concentrations, anaerobic conditions will usually result in undesirable fermentation reactions (undesirable odors and flavors) and a marked deterioration in product quality. In addition, there is a risk of growth of anaerobic pathogens such as *Clostridium botulinum*. Hence, a minimum level of 2 – 3 % O₂ is recommended to ensure aerobic conditions.

6.3 Shrink Packaging

Shrink films, also called as heat-shrinkable films, provide a high degree of free shrink with a controlled level of shrink force over a broad temperature range. Although most plastics exhibit

some amount of free shrink and shrink force at elevated temperatures, shrink films are basically composed of: a) polyvinyl chlorides (PVC and PVC-PVDC copolymers), and b) polyolefins. Usually, these films require temperatures above 100°C to obtain a suitable degree of shrinkage, necessitating the use of hot air tunnels or heat guns. Exceptionally, PVDC copolymer can be shrunk in hot water. Three properties are important when selecting a film for a particular application: (a) wide range of temperature over which a film will shrink and wide softening range, (b) degree of shrinkage (amount of shrinkage may vary from 15-80% depending on polymer composition and manufacturing technique), and (c) shrink tension, which is the stress exerted by the film when it is restrained from shrinking at elevated temperatures. Shrink films are produced by uniaxially/ monoaxially or biaxially orienting a sheet or tube of film by imposing a draw force at a temperature where the film is softened but kept below its melting point, then quickly cooled to retain its physical properties generated during orientation. Orientation occurs between softening point and melting point. The key attributes that are important to shrink films include shrink, sealability, optics, toughness and slip. Shrink properties depends on the onset temperature, free shrink, shrink temperature range and overall package appearance. For sealing properties, one must also consider, ease of trim sealing, trim seal strength, trim seal appearance and thermal lap sealability. For optics, it is important to consider clarity, gloss and haze.

The ultimate goal of shrink packaging is to have a package that is tightly shrunk with no excess material or “ears” on the corners of the finished package. PVC films do not require the same level of temperature control as polyolefins, since they shrink more easily when exposed to heat. The equipment needed to provide a sharp tightly finished package is a shrunk tunnel. In order to have an adequate shrinkage to occur, the package must be exposed to correct temperature and air flow for proper time.

6.4 Active Packaging

Active packaging is defined as the “packaging in which subsidiary constituents have been deliberately included in or on either the packaging material or the package headspace to enhance the performance of the package system”. The two key words are “deliberately” and “enhance”. Implicit in this definition is that performance of the package system includes maintenance and often improving the sensory, safety and quality aspects of the food. Hence, in other words, active packaging may also be defined as “packaging that interferes with the

internal condition of a packaged food product and changes the condition to extend the shelf life or improve food safety or sensory properties in order to maintain the quality”. Active packaging techniques for food preservation can be divided into three categories: scavengers (absorbers), releasing agents and others. Absorbing systems remove undesirable compounds such as oxygen, carbon dioxide, ethylene, excessive water, taints and other compounds. Releasing systems actively add or emit compounds to the packaged food or into the headspace of the package such as carbon dioxide, antioxidants and preservatives. Other systems include self-heating, self-cooling systems, etc.

6.5 Intelligent Packaging

Intelligent packaging is defined as packaging that contains an external or internal indicator to provide some information about the history of the package and / or the quality of the food. In other words, it refers to a package that can sense environmental changes and in turn informs the changes to the users. Such packaging systems contain devices that are capable of sensing and providing information about the functions and properties of the packaged foods and/or contain external or internal indicator for the active product history and quality determination. These devices can be divided into three groups. The first type is the external indicators, which are attached outside the package and include freshness indicator, time-temperature indicators and physical shock indicators. The second type is the internal indicators, which are placed inside the package either in the headspace of the package or attached to the lid such as gas indicators. The third type of devices is the indicators that increase the efficiency of information flow and effective communication between the product and the consumer. These products include special bar codes that store food product information such as directions for use, consumption date expiration, etc. Product traceability, anti-theft, anti-counterfeiting and tamperproof devices are also included in this category. Indicators are called smart or interactive because they interact with compounds in the food.

MAKING VARIABILITY WORK: TOWARDS CONSERVATION OF THREATENED AND NATIVE BREED OF HALARI: THE HALARI DONKEYS

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Livestock Asset vis-à-vis Pastoralism

India's farm animal population includes cattle, goats, buffalo, sheep, donkey, camels, yaks etc.; Around 77 percent of these animals are kept in extensive systems, meaning they are either herded or left to range on their own on common land. Pastoralism represents an alternative perspective and a promising avenue for innovation in addressing a multitude of global challenges. Pastoralism is essentially multifunctional, combining food production and livelihood systems with relatively little investment from outside. Pastoral systems are described as "managing non-measurable uncertainties well beyond the capabilities of formal risk methodologies ... a key service ... foundational to the world economy in times of great uncertainty and complexity". The culture too lends economic value, albeit poorly captured by current mechanisms of appraisal. Among the collage of pastoralist cultures, with its myriad castes and communities, some 46 have distinct pastoralist identities.

Pastoralists have developed a significant proportion of India's livestock breeds: 73 out of 200 officially recognized breeds. These breeds are very special, being on the one hand very alert, resilient, hardy and independent, but also bonded to their herders in a symbiotic relationship. Halari breed of Donkey (breeding tract: Jamnagar and Devbhumi Dwarka districts of Gujarat) is one among them.

Valuing variability – Halari Breed (Donkey)

Halari Donkey is one of the important livestock in the semi-arid landscape of Saurashtra's Jamnagar and Devbhumi Dwarka district of the state of Gujarat. The Bharwad and Rabari pastoralists are the main communities which use this donkey as a pack animal to carry luggage during migration with small ruminants. These pastoralists regularly migrate to other districts. Pastoral women, generally, take care of Halari donkeys. The Kumbhar (potter) community also uses this animal for pottery work in Dwarka in the, Jamnagar region. The animals migrate for

around 8-10 months soon after the monsoon season. The donkeys are well adjusted to the management style of the Dumba/Panchali sheep breeders and the Kahami goat breeders who live a nomadic lifestyle and follow the extensive grazing system. The Bharwad pastoralists maintain the Halari donkey herd with a few elite jacks for breeding. They sell young foals and surplus donkeys at the Vautha donkey fair, an annual event organized in Vautha village of Dholka Block in Ahmedabad District. The fair attracts buyers from different regions of Gujarat and other states. This unique livestock breed is docile in temperament and can walk up to 30-40 km a day during migration.

This elegant donkey from Halar region of Saurashtra is currently in a threatened state and requires immediate steps towards conservation to reverse the declining trend in the population.

On the verge of extinction

A survey of Halari Donkey and its keepers in 2015 found that 1200 individuals of the breed were existing. However, in the recent survey conducted in 2021-22 the number dwindled to 439 individuals in its native tract. After intensive focus group discussions with the Halari donkey keepers' different challenges were brought out. Notable of them was the unavailability of Halari Donkey male for breeding, disincentives to Halari Donkey rearers with no avenues associated with streamlining the livelihoods (based on donkey milk).

In order to revivify on the issues, 154 Halari Donkey breeders across Saurashtra region of Gujarat have collaborated and is democratically evolving a Community Based Organisation inclined towards Halari Donkey Conservation.

Need to look beyond

The National Livestock Mission (NLM) has commenced from 2014-15. The Mission has been designed to cover all the activities required to ensure quantitative and qualitative improvement in livestock production systems and capacity building of all stakeholders. The mission lays emphasis on conservation of threatened breeds of livestock while encouraging community participation and involvement of community in breed conservation. In order to delve further around conservation of the Halari breed of Donkey, it is pertinent to define a road map ahead. Hence, the proposal for a seminar. All this desires concurrence with Halari Donkey breeders to confer upon the following objectives:

1. Constituting an advisory committee with participation of stakeholders for collaborative efforts;
2. Evolving programmes and schemes with dedicated infrastructure for breeding and health care;
3. Enhancing resilient livelihood strategize around Halari Donkey milk economy.
4. Optimizing tenurial security of the Halari donkey breeders with access to seasonal grazing and CFR rights.

Efforts

Community representatives – proposed community institution, Officials from Ministry of Animal Husbandry, Dairying and Fisheries (Govt. of India), New Delhi; Government of Gujarat – State Animal Husbandry department, Scientist - National Research Centre for Equines (NRCE); leading resource persons & representatives from Civil Society organization have all come together to offer an ecosystem for the conservation of Halari Donkey.

Milk Sales

No	Month	Days	Halari donkey milk (lit)
1	Mar-21	19	167.9
2	Apr-21	20	214.85
3	Jun-21	30	316.05
4	Jul-21	15	100.67
5	Mar-22	30	102.5
6	Apr-22	30	203
7	May – 22	30	212
8	June – 22	30	211
9	July -22	30	215
Total			1742.97

REPRODUCTION IN DONKEYS

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Introduction

Donkeys have been valuable to people in different cultures and countries for centuries. Donkeys are used throughout the world as guard animals for protecting small ruminants (sheep and goats), as companion animals, work (pack, pulling wagons, ploughing, etc.). Domestic donkeys usually weigh between 80 and 480 kg, and range between 80 and 160 cm in height. On average, they live 30 to 35 years and can still be actively working even at an advanced age. Donkeys are valued for their easy care, their resistance to diseases and their physical endurance. In the old days, donkeys had many more diverse uses than they do today. They were an important workforce in agriculture, commerce and militia, as well as a source of milk, hide and meat. Although mechanization of agriculture and industrialization in the last century reduced the importance and number of both donkeys and their hybrids, these animals are still heavily used as pack animals and to pull carts in some parts of the country. Currently, the use of this species has diminished. However, an increase in the demand for donkey milk and its by-products has been observed together with that of meat products that are commercialized in the European markets and as specific dietary products.

Puberty

Puberty in healthy, well-fed jennies usually occurs between the first and second year of life, but jennies should not be bred before age 3 years. Studies performed before ultrasonography described donkeys as a long-day seasonal polyestrous species in temperate latitudes. Further reports described the jenny as a non-seasonal annual polyestrous female even in temperate areas of the world.

Seasonality

Seasonality is a controversial subject and is likely influenced by photoperiod combined with other factors such as breed, nutrition, health and environmental conditions. Ovarian activity, pregnancy and parturition appear to be much less seasonal in domestic donkeys than in wild

asses despite the report that melatonin concentrations in jennies and mules are considerably higher (90pg/mL-1) than in mares (24 pg/mL-1) suggesting that melatonin concentrations are genetically determined. The interovulatory interval was shorter during the season of higher sexual activity than during the season of lower activity, corresponding to short rainy and dry seasons, respectively. The authors attributed this to a different influence from the classical photoperiod effect observed in the temperate regions. They consider that owing to forage availability being affected by seasonal rain, nutritional status is the most likely factor affecting ovarian follicular activity.

Estrus Cycle

The estrus cycle length in donkeys is 21-28 days, with the jennet sexually receptive for 5-10 days of this period. The ovulatory follicle size is similar to that in full-size equines and follicles > 25-30 mm in diameter should be considered potentially Ovulatory. The cervical appearance changes with the stage of the estrus cycle, relaxing during estrus, which is accompanied with an increase in vaginal mucous secretions. Jennets may cycle throughout the winter.

Female Sexual Behaviour

A jennet often shows her first heat at 8-12 months of age. Female receptivity is evidenced by the jennet backing up to the jack and making jawing motions with the mouth. Jennets in estrus will kick a jack in the chest (and face if the jack is not careful) for several minutes when receptive. This behavior is required for the jack to achieve full erection. Receptive jennets also squat, wink the vulva, and urinate in the presence of the jack. A receptive jennet may also raise her tail when approached by a jack. Jennets mount each other on occasion, with the estrus jennet on the bottom. Some jennets do not show receptivity when there is no jack present, when nursing a foal, or when another female interferes with the advances of the jack. A jack pursues a jennet in estrus, sometimes very aggressively, and especially when first introduced. He may bite the neck, back, and hind legs and even draw blood. As a consequence, some jacks have to wear a breeding muzzle to avoid injury to the jennet. Jacks usually calm down after a short time (15-30 minutes) and then the muzzle may be removed. It is easy to teach most jacks to mount without biting the jennet by using a chain lead shank over the nose. Vigorous jerking motions on the chain along with verbal commands usually result in a quick learning process for the jack.

Donkeys are intelligent animals and respond well to consistent, firm training methods. External manifestation of sexual receptivity or rejection by jennies occurs upon visual and especially tactile contact with a jack. Jennies in estrus may lower their head with their neck extended forward, open and close their mouth, lay their ears back against their neck, splay their hind legs, raise their tail away from the perineum and present the perineum toward the jack. They usually vocalize after the jack vocalizes and this seems to play a role in the communication between the male and the female in estrus. Winking of vulva lips and eversion of the clitoris before urinating, signs that are frequently seen in mares in estrus, are more discrete in jennies and less frequently seen. However, the most distinctive aspect of estrus manifestation consists in movements of the mouth known as jawing, yawing, or clapping. This is the opening and closing of the mouth with relaxed lips, with head and neck lowered and extended forward and ears laid back against the neck, accompanied by a characteristic sound. They may show an ambivalent behavior moving forward a few strides away from the jack, then suddenly stop and stay in solid estrus stance, with abbreviated double hind-leg lifts reminiscent of kick threats and mild tail swishing and hip swaying. When they are in diestrus, jennies may run away from the jack, turn away and move off rather than stop abruptly, sway their hips side-to-side and away from the jack, clamp the base of the tail against the perineum while swishing the distal portion of the tail, kick out with full extension of both hind legs, make a grunting vocalization and bite the jack. A jennet may back up to the jack after ovulation, but he may not mount again if he has recently bred the jennet by natural cover or through collection for artificial insemination. Normally, jacks mount jennets a few times before fully erect, and some jacks are slow to achieve erection (10-15minutes).

Female Reproduction

The jenny is similar to the mare in several reproductive aspects. They reach puberty at one or two years of age, although showing less seasonality than mares. The vagina is approximately 14 inches long in the average miniature donkey. The cervix lies on the cranial vaginal floor and has a similar appearance to that of the horse with the exception that it often points upwards and is usually longer than that of the mare and with a smaller diameter.

A longer estrus cycle is reported, with a range of 20 to 40 days (average 24 ± 1 days vs. 21 ± 1 days in the mare). Estrus may last 6 to 9 days, with ovulation occurring in the last 24 hours. Dominance is established when follicles are about 25 mm in diameter. Depending on the

reproductive stage, up to 13 follicles of different sizes can be observed per ovary. Follicular growth averages 2.7 mm per day and ovulatory follicle diameter may vary according to the breed.

The interovulatory period is longer than in mares, 24.9 ± 0.7 days for standard jennies and 23.3 ± 2.6 days for mammoth jennies (vs. 21 ± 1 days in the mare). The average life span of the CL ranges between 15 to 20 days (19.3 ± 0.6 in standard Jennies; 17.4 ± 2.6 days in mammoth Jennies. Sometimes corpus lutea may exhibit a homogeneous echotexture the first day after ovulation and in other cases, Jennies show a CL with a non-echogenic central area. Transrectal ultrasound examination is possible with a linear probe taped within a plastic extension in miniature donkeys when the arm is too large to be inserted through the anus. Ultrasound is useful to examine ovarian follicle size for estimation of the opportune time to breed, and then for detection of pregnancy starting at 16 days after breeding.

Field breeding

Jack owners most commonly use field breeding by turning one jack out with as many as 10 jennets. The jack selects the most receptive jennet to breed by checking the herd's manure for pheromones that may be involved in estrus detection. Receptive jennets may also approach a breeding pair to attract the jack.

Hand breeding

Controlled, hand or "appointment" breeding allows for recording of exact breeding dates. The breeding cycle may be coordinated with serial transrectal ultrasound examinations of the ovaries to determine the time of ovulation and the best time to breed. Jacks may be overly aggressive or timid when first training them to mount in controlled situations; however, they are fast learners. Estrus synchronization protocols useful in horses seem to be effective in the donkey. If hand breeding is used it is suggested that the jennet be bred on the second day of estrus and then at 48-hour intervals until the end of standing heat.

Gestation and Foaling

Gestation length ranges between 333 to 395 days, with an average of 371 days. Pregnancy length seems to be affected by foal gender, with cases of longer pregnancies in jennies bearing male fetuses (376 days) compared to pregnancies bearing females fetuses (371 days). Other aspects, such as the year of foaling, the month of ovulation and parturition, the birthweight of

the foal and the age of the jenny, did not significantly influence pregnancy length in Poitou jennies. Thus, duration of gestation became shorter as the breeding season advanced.

Jennies have a diffuse epitheliochorial placenta, as do mares, weighing a 12% of the neonate's birth-weight. However, despite microcotyledons being more complex than those of mares (showing a greater degree of ramifications in the jenny), they are less efficient in terms of kg of foal birth-weight per square meter of feto-maternal contact.

The duration of the normal foaling stages has been reported to be similar between horses and donkeys (first stage: 63 vs 65 min; second stage: 20 vs 17 min.; third stage: 60 vs 58 min. respectively). However, in the normal, mature and viable donkey foal, the average time to stand up (61 min.), to nurse for the first time (101 min) and for elimination of meconium (86 min), were longer when compared to data for horse foals (34 min, 51 min and 63 min respectively).

The foal heat usually occurs between 5 and 13 days postpartum and, did not seem to be affected by environmental conditions. In Martina Franca jennies, estrus duration, follicular development and follicular preovulatory diameter were similar between jennies during the foal heat and jennies in their third estrus after foaling. The pregnancy rate at day 14 was significantly lower in the foal-heat jennies although it increased when ovulation occurred with an interval greater than 12 days after foaling (85.7%).

Male Reproduction

The jack or jackass, like the jenny, shares many reproductive similarities with the horse. However, some differences do exist. Although ultrasonographic features of the reproductive tract are similar, Jack reproductive organs are reported to be larger than those of equine stallions [6]. During erection and ejaculation, the glans penis presents a more pronounced dilation and the accessory glands also are bigger, perhaps due to the con The genitalia of a full-size donkey is similar to that of the full-size horse. The testes of the donkey are relatively large for the size of the animal. They are located in the scrotum and oriented vertically. The penis length of a miniature donkey is approximately 14-18 inches when fully erect, and thus a full-sized equine AV is used for semen collection. The testes and penis of larger sized donkeys are larger than that of comparably sized horses. Although the jack and the stallion have the same accessory sex glands, the ampulla is larger in the jack than in the stallion of comparable size.

Another characteristic of jacks is that they take longer to achieve erection and to ejaculate. They may complete mating in anything between 5 to 30 minutes, which is fairly long when

compared to the 10-11 minutes it takes stallions. Teasing behavior includes vocalization, sniffing the vulva of the female, flehmen and one or more mounts before penile exposure and erection. Although some seasonal differences in libido are observed, there seems to be little to no alteration of the seminal parameters during the winter months.

Male Sexual Behaviour

Vocalization also occurs during this first contact. Thereafter, jacks do amore thorough individual teasing of jennies in estrus. A mount without erection, perhaps preceded by brief naso-nasal contact and sniffing the perineal region of the female, initiated most of the courting. Dismounting occurred after 10 to 30 seconds and courtship proceeded to the occurrence of breeding. During the courtship the jacks nibbled the head, neck, knee and flank and sniffed parts of the jenny's body, including the perineal area. Flehmen responses of the jack were observed after sniffing the perineal area or urine and/or feces on the ground. A striking behaviour that has been observed is the sudden retiring of the male to graze or stand at a relatively short distance from the female. During this period, partial or total letdown of the penis was frequently seen. This period ended with the jack approaching the female again to start another cycle of teasing. At pasture it has been observed that jennies play an active role during the teasing periods, systematically approaching the male. In both management systems used, the male opted to retire from the female to attain erection. This was usually observed in all studies that were carried out. Once erection is reached, the jacks return to the female to breed. At pasture, the female bred was not always the closest one. Once close to the jenny, the jack usually first smelled the perineal area and mounted immediately thereafter. Vocalization by the male was the signal to reinitiate periods of courtship.

Artificial insemination

Artificial insemination (AI) is possible on the farm or with transported fresh, cooled semen. Jacks are easy to train for collection although this technique is not currently used on a widespread basis. Donkey semen is very concentrated and in general has good fertility. It can be handled similar to that of the stallion, and skim milk extenders seem to be useful to keep sperm alive during transport in artificial insemination programs.

Summary

The reproductive anatomy of the male donkey is similar to that of the standard horse. The same applies for the female, including the size of the reproductive tract. Female donkeys have a 21-

28 day estrus cycle with obvious behavioral signs of receptivity when a jack is present. Lactation may stop a donkey from cycling while other females (related or not) may interfere with displays of sexual receptivity and breeding. Pasture and hand breeding are both used in donkeys. Artificial insemination can be performed successfully in donkeys although not widely accepted by owners at this time. Transrectal and transabdominal ultrasound are safe and effective methods for reproductive examinations in donkeys.

References

1. Pugh DG. Donkey Reproduction. Proceedings of the Annual Convention of the AAEP 2002; 113 -114.
2. Ginther OJ. Reproductive biology of the mare. 2nd ed. Cross Plains, Wisconsin: Equiservices, 1992.
3. Tibary A, Sghiri A, Bakkoury M, Fite C. Reproductive patterns in donkeys. Proceedings of the 9th International Congress of World Equine Veterinary Association. Reprinted in IVIS with the permission of the Conference Organizers <http://www.ivis.org>.
4. Taberner E, Medrano A, Peña A, Rigau T, Miró J. Oestrus cycle characteristics and prediction of ovulation in Catalanian jennies. Theriogenology 2008; 70(9): 1489-97.
5. Arthur GH. Endogenous and exogenous control of ovarian cyclicity. In: Arthur's veterinary reproduction and obstetrics. (Noakes, D.E., Parkinson, T.J., England, J.C.W. editors), 8th edition. Saunders Harcourt Publishers, Elsevier Science Limited, pp. 3–51.
6. Derar RI, Hussein HA. Ovarian Follicular Dynamics during the Estrous Cycle in jennies in Upper Egypt. SAGE Hindawi Access to Research. Veterinary Medicine International, 2011, 6.
7. Kebede H, Lemma A, Negussie H. Ultrasonographic studies on ovarian dynamics and associated estrus manifestations of jennies under controlled management, Ethiopia. Trop. Anim. Health Prod. 2012; 44(8): 1965-70.
8. Lemma A, Schwartz HJ, Bekana M. Application of ultrasonography in the study of the reproductive system of tropical Jennies (species *Equus asinus*). Trop. Anim. Health and Prod. 2006; 38, 267–274.

9. Purdy SR. Artificial Insemination for Miniature Donkeys. In: Veterinary Care of Donkeys, Matthews NS and Taylor TS. (Eds.) International Veterinary Information Service, Ithaca NY (www.avis.org), 20-Apr-2005; A2924.0405.
10. Vanderplassche GM, Wesson JA, Ginther OJ. Behavioral, follicular and gonadotropin changes during estrous cycle in donkeys. *Theriogenology* 1981; 16: 239-249.
11. Blanchard TL, Taylor TS, Love CL. Estrous cycle characteristics and response to estrus synchronization in mammoth asses (*Equus asinus americanus*). *Theriogenology* 1999; 52: 827-834.
12. Ginther OJ, Scraba ST, Bergfelt DR. Reproductive seasonality of the jennies. *Theriogenology* 1987; 504 27: 587–592.
13. Henry M, Figueiredo AEF, Palhares MS, Coryn M. Clinical and endocrine aspects of the oestrous cycle in donkeys (*Equus asinus*). *J. Reprod. Fertil.* 1987a; 35: 297–303, Suppl.
14. Guillame D, Zarazaga LA, Malpoux B, Chemineau P. Variability of plasma melatonin level in pony mares (*Equus caballus*), comparison with the hybrid: mules and with jennies (*Equus asinus*). *Reprod. Nutr. Dev.* 2006; 46: 633-639.
15. Galisteo J, PerezMarin CC. Factors affecting gestation length and estrus cycle characteristics in Spanish donkey breeds reared in southern Spain. *Theriogenology* 2010; 74(3): 443-50. Contri A, Robbe D, Gloria A, De Amicis I, Veronesi MC, Carluccio A. Effect of the season on some aspects of the estrous cycle in Martina Franca donkey. *Theriogenology* 2014; 81(5): 657-61.
16. Carluccio A, Gloria A, Veronesi MC, De Amicis I, Noto F, Contri A. Factors affecting pregnancy length and phases of parturition in Martina Franca jennies. *Theriogenology* 2015; 84(4): 650-5.

COLLECTION AND CRYOPRESERVATION OF JACK SEMEN

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The history of semen freezing goes back to discovery of Polge *et al.* (1949) that bull sperm cells can survive freezing (cryopreservation). Only a year later researchers successfully froze stallion spermatozoa, and in 1957 the first pregnancy from frozen stallion sperm was reported in Guelph, Ontario. For economic reasons most of the early research was focused on bull semen, but in the last 15 years the successful and growing use of frozen semen in bulls encouraged many to carry out further research into cryopreservation of equine semen (Leopold Sybil, www.livinglegends.org.au). The utilization and importance of frozen semen is increasing in artificial insemination (AI) as horse breed registries are now allowing foals born through AI (Loomis, 2001) with frozen semen and due to advances in semen cryopreservation techniques. In addition, pregnancy rates have been shown to be equal or even higher after AI with fresh or chilled semen compared to natural mating (Samper *et al.*, 1991). Though, to improve reproductive efficiency when using cryopreserved semen, attention should be given to factors such as the stallion, the quality and handling of the semen, and age as well as reproductive history and management of the mare (Allen, 2005).

Artificial insemination (AI) was first started as early as in 14th century as per Arabic scriptures in the horse. First systematic exploitation of this technique took place with horses around the start of the 20th century. Although it was known by 1776 that cooling semen held the spermatozoa in a dormant state and thereafter followed a huge interest in the physics of cold. Cryopreservation of spermatozoa remained an enigma until the discovery of glycerol. The cooled semen is very successful for short-term storage and use but long term storage by this method is not possible. In order to realize many of the potential advantages of AI, long term storage is necessary. This has become possible by freezing, a system that halts the metabolic processes of the spermatozoa, allowing indefinite storage without loss of fertility. The discovery of cryoprotectant properties of glycerol made cryopreservation of semen possible. As a result, the spermatozoa of many species can now be stored at -196°C in liquid nitrogen for long duration without losing its fertilizing ability. There are several advantages of semen cryopreservation which are listed as under

- Allows the long term preservation of stallion semen.

- Enables the establishment of a semen bank.
- Enables introduction of superior germplasm of stallion even several years after death.
- Enables a major expansion in international trade.
- Plays important role for controlling the sexually transmitted diseases from stallion to mares.

Principle

Cryopreservation of semen is a technique where sperm cells being preserved are exposed to sub zero temperature such as -196°C. This technique is based on principle to minimize biological activities of sperm cells to its least and thus production of negligible amount of waste products; by gradual cooling to subzero temperature. Media which is used to dilute sperm cells helps to supplement energy, nutrient requirement and also resist change in its pH. To save sperm cells from adverse effect of subzero temperature some cryoprotectant/s alone or in combination are added in media used for semen dilution otherwise lead to sperm cell death. However, the sperm cells exposed to low subzero temperature often get damaged to approximately 50 percent during the process of freezing and thawing even after addition of cryoprotectant mainly due to intra and extra-cellular ice crystal formation, solution effect and cell dehydration. Initially the basic procedures for cryopreservation of bull spermatozoa were applied to stallion spermatozoa, but soon researchers realized that stallion spermatozoa are much more fragile and therefore more difficult to freeze and thaw than semen from other species. In the last 20 years scientists from several countries have been working at developing and improving the techniques used to successfully freeze stallion spermatozoa. The use of cryoprotectant such as glycerol, first identified for use with bovine spermatozoa, prevents some of the damage. However, the success of cryopreservation depends upon many other factors, including interactions between cryoprotectant, extender, cooling rate, warming rate and packaging, as well as individual variation, which appear to be particularly significant in the stallion. Some loss in spermatozoa viability must be expected, to the processing procedures prior to freezing as well as the actual freezing process.

Collection of semen: Semen is collected by means of artificial vagina (AV) over an estrus mare/jenny or dummy mare. In case dummy is not available and using an estrus female, her tail is bandaged; perineal area is washed and cleaned after proper restraining. The operator holding AV and the animal handlers should be alert and take necessary precautions to protect themselves from mare as well as stallion. Use of helmets during semen collection is an added precaution

while stallion is mounting and dismounting. The penis of the jack/stallion is washed with luke warm water before mounting. Just after dismounting penis is given flush of mild betadine/antiseptic solution. Ejaculation is completed in 15 to 20 seconds marked by pulsation at the base of penis and flagging of tail.

The AV is prepared by assembling its parts and filling hot water within the space provided through nozzle with help of funnel. The optimum temperature of AV is maintained 42 to 45°C and lubricated well with liquid paraffin or vaseline before use. Higher temperature of AV may cause irritation to stallion penis and cause damage to sperms. The pressure inside AV is such that the stallion is able to penetrate penis with ease. A pre-warmed graduated collection bottle covered with thermo-jacket is attached to one end with AV to accumulate an ejaculate and to prevent sperm from thermal shock. Ejaculation occurs into the lumen of the AV lined with a disposable plastic liner and is collected into graduated collection bottle. There are three major types of AV's such as Colorado, French (INRA) and Missouri model that satisfy these criteria in slightly different ways. Because of large size, comparatively heavier and vigorous thrust by the stallion at ejaculation, the AV used has a handle to hold it firmly.



Fig. Preparation of Artificial vagina and Semen collection from a Jack

Removal of gel and evaluation: Immediately after collection, remove of gel fraction by filtering through gauge or fit IMV gel filter in AV to get gel free semen. Small sample of gel free semen is evaluated for its quality i.e. initial motility, progressive motility, pH, sperm concentration, live-dead count and abnormality of sperms etc.

Extenders used for frozen semen: Selection of extender for cryopreservation of semen is a very important for successful freezing. After observing deleterious effects of seminal plasma on semen freezability, double diluents procedure was proposed. It is common practice today that

the preparation of semen for cryopreservation involves the use of two extenders, a primary extender for initial dilution, which is aspirated off after centrifugation, prior to the addition of a secondary extender and freezing. There are numerous primary or secondary extenders in use with varying success rate.

Primary extenders: The prime function of a primary extender is to maintain motility, but it also acts to protect spermatozoa during the process of centrifugation. There is no requirement of cryoprotectant in such extenders. Examples of primary extenders are BSA primary extender (Samper, 1995), Glucose-EDTA primary extender-I (Cochran *et al.*, 1984), HF-20 primary extender (Nishikawa, 1975), skimmed milk and sugar primary extender (Pickett and Amann, 1993) and EDTA-Glucose primary extender-II (Cochran *et al.*, 1984). National Research Centre on Equines using Glucose-EDTA primary extender-I in our studies as reported by Cochran *et al.* (1984).

Secondary extenders: Many of the secondary extenders are similar to the primary extenders used but in order to protect spermatozoa from cold shock, the addition of a cryoprotectant is needed. Most of the extenders use glycerol as the major cryoprotectant. The use of glycerol may be detrimental to spermatozoa viability. A compromise has to be reached in regards to concentration of glycerol (3-5%) and the length of the time that the glycerol is in contact with the spermatozoa prior to freezing, in order to maximize the beneficial affects of glycerol as a cryoprotectant but minimize the toxic effects. Nagase (1967) reported that the optimum effect of glycerol was obtained with an equilibration time of 3-5 hour. National Research Centre on Equines using glucose-lactose-EDTA egg yolk as secondary extender as reported by Cochran *et al.*, (1984).

Dilution of semen: The aim of dilution is to store semen samples at appropriate concentration and a sufficient volume for immediate insemination without any further treatment. Adequate extension is ensure maximum sperm survival whereas excessive dilution results in low concentration that require large volume to be inseminated for acceptable fertilization rates. Usually 400-500 million progressive sperm per insemination/4 ml diluted semen is required in order to have optimal fertilization. The sperm concentrations of 200 millions/ml after final dilution with modified secondary extender are recommended. The recent development in horse AI is low dose insemination (5 to 100 million progressive sperm) to minimize the amount of semen used.

Dilution rate depends upon the concentration and motility of the semen sample. Adequate extender is required to ensure maximum survival rate and in general, the greater the dilution, the greater is the survival potential (Samper *et al.*, 1988). However, excessive dilution results in low semen concentration and it may require large volumes to be inseminated to ensure that adequate numbers of spermatozoa are inseminated for acceptable fertilization rate. It is generally agreed that 100 to 500x10⁶ spermatozoa (normally 250x10⁶ are used) are required in order to obtain optimal fertilization rates (Pace and Sullivan, 1975). Insemination of volume in excess of 100 ml has been reported to be associated with depressed fertility rate (Rowley *et al.*, 1990).

Filling of straws: Mostly, the diluted semen is packaged into 0.5 or 0.25 ml straws. Some laboratories practice a single dose in 2.5 or 5 ml macro tubes before freezing. The volume of semen and size of packaging is related to convenience and the freezing success. There are specific cooling rates to perform while semen freezing and once frozen, the straws can be stored in liquid nitrogen (-196°C) for an indefinite period.

Freezing of semen in programmable cryo-freezer

The freezing rate or rate of cooling for semen depends upon the method of processing and storage. The straws were loaded in automatic cryo-freezer for cooling as per following protocol.

- Cooling of semen @ 0.3 °C min⁻¹ from 18 °C to 5°C. Hold for 10 minute at 5°C.
- Cooling @ 10°C min⁻¹ from 5°C to -15°C.
- Cooling @ 19°C min⁻¹ from -15°C to -100°C. Hold for 10 minute.
- Plunge into LN₂ container for storage.

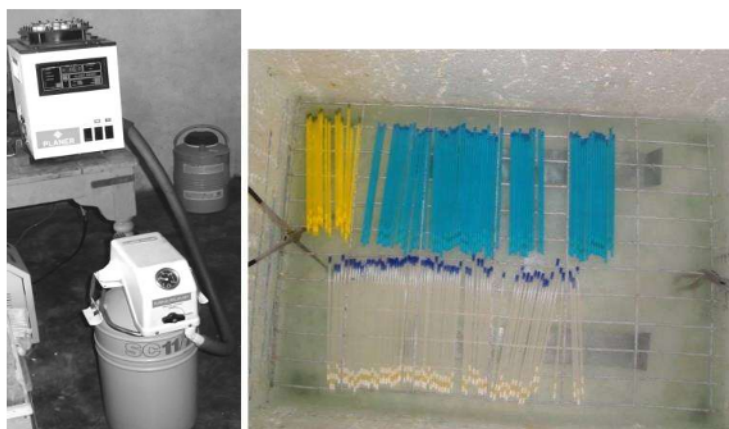


Fig. Programmable Freezer Fig. Vapour freezing/Customized freezing technique.

Semen freezing on LN₂ vapours

Method of semen cryopreservation was already explained in the above part but it needs programmable semen cryo-freezer / bio-freezer. However, another easy and simple method of semen freezing are available for laboratory as well as field conditions, where there is no need of cryo-freezer / bio-freezer. This technique is easier over earlier explained method as it consumes less LN₂ for the charging; also the cryo-freezer is a costlier instrument and consume more LN₂. In this technique, we put the semen filled straws on LN₂ vapours for 8- 12 minutes at a certain height and then directly plunge in the liquid nitrogen and finally shift these straws into LN₂ filled container for storage. Even small veterinary hospitals can cryopreserve the semen using this technique without involving sophisticated instrument like cryo-freezer.

References

- Cochran, J. D, Amann, R. P, Froman, D. P. and Pickett, B. W. (1984). Effect of centrifugation, glycerol level, cooling to 5°C, freezing rate and thawing rate on the post thaw motility of equine spermatozoa. *Theriogenology* 22, 25-38.
- Graham, J. K. (1996). Methods for induction of capacitation and the acrosome reaction of stallion spermatozoa. *Veterinary Clinics of North America, Equine Practice* 12(1), 131-147.
- Loomis, P. R. (1993). Factors affecting the success of AI with cooled, transported semen. *Proceeding of the 38th Annual Convention of the American Association of Equine Practitioners* 2, pp. 629 - 647.
- Mazur, P. (1984). Freezing of living cells. Mechanisms and implications. *American Journal of Physiology* 247, C125 - C142.
- Nagase, H. (1967). Studies on the freezing of stallion semen. II. Factors affecting survival rates of stallion spermatozoa after freezing and thawing and results of fertility trial. *Japanese journal of Animal Reproduction* 12, 52-57. *ABA* 35, 195.
- Nishikawa, Y. (1975). Studies on the preservation of raw and frozen horse semen. *Journal of Reproduction and Fertility, Supplement* 23, 99-104.
- Pace, M. M. and Sullivan, J. J. (1975). Effect of timing of insemination, numbers of spermatozoa and extender components on the pregnancy rates in mares inseminated with frozen stallion semen. *Journal of Reproduction and Fertility, Supplement* 23, 115-121.
- Pickett, B. W. and Amann, R. P. (1993). Cryopreservation of semen. In *Mckinnon, A.I. and Voss, E.L.(eds) equine Reproduction Lea and Febiger, Philadelphia*, pp. 768-789.

- Polge, C., Smith, A. U. and Parkes, A. S. (1949). Revival of spermatozoa after vitrification and dehydration at low temperature. *Nature* 164, 666.
- Rowley, M. S., Squires, E. L. and Pickett, B. W (1990). Effect of insemination volume on embryo recovery in mares. *Equine Veterinary Science* 10. 298-300.
- Salisbury, G. W., Van Denmark, N. L. and Lodge, J. R. (1978). Part 2: The storage and the planting. In: *Physiology of Reproduction and AI of Cattle*, 2nd edn. W.H.Freeman, San Francisoco, pp. 187-578.
- Samper, J. C. (1995). Stallion semen cryopreservation: male factors affecting pregnancy rates. *Proceedings of the Society for Theriogenology*. San Antonio Texas, pp. 160-165.
- Samper, J. C., Loseth, K. J. and Crabo, B. G. (1988). Evaluation of horse's spermatozoa with sephadex filtration using three extenders and three dilutions. In: *Proceedings of the 11th International Congress on Animal Production and Artificial Insemination*, June 26-30, Vol. 3, Brief communications, 294. University College Dublin, Republic of Ireland.

DONKEY FARM MANAGEMENT PRACTICES FOR OPTIMUM PRODUCTION

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The equids have been a symbol of power, strength, bravery and national pride as they provided power of fast movement and transport since ancient times. Our country is home track to seven distinct indigenous horse (*Equus caballus*) breeds, namely Marwari, Kathiawari, Katchi-Sindhi, Zanskari, Bhutia, Manipuri and Spiti as well as the indigenous donkeys (Spiti and halari) and wild asses. Equines being an important animal for draught and transport play a significant role in rural economy and constitute source of income for sizeable underprivileged section of society in many parts of the country. The ponies, mules and donkeys have unique utility in hilly areas as pack and riding as well as in sports. Advent of mechanization has decreased the utility of equids drastically and at a higher pace.

Equine Scenario in India

The equine population of the country is dwindling at a very fast rate. The 19th and 20th Livestock Census (2012-2019) data reveals that the population of horses, donkeys and Mules in the country is 0.34 m, 0.12 m and 0.08 m and it has gone down by 45.58%, 61.23% and 57.09% respectively. Collectively this amounts to 52.71% reduction in the population of equines.

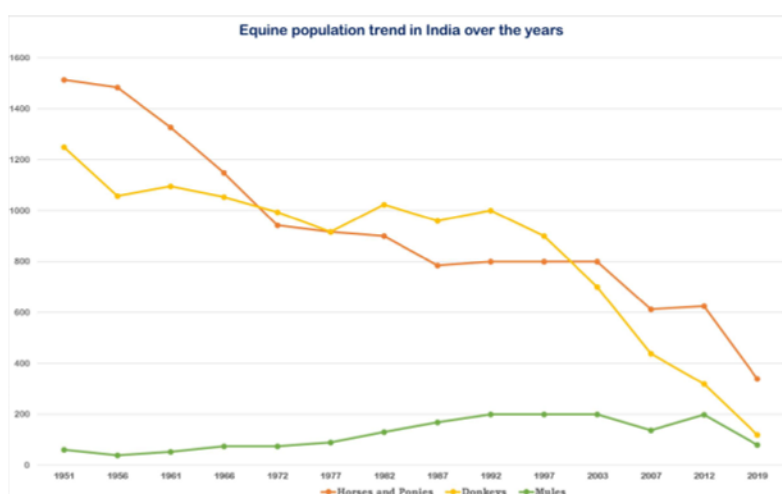


Fig. 1. Equine population trend in India

Approximately, 98% equines in India contributes to the employment and income of the poor farmers and landless laborers mainly through utilization of equines in cart and carriage. The remaining about 2% of the equine population is owned by elite sections of society and is used for sports such as racing, polo and for national security purpose by military and paramilitary forces.

Tab.1. Population trend in Indian horses and ponies

Horses, Ponies, Mules & Donkeys Population 2012 and 2019

Category	Population (In million) 2012	Population (In million) 2019	% Change
Horses & Ponies-Total	0.62	0.34	-45.58
Mules-Total	0.20	0.08	-57.09
Donkey-Total	0.32	0.12	-61.23
Total	1.14	0.54	-52.63

Tab 2. Equine population in major states of India

Horses & Ponies Population 2012 & 2019 of Major States

S.No.	States	Population (In Lakhs) 2012	Population (In Lakhs) 2019	% Change
1	Uttar Pradesh	1.52	0.76	-50.14
2	Jammu & Kashmir	1.44	0.63	-56.17
3	Rajasthan	0.38	0.34	-10.85
4	Bihar	0.49	0.32	-34.13
5	Gujarat	0.18	0.22	19.42
6	Maharashtra	0.37	0.19	-49.33
7	Punjab	0.33	0.14	-56.66
8	Madhya Pradesh	0.19	0.13	-29.48
9	Assam	0.14	0.13	-9.68
10	Haryana	0.37	0.10	-73.58

Donkey management:

Management of any livestock species is important and the same applies to the donkeys and other equines too. The equines are companion animals. These animals require attention and expect love and affection from the accompanying person. One should understand these basic

things before going closer to it. However, apart from scheduled vaccination, health care, nutritional care, the housing also need proper attention. Providing enough space, looking at the compatibility of the animals with each other when housed in same enclosure, physiological status, age, sex etc. needs attention. In order to reduce the stress, the housing should be such that it protects them from extreme heat during summer and extreme cold during winter. This is especially more important when someone is rearing the animals in *ex situ* conditions, such as rearing of Poitou donkeys in Rajasthan having extremely hot climate and vice versa. Quality of water, availability of green fodder, proper amount of concentrate, macro-and micro-minerals is also very important. Daily grooming and exercise is also very important. Housing at elevated -dry places having proper drainage is also very much important. Reptiles, especially the snakes, eat rats which are generally found close to the feed and fodder go downs. So, if possible, the feed storage should be at a distance from the barns of equines.

Likewise the management of different categories/ age group of donkeys some important management practices should be followed:

Care of new born foal: The proper care of newborn foal is actually started before its birth because the good health of newborn foal depends on health and body condition of dam. Therefore, it is necessary to provide good quality feed and fodder especially during last quarter of gestation, some extra concentrate should be offered to pregnant Jennies to gain their body condition which helps in milk production for feeding the foal. The advanced pregnant donkey mares should be separated from other donkey mare to avoid any type of injuries. The jenny should be shifted to cleaned/disinfected foaling shed near to foaling dates. The jenny should be closely watched by the attendant for foaling. Generally foaling takes place without any help but attendant should be vigilant and if any difficulty in foaling, he should do the needful and if there is serious problem the trained veterinarian should be called immediately. Just after foaling, the nostrils of newborn foals should be cleaned with clean soft cloth for proper breathing and properly examine the foal. The umbilical cord should be cut with sterilized blade and tincture iodine may be applied properly to avoid any type of infection from surroundings. The newborn foal should be fed first milk/ colostrum as early as possible.

Normal foaling parameters of a jenny:

- ✚ Normal gestation 350-370 days
- ✚ Normal birth within 30 min. with no manipulation
- ✚ Suckle reflex within 20 minutes
- ✚ Standing within 2 hours

Nursing within 3 hours

Neonatal examination: A newborn foal is less than one week old. The normal rectal temperature of newborn foal is 99.5-102°F, which is higher than an adult donkey. Foal does not regulate body temperature very well for the first few days of life, it is therefore, important to provide foal proper protection against the existing outside weather conditions and environment.

A foal is considered hypothermic when its rectal temperature is less than 99°F. It will expend a lot of energy trying to keep body warm and thus become weak quickly. The gastro-intestinal motility reduces markedly if the foal is cold. Such foals neither digesting milk and nor absorbing energy and calories. They become weak and dehydrated, and body condition rapidly deteriorates. This can happen in just a few short hours after birth. Therefore, foal should be kept dry and warm. In summer season hot environment, foal can become hyper thermic (body temperature greater than 103°F) and heat stressed. Foal start hyperventilating to blow off extra heat. Since newborn do not regulate its body temperature, they do not sweat in response to hot outside temperatures. Hyperventilating also expends a lot of energy and the foal can quickly become dehydrated. If the body temperature is greater than 104°F, repeated cool water baths and shade should be implemented immediately.

A septic foal may have a fever, but its body temperature usually is not as high as a hyper thermic foal. Septic foal has signs of illness, like diarrhea, weakness, decreased appetite, depression, and swollen joints or navel. A normal foal passes its first feces (meconium) a dark brown, firm fecal balls within three hours of birth and continue to pass multiple small piles of meconium. All of the meconium should be passed within 24 hours. If a foal show sign of straining a lot, Fleet enema should be given to assist the foal to pass the meconium. When all of the meconium has passed, foal start to defecate a thick, pasty, orange colored feces which is a good sign indicating that milk has made its way through the entire digestive tract. In newborn foal, lax tendons are found commonly when the muscle/tendon unit is weak resulting in low fetlocks and pasterns, and this condition do not need veterinary attention. Contracted tendons need veterinary attention depending on the severity, when the pasterns, fetlocks, and/or knees are extremely upright or buckled forward. The cause of this is due to the mal positioning of fetus in the uterus, a few days of exercise and stretching in a small paddock will usually correct the problem.

Colostrum: Soon after foaling donkey mare produces a thick milk, which is called colostrum. Colostrum is very important for new born because it is rich in antibodies, protein and calories.

These antibodies protect the foal against environmental diseases for the first few months of life.

Foal risk factors

The following are risk factors in the foal that may lead to problems

- ❖ Foal doesn't stand or nurse within normal time period
- ❖ Lack of suckle or poor affinity for mare cause is Neonatal Maladjustment Syndrome
- ❖ Colic
 - Diarrhoea - foal should not have diarrhoea the first few days of life.
- ❖ Abnormal temperature, heart rate and respiration rate
- ❖ Swollen joints or navel - a sign of septicemia
- ❖ Navel doesn't dry up/stays moist, maggoted wound

Common Problems in Newborn Foal

Septicemia

The common cause of newborn illness is septicemia, which is very serious, and life threatening for a foal. The signs of septicemia are lethargy, diarrhoea, hypothermia, fever and weakness, cessation of suckle reflex, milk face when a foal bumps the mare's bag but does not suckle and milk squirts all over the foal's face. Septicemia may occur when a foal is exposed to bacterial infection through umbilicus, placenta, respiratory or digestive tract and most common causative agents of septicemia are *E. coli*, *Actinobacillus*, *Klebsiella*, and *Streptococcus*.

Ruptured Bladder

A ruptured bladder may occur during the foaling process. When the foal is pushed through the birth canal, the bladder may tear internally inside the foal. It is common in colts (90%). The foal appears normal at birth, but depending on the size of the tear, the foal will subsequently show signs of uroperitoneum (urine in the abdomen) between days 1 and 7 of birth. The signs of ruptured bladder are colic, depression, abdominal distension, and urine dribbling. The foal may or may not be able to urinate, while some can urinate, but tend to strain.

Failure of Passive Transfer (FPT):

Failure of passive transfer is the failure of the transfer of immunoglobulins (IgG). Such foal's IgG levels are less than 400 mg/dl. IgG levels between 400-800 mg/dl are considered partial failure of passive transfer. The causes of failure of passive transfer are poor colostrum quality,

failure to ingest enough colostrum, poor mammary gland development, or the mare leaking the colostrum before foaling. If the foal's IgG <400 mg/dl, the foal has a 25% chance that it will contract a disease if exposed. If the foal's IgG < 200 mg/dl, the foal has a 75% chance it will contract an environmental disease if exposed.

Management of Orphan foal:

Foal whose dam has not accepted it or has passed away due to one or other reason, such foal can be provided cow's or goat's milk as an alternative to mare's milk, but better results are usually obtained with the use of milk replacers specifically designed to replace the dam's milk. Goat milk can be used successfully since they are the perfect "foster mother" for most mammalian species. Goat's milk tends to be more palatable to foals than cow's milk and causes fewer digestive upsets. A nurse mare, preferably with a good disposition, is best for the overall care of an orphan foal. The amnion and/or placenta of a mare who has lost a newborn foal can be placed over the orphan foal to increase the mare's acceptance of the foal. The mare and foal should not be left unattended until the mare has accepted the orphan; physical or chemical restraint of the mare may be required initially and repeated on several occasions before she will accept the new foal.

Artificial mare's milk diets and goat's milk have also been used successfully to feed orphan foals. Foals should be fed every 1-2 hr for the first 1-2 days of life, then every 2-4 hr for the next 2 week at the rate of 250-500 ml per feeding, using a warmed milk container and an artificial nipple. Of the various artificial nipples available, those designed for use by lambs are best suited for foals. The feeding intervals may be lengthened gradually after 2 wk; however, the amount per feeding also should be increased so that the foal consumes 10-15% of its body wt/day. A foal should be encouraged to drink freshly prepared milk out of a bucket, *ad lib*, early in life. After 1 mo, the foal can be encouraged to eat grain mixes (with 18% crude protein designed for growing foals) and good-quality hay in addition to the milk or milk replacer. The foal can be weaned off the milk replacer at 3 months of age. Fresh water should be available to the foal at all times from birth

Nutritional Management of growing foal:

An important role in early foal management is creep feeding as foals increase in age, their dependence on solid food increases while milk intake decreases. The donkey mare's milk is providing only about 50 percent of the protein and energy the three month old foal requires. It

is necessary to provide creep feed to the foal to meet the foal's nutrient demand for optimum foal growth. Foals may be offered creep feed as early as two to three weeks of age. However, very little consumption will take place at this time. Initially, foals will start to nibble and play with the creep feed before reaching normal consumption levels. Fresh creep feed should be put into the feeder daily. Creep feed left in the feeder that day can be fed to mares or other horses if it has not become sour or moldy. The major considerations of a good creep feed include high quality protein, moderately high energy levels and adequate calcium and phosphorus amounts in the proper ratios. Presented below is a creep ration that is highly palatable and nutritionally adequate

Example Creep Feed Ration

Ingredient	% of Ration
Oats, crimped	50.5
Corn, Cracked	23.0
Soybean Meal	20.0
Molasses	5.0
Ground Limestone	0.9
Dicalcium phosphate	0.6
	100.0

Weaning:

Weaning of foals can be done at the age of 4-6 months depending on the purpose of rearing the mares. The foals should be separated in different pens from their dams. The care should be taken before separating the foals from their dams, the foals should have habit of eating of feed and fodders well before separating. Creep feeding should be adopted for feeding the foals at the age of 1.5 to 2 months to avoid weaning stress to foals. The male and female foals should be housed in separate pens in groups after the age of six months. The special attention should

paid to newly weaned foals. The females can be housed in-group of 10-15 animals depending on the size of paddock. The male horse should be housed in separate pen after the age of one year.

Jack/Stallion management

Breeding stallions in efficient breeding programs require high maintenance and have certain management needs. Like foal management, care of stallion starts with very early age. True to breed good colt having proper confirmation should be selected for future stallion. The proper feeding of selected colt with the objective of optimum growth should be taken. The selected colt can be fed with extra milk and creep feed having 18-19 % crude protein for gaining proper body weight and measurements. All routine management practices should be followed *i.e.* exercise, grooming, hoof care, deworming and vaccination. The colt above one-year age should be housed in separate pen having sufficient covered and open area and well ventilated covered area.

At the age of three years male horse attain almost full height and body weight (95 %) and able to cover the mares but it is recommended to fully use the stallion as breeding purpose after completing 3.5 to 4 years of age. However, the stallion should be trained for breeding as well as for semen collection well before attaining the four year age. Horse breeding enterprises are very diverse. Most people only own broodmares and contract breeding with stallion owners. Stallion owner enterprises range from those with a single stallion that may breed fewer than five mares per year to operations that stand several stallions and breed hundreds of mares annually. Breeding methods include in-hand natural mating and artificial insemination with fresh, cooled, or frozen semen.

Although exercise has not been shown to increase stallion semen production, most stallions will respond behaviorally and appear healthier when allowed daily access to mild exercise. Stalls are commonly connected to a turnout area to provide self-exercise and visual contact with other horses. Stallions in stalls without a connected turnout should be routinely allowed access to an individual turnout paddock or mild exercise such as hand walking.

Breeding / covering of mares:

Care of Jack/stallions at the time of natural covering / semen collection is also very important. It should be ensured that mare coming for natural covering or dummy should be free from any type of infection and cleaned properly before mounting by stallion. The mare should be

properly restrained by applying breeding hobbles to avoid injuries to stallion as well as handler. The prepuce of stallion should be properly washed and cleaned before mounting and also after covering/ semen collection. The recommended frequency of semen collection and natural covering is alternate day. Overuse can cause decreases in sex drive, observed by the stallion's disinterest in breeding, longer mounting times required before breeding, or lack of ejaculation during breeding.

Estimating the Time to Inseminate/ natural covering the broodmare/jennies

Once in the breeding season, a mare will cycle every 21 days until she is bred or enters the anestrus or non-cyclic season. Each cycle will have a 5- to 7-day estrus period. During this period, a mare will build follicles on her ovaries, of which one will eventually ovulate a day to two before she goes out of estrus. Highest conception rates are most likely when mares are inseminated 1 or 2 days prior to ovulation up to the time of ovulation. Insemination outside that time frame reduces the chance of conception. As follicles grow, so will the mare's acceptance to a stallion's sexual advances. Because of this nature, teasing a mare is the most common detection tool for determining timing of insemination (see Table 1).

When in the presence of a jack/stallion, the majority of jennies/mares in estrus will exhibit one or more of the signs characteristic of a teasing score of 3 or greater. Mares showing a teasing score of 3 or greater are covered. However, relying on this behavior alone may require mares to be serviced multiple times in one estrus because mares may show interest in stallions without being within one or two days of ovulation. Or a mare with a lack of estrus behavior may not be bred, even though she does ovulate. Because of the variations between estrus behavior and time of ovulation, efficient use of stallions requires that teasing is combined with palpation and use of ultrasound. This is especially important when servicing a large number of mares, as limits to semen production or sex drive will place limits on how often the stallion can be collected. When approaching ovulation, palpation and ultrasound can detect changes in the tone of the cervix and uterus, and level of follicular activity and ovulatory follicle size, all signs that increase the accuracy of estimating the day of ovulation.

Teasing also influences the Jack/ stallion's behavior and the composition of semen. The need to tease a stallion to increase sex drive will depend on the individual nature of the stallion. Teasing influences total volume of the ejaculate more than the number of spermatozoa per ejaculate. This increase in volume results from increases in accessory sex gland fluid that does

not contain spermatozoa. The number of mares that can be booked successfully to a stallion depends on the stallion's spermatozoa output, the stallion's sex drive, and the number of times each mare must be bred. A common booking limit is 30 to 40 mares per season with natural mating. This number can be larger if the manager utilizes palpation and ultrasound to accurately determine ovulation time in mares.

General Management practices of equids:

Equids are very sensitive and agile animal. It can be saved from many diseases by proper maintenance and proper housing. Equids shelter should always be open and ventilated and the direction should be such that enough light keeps coming in it. Therefore, the equids shelter should be built in the north-south direction. The room of the shed should be pucca (made of bricks) and the floor should be solid and rough so that it can be cleaned properly and can be washed with water from time to time, for this the slope of the floor should be such that there is no water Stop and water should also be allowed to drain. Having a rough floor also reduces the chances of the animal slipping. If the floor is unpaved, the top 6-inch soil layer should be changed twice a year.

It is necessary to have enough open area in the stables, which is raw, because the horses have a habit of rolling in the raw, due to which they feel energized and also remain physically healthy by roaming freely in the open. The boundary wall of the open area of the stable can be made of wooden batons or GI pipe. The boundary of the horse house should never be barbed wire, mesh or bushes, this increases the chances of injury to the horse. There should be no peg, nail or sharp object in the stud and the corners etc. should be made in roundness. If the horse is to be tied in the enclosure, iron rings/rings should be used, which are in the wall up to shoulder height. The use of wooden pegs on the floor increases the chances of injury to the horse. Do not put any kind of piece in the enclosure of the horse, sometimes there is a fear of the piece getting stuck in something which can hurt the horse. Proper arrangements should be made to protect the horses from heat or cold. In the summer season, fan/cooler can be used and cold water can also be sprinkled in the enclosure as per the need. It is very important to clean the water and fodder / grain drains regularly. Clean and clean drinking water should always be available. While managing the electricity in the horse shed, care should be taken that the power cords and switches etc. are away from the reach of animals so that any kind of accident can be avoided. If equine keepers want to keep more animals, keep in mind that the size of the stable

should be such that the animals can see each other, so that they do not feel lonely. I continued to have excitement towards the female and she remained excellent in breeding.

The gestation period is from conception to delivery. The average gestation period is 340 days in mares and 365 days in warmblood mares and 360 days in donkeys. Pregnancy is detected in female horses by ultrasound method only 14 - 15 days after matching/artificial insemination. Pregnant female need high amounts of vitamins, minerals and calcium during gestation, especially in the last quarter of pregnancy. During pregnancy, vaccination and anti-parasitic drugs should be given to prevent diseases so that newborn equines.

The pregnant female horse should be kept separate from the rest of the animals during the last days of pregnancy and kept in the foaling house. Straw/dry grass/bread/wood sawdust etc. should be laid as a bed so that the newborn equine is not injured at the time of birth. After the birth of a newborn horse baby, a medicine called tincture iodine should be applied on its navel. This medicine should be used twice a day for a week, otherwise the risk of infection in the navel increases. The size and shape of the stud is considered appropriate:

Animal/House	Room L x B x H (feet)	Open Area L x B (feet)	Door size B x H (feet)
Horse stallion	14 x 14 x 10	50 x 50	5 x 8
Mare	12 x 12 x 10	40 x 12	5 x 8
Pony/Mule/Donkey	10 x 10 x 10	40 x 12	4 x 8
1 year old foals	10 x 10 x 10	30 x 12	4 x 8
Mating paddock	-----	24 x 24	-----
Foaling Room	16 x 16	-----	5 x 8

The following things are of special importance in the health of horses:

1. Horse Nutrition
2. Exercise
3. Grooming
4. Hoof care
5. Internal and external parasites
6. Vaccination

Equids Nutrition:

Equids are pure vegetarian animals with a simple stomach. Feed/feed given to equids plays an important role for their physical development and health. Some important things regarding horse diet are as follows:

Equids diet is mainly divided into two parts, feed and fodder. Grain mixture is made by mixing a variety of cereals (oats, barley, millet, maize, etc.), cereal by-products (wheat bran, rice polish), legume seeds (gram) and mineral mixtures and common salt. Cereals are rich in starch, phosphorus and potash, but low in fiber, sodium, chloride, calcium and protein. It is a good source of Vitamin B and E. The grain should be soaked in water for 3-4 hours at night during summer and 6-8 hours in winter. This makes the grain soft and easy to eat. Fodder can also be divided into two parts – dry and green fodder. Dry fodder such as sorghum, oats, sorghum, millet husk, moth husk and maize etc. It is the material left over from all crops in which nutrients are less. Oats, sorghum, Lucerne, barley etc. are used in green fodder. The amount of protein, vitamins and minerals is high in green fodder. Hay is also used as dry fodder. It is harvested before the crop is ripe and is rich in nutrients. Never feed dirty, wet, rotten and stale food to the animal, it increases the chances of colic, spoilage of the animal and the animal becomes ill.

The amount of food is decided according to the physiological need and use of the horse such as physical growth, exercise, reproduction, pregnancy, etc. The amount of grain, hay and fodder (green/dry) in the diet is the workload of the animal and should be given as per the need. Feeding too much hay can lead to abdominal pain and lameness due to overfeeding of the animal. Always give food to the animal at the appointed time. Do not give the animal anything to eat immediately after exercise. Pregnant females should not be fed too much berseem because it contains high amounts of estrogen salt, which can lead to miscarriage. Never make sudden changes in the diet of horses, this can lead to colic (abdominal pain) and death. If necessary, change the diet gradually at an interval of 7-10 days. Diet control is very important in horses as they often overeat and become prone to colic, lameness and obesity, unlike donkeys and mules that do not overeat. The body of a healthy animal is light green in color, the presence of a brownish color or a more foul smell indicates that the horse's digestive system is not correct and he is suffering from digestive problems. If horse is found eating lid/wood, it is due to deficiency of essential elements (minerals) in the body. The diet should contain proper amount of minerals, salts and vitamins. Adult horse should have 30-50gm per day. One percent of the gram mineral mixture and one percent iodized common salt should be mixed in the grain and

fed.

The following are the dietary parameters for the proper health of horses:

Composition of concentrate feed:

Oats	40%
Gram	30%
Wheat bran	27%
Mineral mix & common salt	3%

Grains (Quantity in Kilograms):

S. No.	Description of animals	Adult	2-3 yrs	1-2 yrs	6-12 m	3-6 m
1.	Horses	4 kg	3 kg	2 kg	1.25 kg	0.75 kg
2.	Pony / Mule / Exotic donkey	2 kg	1.5 kg	1 kg	0.75 kg	0.5 kg
3.	Indigenous donkey	1 kg	1 kg	0.75kg	0.5 kg	0.25 kg

Dry Fodder (Quantity in Kg):

S. No.	Description of animals	Age group				
		>3 yrs	2-3 yrs	1-2 yrs	6-12 m	3-6 m
1.	Horses	9 kg	7 kg	5 kg	3 kg	2 kg
2.	Ponies / Mules / Exotic donkeys	7 kg	5 Kg	4 Kg	2 Kg	1 kg
3.	Indigenous donkeys	5 kg	4 kg	3 kg	2 kg	1 kg

Note: 1 kg dry fodder should be reduced by giving 3 kg green fodder

Exercise:

To keep the equids healthy, regular exercise is necessary. If the animal has not been exercised for several days, then start with light exercise. And the hooves are also strong and they remain healthy. Exercise can be done in two ways, first by circular/circular ring and second by horse

riding. In the circular exercise, the animal is run in a circular/circular ring of 15-20 feet size for 15 minutes clockwise and 15 minutes anti-clockwise. It is a matter to keep in mind that immediately after exercise, the animal should be walked for at least 15 minutes.

Grooming:

Regular grooming of equids is very necessary and beneficial. Massaging the skin of the body increases blood circulation, as well as keeps the hair clean and shiny and also protects against parasites and skin diseases. But the external parasites present, if any, should be killed immediately. The hair should be cleaned of the whole body, such as the mane, the hair of the tail, the legs as well as the nostrils. Each animal should be groomed at least once a day. is essential

Hoof care:

There is a saying in English "No foot no horse". Hoofs have an important place in equids. Any equids becomes useless when the hoof is damaged, so the hooves should be cleaned and taken care of regularly. Regular cleaning of the hooves prevents infections and diseases. Lack of regular cleaning and standing in filth (feces and urine) and accumulation of dirt in their hooves leads to thrush, hoof abscess and lameness. The hoof should be trimmed every 6–8 weeks. Consult a veterinarian if any cracks appear in the hoof.

Internal and external parasites:

Intra-parasites (worms of the stomach and intestines) are commonly found in horses. These parasites feed on horse blood for their growth, reproduction and growth. Due to which the horse becomes weak and there is a lack of blood. And its growth stops and the level of production capacity falls. Despite giving good nutritional supplements, it's working capacity and fertility decreases. The following symptoms can be clearly seen in such horses –

- loss of appetite
- having diarrhea
- constipation
- frequent abdominal pain
- anemia

In horses, these parasites enter the body by eating fodder containing eggs and drinking water. To keep the horses free from these parasites, at least four times a year, at an interval of every

3 months, the drug should be given to make the parasite free. The medicine should be changed every time, do not give the same medicine again and again. Because at that time there is more larvae and sludge of parasitic germs in the grass. Horses should not be fed water directly from the pond and should never be allowed to eat the green grass around the pond and canal. Prevention of external parasites such as ticks, flies, lice and mosquitoes etc. is very important. There is a risk of causing many diseases from them. Therefore, from time to time, external parasiticides should be sprayed on animal and stables.

Vaccination:

Table of Useful Vaccines in Horses:

Sr No.	Disease	Age at Ist vaccination	Booster/re-vaccination	Route	Dose
1.	Rabies	3 months	Annually	Intra muscular	1 ml
2.	Tetanus	3 months	Twice a year	Intramuscular	3 months – 1 ml 1 – 2 year – 2 ml 2 – 3 year – 3 ml >3 year – 5 ml
3.	Equine Influenza vaccine, NRCE, Hisar	Twice a year, at a gap of 2-4 weeks	Annually	Intramuscular	1 ml
4.	EHV-1 vaccine NRCE, Hisar	5 months of gestation	7 & 9 months of gestation	Sub cutaneous	2 ml

Farm Bio-security:

It is very important that strict bio-security measures should be adopted at horse farm. In the premises of the horses housed, no outsider animal should be allowed to enter. There should be minimal movement of the human and vehicle or be restricted. Dung/excreta should be disposed at a far distance from the stable premises. Any stranger if is allowed to enter in the premises should walk through Disinfectant and with proper washing of hands with antiseptic solutions.

BASIC DONKEY HEALTH MANAGEMENT

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Donkeys must be observed at regular intervals for any abnormal behaviour. Behaviour, normal stimuli responses and normal vital sign from each individual animal should be observed carefully. Record keeping of all the parameters is key.

Vital Signs:

Measurements of a donkey's body function and are good indicators of the donkey's overall health.

Common vital signs are temperature, pulse rate, respiration rate, gut sounds, mucosal color/capillary refill time and skin pliability.

1. Temperature:

Normal temperature is 100°F or 38°C, which may vary from 99.5 – 101.5°F. Exercise, excitement, hot weather, illness and pain will raise the temperature. Shock and very cold weather will decrease the temperature.

2. Pulse rate:

Donkey should be calm, rested and relaxed for most accurate results. To determine pulse gently press fingers against an artery. Count the beats for 60 seconds.

Normal Resting Pulse Rates (in Beats Per Minute):

Age	No. of beats/ minute
2 weeks old	up to 100
4 weeks old	70
Yearling	45 – 60
2 Years old	40 – 50
Adult	30 – 40

3. Respiration rate:

Normal respiration is 8 - 16 breaths per minute. To determine watch the nostrils or flanks. Nostrils flare and contract with each breath. The respiration rate should not exceed the heart rate.

Pulse/Respiration Ratio: Normal Ratio is 4:2 or 2:1. A more significant measure of stress than each of the actual figures alone. Respiration exceeding pulse is an indicator of serious stress

4. Gut sounds:

It can help you diagnose a sick donkey. Caused by the normal contracting and relaxing movements of the digestive tract during the digestion process. Place an ear on the flank area or use a stethoscope. Abnormal Absence of gut sounds.

5. Vital Signs:

Mucosal Color is an indicator of blood circulation. Several mucous membranes can be checked like the inner lips, gums, inside the vulva and nostrils, which should be moist and pink. Determine capillary refill time by pressing your thumb on the donkey's gum and then releasing it.

It should take ~2 seconds for the blood and normal color to return to the area.

6. Skin Pliability:

It is a test for dehydration. Pinch a fold of skin on your donkey's neck and release it. It should quickly flatten back in place. If the donkey is dehydrated, the skin will flatten slowly or tend to stay in a fold.

Other Factors to Consider: there are some other factors also which indicates the body condition and health of an animal.

- Bodily fluids colour and consistency (feces, urine, saliva & sweat)
- Body condition and weight
- Movement/gait
- Hair coat
- Hoof condition
- Feeding habits
- Behaviour

Parasite control:

Parasites are blood suckers and have a life cycle in body. They damage the intestinal vasculature and mucosa which causes gastro-intestinal disturbances, including impactions, motility disorders, diarrhea, and peritonitis. Migrating ascarids contribute to pulmonary inflammation in foals. The principal internal parasites of equids are nematodes. With the advent of more effective anthelmintics, the nematode species of importance have shifted from large

strongyles to cyathostomes. There is no single parasite control program that is ideal for all equids. Age of the equine, population density, and pasture size and quality can affect choice of programs. As equine age, they develop resistance to reinfection with certain parasites, such as *Strongyloides westeri* and *Parascaris equorum*. Deworming can be carried out 4 – 6 times a year with different dewormer every time.

Deworming Schedule:

Month	Dewormer
January	Ivermectin
March	Oxfendazole
May	Moxidectin
July	Pyrantel Pamoate / Praziquantel
September	Fenendazole
November	Ivermectin

Vaccination Schedule:

Equids should be vaccinated to prevent them from exposure of deadly diseases.

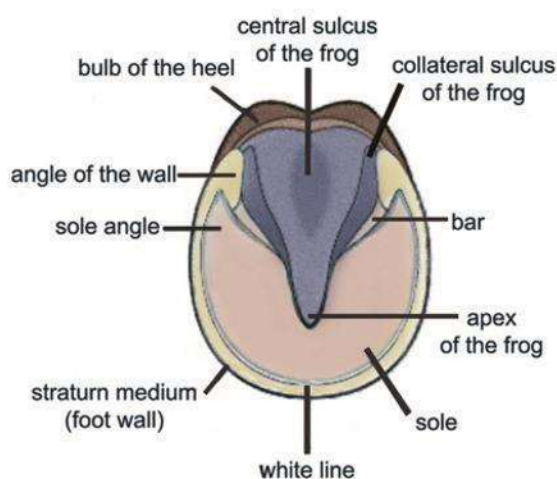
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1.	Tetanus	3 months	Twice a year	Intra muscular	3 months to 1 year – 1 ml 1 – 2 year – 2 ml 2 – 3 year – 3 ml >3 year – 5 ml
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3.	Equine Influenza vaccine (NRCE, Hisar)	Twice a year, at a gap of 2-4 weeks	Annually	Intra muscular	1 ml

4.	EHV-1 vaccine (NRCE, Hisar)	5 th month of gestation	7 th & 9 th months of gestation	Sub cutaneous	2 ml
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Hoof Trimming

Donkey hooves are smaller, steeper and flexible but tougher than horse hooves. Hoof wall is thick right to the heels, frog extends further rearwards than the hoof capsule, which provide maximum impact protection on hard grounds and sole is relatively thicker and concave. Donkey feet are much more efficient than horses at absorbing water and can become soft, so they often suffer from foot diseases.

Parts of a hoof:



Donkeys require a foot trim every 6-10 weeks, but care should be taken with older donkeys who might struggle to lift their limbs.

A hoof stand can be used for proper hoof trimming:



1. Remove any debris from hoof by using a hoof pick



2. Use nippers to cut the overgrown hoof wall

Rims off the excess hoof wall.



3. Use a hoof knife to remove any debris and trim the sole of the hoof



4. Use rasp to smooth out the edges and clean up any rough edges around the hoof



Donkey hoof problems:

1. Seedy Toe:

In this condition, white line area becomes weak and crumbly, and is rarely painful unless there is extensive hoof instability. There are chances of foreign objects and organisms to get enter when there is widened white line. Under the horny part of the hoof dirt or little stones can get stuck in the space, causing pain or an infection.

By cutting out the affected part of the hoof wall seedy toe is corrected. Discoloured material and dead part are removed to allow the new healthy horn to grow. To reduce the chance of infection exposure to dirt and germs is necessary to minimize. Once corrected donkey should be housed in clean, dry ground until fully recover.



2. Thrush:

Thrush is an infection of the underneath of the foot, which affects the frog. It has a distinctive, unpleasant smell. It is associated with keeping the donkey in wet conditions.



Thrush is prevented by keeping the feet clean and dry as much as possible.

3. Laminitis:

[Laminitis](#) is a very painful condition often with irreversible consequences. There are several causes of laminitis too much rich food, infection or pregnancy. There is destruction of the support mechanism of the toe bone within the hoof, and toe bone can rotate or move downwards. In this condition donkey is unwilling to walk and lay down more than usual.



Donkey suffering from laminitis should be placed in a well-bedded stable, with easy access to food and water. The laminitic foot should be trimmed frequently. Thick cotton wool and bandages can be used to pad the entire sole.

4. Foot abscess:

It is a type of wound that penetrates the sole or white line. It develops in the foot when the wound is infected and pus builds up. It may become a cause of [Tetanus](#). A Veterinarian should be consulted for treatment.



Dental care:

Dental disease is recorded as being the second most common clinical problem encountered in the donkey, after disorders of the feet. It has the potential to cause significant pain, which may go unrecognised in these stoic animals. Routine dental care reduces the incidence of dental disease. Sharp teeth and uneven dental wear predispose to tongue and cheek lacerations, poorly masticated feed, decreased feed intake, choke, and weight loss, and may contribute to mouth pain when the donkey is bridled. Donkeys are stoic, and often won't show any sign of

something being wrong. Frequent dental examination allows early detection of tooth root abscessation before more serious complications eg, sinusitis develop. Dental ailments are some of the most common medical issues in donkeys. They may break, or even lose teeth in the course of their life. Owners should watch out for weight loss, difficulty chewing, excessive drooling or nasal discharge, and bad breath.

Donkeys have two sets of teeth in their lifetime. The first set are called 'deciduous teeth', which erupt (appear above the gums) in the first few weeks of life. The deciduous teeth gradually fall out and are replaced by a permanent set of adult teeth. Donkeys have a full set of permanent teeth at around five years old. Donkeys may have up to 44 teeth. Because they are continual grazers and thrive on a diet of highly fibrous/abrasive food, donkeys have developed teeth that are designed to wear constantly.

How to spot problems with your donkey's teeth:

Watching for signs will help you identify dental problems at an early stage. If your donkey becomes reluctant to eat, it may be because they are associating eating with pain. Problems with picking up food and chewing can lead to weight loss because your donkey is not getting the nutrients they need.

Signs of dental problems include:

- behavioural change
- slow or intermittent eating (sometimes complete reluctance or inappetence)
- weight loss
- difficulty chewing, or dropping balls of food ('quidding')
- difficulty grazing
- excessive saliva (drooling)
- strong-smelling mouth
- discharge from the nose (thick snot)
- colic.

Common dental conditions of donkeys:

1. sharp points on the teeth ('overgrowths')
2. diastema (gaps between teeth where food can become trapped)
3. periodontal disease (gum disease)
4. missing teeth
5. fractured (broken) teeth.

PREGNANCY DIAGNOSIS USING ULTRASOUND TECHNIQUE METHOD

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Ultrasonography, developed originally for observation of pelvic organs in humans, is now being used for early diagnosis of pregnancy in horses (around Day-14 of gestation) and thus has become the most common and reliable method. Economic losses are reduced by early identification of non pregnant farm animals, which can either be rebred or treated for prevailing gynaecological problem.

Use of ultrasound for pregnancy diagnosis in the jenny

Using ultrasound enables detection of pregnancy as early as 11 days post-ovulation. Most veterinarians, however, prefer to check jenny between 12 and 15 days post-ovulation, when the embryonic vesicle is larger and easier to find. The experienced veterinarian will follow every inch of the uterine horns and body to find the early embryo (which can be as small as a quarter of an inch in diameter) and to rule out the presence of twins for early twin reduction. Ultrasound also allows to monitor for normal growth and health of the embryo. If early embryo loss is detected, one can arrange to rebreed the jenny before the end of the breeding season.

A peculiar fact about pregnancy in the jenny is that the early embryo travels all around, covering every inch within the uterus, from days 7 to 16. This is a very important event in what we term *maternal recognition of pregnancy*; basically, the embryo is asserting its presence by preventing prostaglandin release from the endometrium (uterine lining), which would induce the jenny to come back into heat, and thus the pregnancy would be lost. This is why when we perform early pregnancy diagnosis (before day 16) the embryo can be located anywhere in the uterus and thus it is very important to be thorough and systematic with the ultrasound examination. At around day 16, the embryo becomes too large for such movement and gets lodged at the base of one of the uterine horns, where development progresses.

Ultrasounds machine

Principle: Most diagnostic ultrasound machines use the principle of brightness modulation (B-mode) where the returning echoes are displayed as dots, the brightness of which is proportional to their amplitude.

Diagnostic ultrasound utilises sound frequencies between 2 and 10 MHz. Linear array B-mode real-time ultrasound scanners are the most commonly used for examination of the reproductive tract of jennies. An ultrasound machine has two important parts: A transducer or probe and a processing computer with a monitor.

A real-time scanning probe is passed over the abdominal wall that transmits two-dimensional images to an oscilloscope. Ultrasonic waves are reflected at different levels of energy by tissues of varied density. Dense tissues, like bone, readily reflect waves (bats have used echo-location to navigate for ages). As pregnancy progresses, virtually every organ can be monitored.

Ultrasounds travel best through fluids giving rise to a pitch-black image on the monitor. The early equine embryo appears like a small vesicle or bubble filled with fluid (yolk sac stage), and therefore on our ultrasound screen we see a black circumference within the lumen of the uterus. After day 21 of the pregnancy, the embryo proper can be seen within a fluid-filled vesicle. Structures within the fetus appear in different shades of gray and the bones appear bright white.

Ultrasound terminology:

- Tissues that markedly reflect sound (such as bone and metal) appear white on the ultrasound screen and are called echogenic.
- Tissues that transmit sound (such as fluid) appear black on the ultrasound screen and are called anechogenic (or anechoic).
- Tissues that allow some transmission and some reflection (such as most soft tissues) appear as varying shades of grey and are called hypoechogenic or hyperechogenic depending upon their exact appearance. For example, ovaries usually contain follicles (which are anechoic), and may contain luteal structures (which are relatively echogenic - varying shades of greywhite).

Reasons for early pregnancy diagnosis

1. It is very important to be able to tell if a jenny is pregnant for management and husbandry reasons:
 - i. Pregnant jennies should be managed differently from non-pregnant jennies.

- ii. Pregnant jennies will require further examinations to monitor the development of the pregnancy.
- iii. It is important to confirm the absence of twin pregnancies and monitor for early embryonic or foetal death. Twin pregnancies are bad news in the jenny and if pregnancy is diagnosed early enough, something can be done about the twin pregnancy.

2. It is just as important to diagnose the non pregnant jenny as soon as possible. Some jennies have prolonged luteal activity {previously termed 'persistent corpus luteum' (PCL)} and the maintenance of circulating progesterone concentrations in these non-pregnant jennies means they do not return to oestrus themselves.

Diagnosis of early pregnancy using ultrasound:

Day 10-11

The equine embryonic vesicle can be reliably detected at day 11 when sufficient anechoic yolk sac fluid has developed.

Jennies are not usually scanned as early as Day 11 because it is possible to miss the conceptus if scanning conditions are not ideal and the ovulation date is not accurately known. If there was an ovulation one or two days after the first ovulation, any pregnancy arising from this later ovulation would be too small to be detected.

Day 14



The 14 day conceptus is 13 to 18 mm in size and lies centrally in the uterine body. Although pregnancy diagnosis is highly accurate even at this early stage, it is important to be aware of the possible confusion caused by uterine cysts and the presence of twin conceptuses. Ideally one would have performed an ultrasound examination before breeding the jenny, but this is not

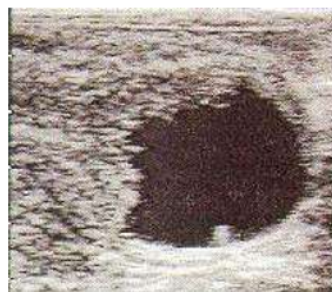
always possible. If the first scan is performed at Day 14 or 15, then it is possible to return the next day in cases of confusion and see if the pregnancy has changed position or grown in size. This should allow differentiation from a cyst before the pregnancies have a chance to become unilaterally fixed.

Day 16



By day 16 of pregnancy the vesicle is normally fixed at the base of either the left or the right horn. The shape is still regular, but more ovoid than strictly spherical.

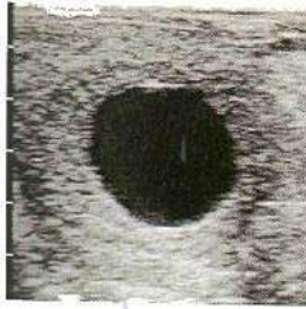
Day 22



At day 22 of pregnancy the conceptus is irregular in outline. This irregular shape is normal and is not an indication of imminent pregnancy loss. It is important to have an accurate history of the age of the pregnancy because small for age vesicles can indicate impending early embryonic death. All embryos should be detectable by day 24.

From this stage of pregnancy onward, morphological changes are also used to age the pregnancy. These include shape changes, the location of the embryo within the trophoblast and the relative sizes of the yolk sac and allantois.

Day 24



By day 24 of pregnancy, the heartbeat can normally be detected as a flickering movement in the middle of the echoic embryo around this stage of pregnancy. There is emergence of the allantoic sac as a small anechoic area from beneath the embryo. Over the next few days, the development of the allantois will lift the embryo dorsally and the yolk sac will gradually reduce in size. This change in ratio of the yolk sac to allantois is an important feature in ageing pregnancies. It is important to recognise the embryo and identify a heart-beat because the irregular shape of the vesicle is easily confused with an endometrial cyst.

Day 28-29



In the day 28-29 pregnancy the apposition of yolk sac and allantois results in an ultrasonically visible thin line normally orientated horizontally. The embryo is visible as an echoic mass on this line. There is enlargement of the allantoic sac such that the two sacs are approximately equal in size. The embryo is highly echogenic and is visible on the line separating the allantoic and yolk sacs and the heartbeat can be clearly seen.

Day 33



By day 33 the embryo is usually in the dorsal part of the vesicle often at a "2 o'clock" position. The volume of the allantois greatly exceeds that of the yolk sac. The aim of this examination is to confirm that a single conceptus is developing normally. If there is failure of normal development or if twins are detected, it is usually possible to terminate the pregnancy and re-breed the jenny. However, if examination is delayed until after day 33, the endometrial cups may have developed and even if pregnancy is terminated, eCG production may continue for a variable time, sometimes preventing normal oestrous cycles for the rest of the breeding season.

Day 40



By day 40 of the pregnancy the allantois surrounds the yolk sac which has almost disappeared. The allantoic membranes are nearly opposed. The embryo is approximately 17 mm in length.

Day 45



By day 45, the foetus has descended approximately two-thirds of the way towards the ventral part of the allantois. The developing head of the foetus is recognisable.

Day 60

By day 60 of pregnancy the diameter of the conceptus exceeds the scanning width of the ultrasound probe. Considerable foetal motility is obvious.

Ovarian Examination

Examination of the ovaries is important at every examination for pregnancy to provide information on the number and appearance of the Corpus Luteum (CL). During the examination of pregnant jennies between 40 and approximately 150 days, large follicles and supplementary CLs are frequently found in addition to the primary CL. The supplementary CLs are often as large as 6 to 8 centimetres, with thick echoic walls and anechoic centres crisscrossed by echoic trabeculae.

Pregnancy Diagnosis Protocol (When Ovulation Time is known)

(Day of ovulation is Day 0)

First exam	Day 14 to 15
Second exam	Day 24 to 27
Third exam	Day 33 to 35
Autumn exam	October

RECORD KEEPING AT DONKEY FARM

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For good livestock management in an animal farm, records maintenance plays a very essential role. It would be difficult to make right decisions if no records are maintained and farmers have to depend on their memory to make any decision, which is not advisable. Moreover, the decisions made on the basis of memories can become unreliable as the days pass. It becomes easier to record the performance of an animal if that animal has identification tags or cold branded numbers.

Therefore, animal performance record and identification marks are required, always. For donkey dairy farming, there are many useful records like production and financial transaction, which should be maintained. The input and output cost on maintain donkey farm can be assessed if all records are maintained in a proper way. This can also show that how a farm is maintained in comparison to other farms in which records are not maintained at all. The strength and weakness in maintaining farm can be easily determined.

Benefits of record maintaining at donkey farm:

- On the basis of records maintained it becomes easier to evaluate an animal for the selection and culling of non-productive animal.
- The disease status of the herd and abnormal conditions that leads to loss in body weight, loss in milk production etc. can be judged. Records helps in finding the commonly occurring diseases in a herd and to design precautionary measures like vaccination, deworming etc.
- Records also helps in preparing pedigree and history of animals. To prepare breeding plans to check inbreeding, selection of superior jack and jennet breeding records plays an important role. Progeny testing of superior jacks can be assessed if records of that particular jack is maintained.
- The economics of a donkey farm can be ascertained since records helps in analyzing feeding cost and benefits from animal product outputs. Hence helps to formulate economic feeding strategies for optimal productions. Helps in estimating the cost of

milk production. Helpful in comparing the efficiency of labour and herd with other farms.

- Helps in Herd can be better supervised and managed with the help of records. Also to compare the herd performances in different years to determine the amount of profit/loss annually and on that basis setting future plans for the farm.
- Proper prices of animal meant for purchase and sale can be calculated effectively.

Types of records to be maintained at donkey farm:

1. **Livestock register** : This register records the total number of the animals at the farm along with their identification number, date of birth, sire number, dam number, foal and its sex, date of foaling, date of purchase, date of sale/auction/death etc.
2. **Foaling register**: This register maintains the records of foaling that take place in the farm. It maintains dam and sire number of the foal, foal number, sex and its date of birth, birth weight and any other remarks like type of foaling (normal/dystocia).
3. **Daily milk yield register**: This register records the daily milk yield performance of the jennies.
4. **Body weight & Measurement register of foal**: this record maintains the weight and body measurement (height & length) of the young foals at different intervals to the age of 2 years.
5. **Feed Fodder Register**: This register records the amount of concentrate, dry fodder, green fodder, mineral mixture and common salt given to the animals daily and the total quantity of these consumed per day.
6. **Treatment register**: It maintains the record of the sick animals along with history, symptoms, disease diagnosis, treatment given and details of the veterinarian who attended the case.
7. **Breeding register**: This register maintains the details of breeding practices in the farm such as jenny number, date of calving, date of estrus and artificial insemination / natural covering given with jack number, pregnancy diagnosis records, expected date of foaling, actual date of foaling, number allotted to the new born foal etc.
8. **History sheet**: This maintains animal number, breed, date of birth, sire and dam number, lactation yield records, date of drying, treatment, vaccination, deworming, date of disposal/death, cause of disposal etc.

Training on
Entrepreneurship Development Programme on Donkey
Farming

September 02 - 04, 2022

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