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editorjiva@gmail.com

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Contents

REVIEW ARTICLES --- 05-26

1. Factors affecting fatty acid composition of farm animals and its impact on human health 05
John Abraham, Ramesh Saravanakumar. V and Purushothaman, M. V
2. The success story of RKVY- SLBP Calf Feed Subsidy Scheme implemented in Ayyappancovil Grama Panchayath, Idukki 15
Arun Kurian and Josephine Francis
3. Lameness in Dairy Cattle: Nutritional Approaches For Prevention And Management 18
Axsa P. Thomas and M. T. Dipu
4. Meat From Laboratory-not A Fantasy Anymore 23
Sreekumar.T.R and Mohd Matin Ansari

RESEARCH ARTICLES --- 27-52

1. Studies on the influence of Fat Percentage of Milk on Nisin Activity 27
Radha, K. and Anna Anandh, M.
2. Hepatoprotective activity of methanolic extract of *boerrhaviadiffusa* L. against Carbon tetrachloride (CCl₄) induced hepatotoxicity in rats 32
Mini Bharathan and Joy .A.D
3. Effect of phytase supplementation in swine rations on bone and carcass characteristics 37
Shyama, K., Gangadevi, P. and Syam Mohan, K. M
4. Comparative Morpho-histology of Muzzle in Deer and Goat 46
Maya, S., Chungath, J.J., Ashok, N., Lucy, K.M., Sreeranjini, A.R., and Indu, V.R.
5. Reproductive Characteristics in Triple Cross Cattle 50
Ramesh, J. Padodara and Arya, J. S

Contents

CLINICAL ARTICLES	53-63
1. Visceral Gout in Poultry - A Report <i>Deepa Chirayath and Rejitha, T. S.</i>	53
2. Hyperplastic Prostatitis in a Jack Russell Terrier - A Case Report <i>Ambily, V.R., Kanaran, P.P and Usha Narayana Pillai</i>	55
3. Pre Partum Cervico Vaginal Prolapse in a Rabbit - A Case Report <i>Ambili John, Upasana Ratnakaran, M.P. Unnikrishnan and B. Bibin Becha</i>	57
4. Surgical Management of Bilateral Mandibular Fracture in a Dromedary Camel <i>Jayamohan. T.V., Rafeek. A.K., Anilkumar.V.T. and Baby.P.G.</i>	59
5. Occurrence of foot disorders in elephants <i>Giridas, P.B., Usha Narayana Pillai and Alex, P.C.</i>	62
FROM EDITOR'S DESK	64-66

FACTORS AFFECTING FATTY ACID COMPOSITION OF FARM ANIMALS AND ITS IMPACT ON HUMAN HEALTH

John Abraham¹, Ramesh Saravanakumar. V² and Purushothaman, M. V³

¹Assistant professor, Department of Livestock Production and Management
College of Veterinary and Animal Sciences, Pookode

² Professor and Head, Department of Livestock Production and Management

³ Professor and Head, Department of Animal Nutrition,
Veterinary College and Research Institute, Namakkal,
Tamil Nadu Veterinary and Animal Science University

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ABSTRACT

The fatty acid composition of farm animals is affected by species, breeds diet and differs between tissues. The levels of fat tissue in the carcass and meat are important underlying factors because fatty acid composition changes as fat is deposited. The fatty acid composition of pork can be readily modified by diet since fatty acids are deposited unchanged by digestion. In ruminants, the rumen is a barrier to the incorporation of Poly Unsaturated Fatty Acids (PUFAs) into meat although the effect of grass diet in increasing proportions of n-3 PUFA and possibly Conjugated Linoleic Acid (CLA) is an interesting area of current research, leading to more desirable meat products for the consumer. Poultry meat with skin not only contain more total fat but also have a greater proportion of monounsaturated fatty acids (primarily oleic acid) and lower proportions of saturated and poly unsaturated fatty acids than muscle alone. Poultry represents a muscle food in which diet can be used to increase the concentration of bioactive fatty acids in the final product. Increasing n-3 fatty acids in poultry products by dietary supplementation of marine lipid is possible. However, such process should not change the physical and chemical properties of muscle. Muscles containing high concentration of PUFA have lipids with lower melting point

leading to muscle with soft and even liquid fat, eventually leading to consumer rejection. The fatty acid composition of meat is important for human health reasons and also has crucial effects on meat quality. The effect of diet on fatty acid composition and genetic effects on fatty acid composition on cattle, sheep, pig and chicken are discussed in detail. The impact of animal fatty acid on human health and future prospects are also discussed.

Keywords: Animal fat; fatty acid composition; diet

Fatty acid composition varies between species of livestock and also tissue sites in the body. Fatty acids are located mainly in adipose tissue commonly termed as “fat”. Ruminants (cattle and sheep) contain relatively high amounts of saturated fatty acids (SFA) and are low in polyunsaturated fatty acids (PUFA). Fatty acid composition is greatly influenced by production factors such as animal diet, age, weight, sex and breed. Fatty acid composition also affects the texture, juiciness, sliceability, stability and flavour of meat (Tye *et al.*, 2006). Fatty acids in adipose tissue and in muscle membranes also contribute to meat flavour, providing volatile degradation products during cooking.

Type of fatty acids

The fatty acids in animals are mainly of medium to long chain length that is they have 12 to 22 carbon atoms in the molecule, with the basic structure of $\text{CH}_3\text{-(CH}_2\text{)}_n\text{-COOH}$. Small amount of shorter chain length C8-C10 are present in lamb fat.

About 40% of fatty acids are saturated, that is each carbon has two hydrogen atoms attached, about 40% have one double bond (monounsaturated fatty acid, MUFA) where adjacent carbon atom is attached to only one hydrogen atom each and a smaller proportion, about 2% -25% have more than one double bond (poly unsaturated fatty acid, PUFA). Fatty acids are commonly labelled according to carbon chain length and the number of double bonds, for example linoleic acid is labelled as 18:2 being 18 carbons in length and containing two double bonds. Double bonds are either of the more common cis-type, in which the hydrogen atoms point in the same direction or of the trans-type, in which they point in opposite directions, resulting in a straighter molecular configuration

Oleic acid (18:1 cis-9) is the major fatty acids in all meat, contributing over 30% of total fatty acids. The length, degree of unsaturation

and configuration of the fatty acid molecule influence the physical properties such as melting point. The longer the chain length and fewer the number of double bonds present in the molecule, the higher the melting point. Saturated and trans fatty acids have a higher melting point than unsaturated and 'cis' fatty acids.

Fatty acids in ruminant tissue are more complex than those in non-ruminants, containing higher portions of 'trans' fatty acids. Fatty acids with an odd number of carbon atoms act as precursor for fatty acid synthesis, e.g. (C15 and C17). Fatty acids with branched chains derived from amino acids, leucine, valine and isoleucine (i.e. 4-methyl octanoic acid, C8: 0 and 4-methyl nonanoic acid C9:0) and fatty acids with conjugated double bonds (i.e. the bonds are on adjacent carbon atom rather than being separated by CH₂ group) are found in ruminants. These variations are a results of the action in the rumen that degrade plant structures and dietary fatty acids, producing a wide range of products, some of which are absorbed in the small intestine and incorporated into tissue lipids. An important group of fatty acids in ruminants are the Conjugated Linoleic Acids (CLAS) with 18 carbon and 2 conjugated double bonds. These have been shown to have

Table. 1 Effect of chain length and configuration of double bonds on the melting point of fatty acids

Increasing Chain Length		Increasing Unsaturation	
Fatty Acid	Melting Point °C	Fatty Acid	Melting Point °C
Lauric acid, 12:0	44.2	Stearic, 18:0	69.6
Myristic acid, 14:0	54.4	Elaidic, 18:1 <i>trans</i> -9	43.7
Palmitic acid, 16:0	62.9	Oleic, 18:1 <i>cis</i> -9	13.4
Stearic acid, 18:0	69.6	Linoleic, 18:2	-5.0
Arachidonic acid, 20:0	75.4	Linolenic, 18:3	-11.0

a range of physiological action in the body of the animals and meat consumers.

The SFA (Saturated Fatty Acids) in meat can be derived from the diet, produced in the rumen from unsaturated dietary fatty acids or synthesised from glucose or acetate in liver or adipose tissue.

MUFA (e.g. 18:1 cis-9) are mainly formed in adipose tissue from SFA by the action of desaturase enzyme, for example, delta-9-desaturase from oleic acid (C18:1 cis-9) from stearic acid (18:0) and palmitoleic acid (C16:1 cis-9) from palmitic acid (16:0). This same enzyme complex forms the main CLA isomer, cis-9, trans-11 CLA from 18:1 trans 11 which is produced in the rumen. Most CLA formation occurs in adipose tissue (the mammary gland in the case of lactating animals) but some occurs in the rumen.

PUFAS are of the n-6 or n-3 type that describes the position along the carbon chain from the methyl end where the first double bond is inserted. The n-6 fatty acid, present in the largest amount is the linoleic acid (18:2n-6) which is an essential fatty acid, i.e. it is derived entirely from the diet. (e.g., oil seeds and grams). The animal possesses desaturase and elongase enzymes that can convert 18:2 to longer-chain n-6 fatty acids such as arachidonic acid (20:4n-6). Similarly, the most common n-3 fatty acid is linoleic acid (18: 3n-3) which is present in the leaves of plants and grasses. This fatty acid can be converted to long chain n-3 fatty acids such as eicosapentaenoic acid (EPA; 20: 5N-3) and docosahexaenoic acid (DHA, 22: 6n-3). There is competition between 18: 2n-6 and 18:3n-3 for conversion to the long-chain PUFA because the enzymes are shared. Evidence suggests that 18:3n-3 is the preferred substrate, but the presence of much more 18: 2n-6 usually results in greater synthesis and deposition of long-chain PUFA derived from this fatty acid (William and Burdge. 2006).

These long-chain n-6 and n-3 fatty acids have important physiological roles in the body through their conversion to eicosanoids, which among other actions control thrombosis and tissue inflammation.

Ruminants and non-ruminants species differ greatly in their proportions of PUFA in tissue and meat, whereas these are hardly changed by digestion in pigs and poultry and are incorporated directly into the adipose tissue. In ruminants they are extensively hydrogenated by micro-organisms in the rumen. The microbial action results in generally low levels (10% or less) of dietary PUFAs being available for absorption into body tissue after passing through rumen.

The fatty acids in meat are found in two main lipid classes, neutral triacylglycerol (storage roles) and more polar glycerophospholipid (structural and metabolic role). The former is the main lipid component (>90%) of adipose tissue in mature animals (visible fat) and the latter, a constituent of cell membrane, contributes between 10% and 40% of the total fatty acids in muscle. Phospholipid has a much higher concentration of PUFA than triacyl glycerol. For example, pig loin muscle contained neutral lipid (triacyl glycerol) 12% and phospholipids 34% 18:2n-6, respectively as reported by wood *et al.*, 2005.

As meat animal grow towards the point of slaughter, they deposit increasing amounts of fat even within the muscle (marbling fat) in the carcass. This results in an increasing ratio of triglycerol to phospholipids, producing a lower concentration of PUFA in total lipids. A clear picture of the effect of production factors on fatty acid composition can therefore only be obtained by analysing triacylglycerol and phospholipids separately.

Species Effect on Fatty Acid Composition

The fatty acid composition of longissimus muscle of beef, mutton, pork and chicken are given in the table.

The results in Table. 4 show that beef and mutton have higher pro portion of most SFA than pork and chicken. Conversely, chicken and pork is much higher than beef and mutton in the main PUFA 18:2n-6. Values for 18:3n-3 is more similar between the species, reflecting its presence at a high level in grass and forages. Although a high proportion (about 90%) of 18:3n-3 in the diets of ruminants is normally hydrogenated in the rumen (Scollan *et al.*, 2001), some do escape to the duodenum, to be

Table: 2. Fatty Acid Composition of Longissimus muscle from beef, mutton, pork and chicken

Fatty Acid	Percentage of Total Fatty Acids				
	Beef	Mutton	Pork	Chicken	
				No Skin	With Skin
12:0	0.08	0.31	0.12	--	0.1
14:0	2.66	3.30	1.33	0.8	0.9
16:0	25.0	22.2	23.2	23.3	23.3
16:1 <i>cis</i>	4.54	2.20	2.71	3.3	6.3
18:0	13.4	18.1	12.2	10.8	6.3
18:1 <i>trans</i>	2.75	4.67	ND	ND	ND
18:1 <i>cis</i> -9	36.1	32.5	32.8	28.3	37.3
18:1 <i>cis</i> -11	2.33	1.45	3.99	2.24	3.22
18:2n-6	2.42	2.70	14.2	18.3	20.6
18:3n-3	0.70	1.37	0.95	0.8	1.0
20:3n-3	0.21	0.05	0.34	ND	ND
20:4n-6	0.63	0.64	2.21	5	0.6
20:5n-3	0.28	0.45	0.31	0.8	0.1
22:4n-6	0.04	ND	0.23	ND	ND
22:5n-3	0.45	0.52	0.62	0.8	0.1
22:6n-3	0.05	0.15	0.39	1.7	0.2
Total fatty acids (g/100g muscle)	3.8	4.9	2.2	1.65	11.07

ND-Not Detectable, Source: Enser M. *et al.*, 1996. *Meat Science*, 42, 443-456

absorbed and deposited in tissues as in pigs. The proportion of long-chain PUFA is also similar between the species, except for 20: 4n-6 that is high in pigs because of the high concentration of its precursor 18: 2n-6.

Enser *et al.*, (1996) analysed the subcutaneous adipose tissue removed from the carcass and reported that small proportion of the C20-C22 PUFA were present in pork and these were not detected in beef and mutton, reflecting the lack of incorporation of the long-chain PUFA into ruminant triacylglycerols.

In comparison of different species, Rosell (2001) found that broiler chicken meat fatty acid composition was similar to that of pork, although the proportion of the 18:2n-6 was much higher, that is 18.9% against 9.5%. Rule *et al.* (2002) also found a high value for the chicken 18:2n-6 in a comparative study, 17% of the total fatty acids in breast muscle. Among other species, difference is in the very high value of 18:3n-3 in horse muscle adipose tissue (Robb *et al.*, 1972).

Dietary effects on fatty acid composition

Compared with other production factors, diet has the largest effect on fatty acid composition in all species, particularly in the monogastrics.

Pigs

Studies conducted in the United States show clearly the effects of different oil sources in the diet on the fatty acid composition of pork (Ellis and Isbell, 1926). Different diets produced different commercial grades of subcutaneous fat tissue, from brewer's waste that produced hard fat to soybeans grazed in the field, which produced oily fat. These effects were due to the incorporation of relatively saturated fat from brewer's waste into body fats to a large amount of highly unsaturated fat as in the case of grazed soy beans. The fatty acid most affected by diet was 18:2n-6, which was 1.9%

and 30.6% of total fatty acids in subcutaneous fat of pigs, fed brewer's waste and grazed soybeans respectively.

A review by wood (2005) showed that 18:2n-6 from oil source such as soy bean meal is incorporated into muscle and adipose tissue in direct proportion to its concentration in diet. Similar results were found for other PUFA source e.g. linseed, which contain a high proportion of 18:3n-3 (Enser *et al.*, 2000) and fish oil which contains the long-chain n-3 PUFA, EPA and DHA. (Irie and Sakimoto, 1992)

Feeding SFA does not raise their proportion in muscle and adipose tissue as much as feeding PUFA. This is because of a lower incorporation into lipid and elongation and desaturation of these fatty acids into other SFA and MUFA. Comparison of papers published in the 1970's and 1980's with more recent ones show that the level of 18:2n-6 in pig muscle and adipose tissue has greatly increased during this time. This is partly not only because the oil content of diet has increased to promote faster growth but also due to lower carcass fat levels leading to softer fat tissue in modern lean pigs compared with the fatter pigs of former years.

Cattle

Despite the hydrogenating effect of the condition of the rumen on dietary PUFA, small but significant amounts of fats enter the duodenum to be absorbed into blood and is delivered to tissues. A recent study conducted by the university of Bristol and the institute of Grass land and Environmental Research (IGER) compared fatty acid composition of cattle fed either a grain based concentrate diet and a grass silage diet. The concentrate diet raised the proportion of 18:2n-6 and 20:4n-6, where as the grass silage diet increased levels of the n-3 PUFA 18:3, 20:5, and 22:6. All these changes reflect the fatty acid composition of the

diets. The increased concentration of 22:6n-3 in the grass silage group was significant since the provision of more 18:3n-3 does not always result in the higher levels of 22: 6n-3 in tissue. (Scollen *et al.*, 2003). The higher levels of SFA and MUFA in the grass silage group can partly be explained by overall greater fatness resulting from denovo fatty acid synthesis in cattle. When cattle are fed concentrate alone, the rate of PUFA passage through the rumen is rapid limiting the access to microorganism action, compared with a mixed forage/ concentrate diet. This helps to explain the high values for muscle PUFA proportion seen in young bulls fed a barley diet (Enser *et al.*, 1996).

The concentrate portion of beef cattle diets can be fortified with dietary oils with positive effects on muscle and adipose tissue fatty acid composition. Scollen *et al.* (2001) fed linseed and fish oil to increase proportions of 18:3n-3 and the long chain n-3 PUFA, respectively.

The effects of dietary oils on meat fatty acid composition are enhanced if they are protected from bio-hydrogenation in the rumen. Chemical protection can be achieved if protein in the diet is treated with formaldehyde, which results in a matrix structure with in which dietary fatty acids are encapsulated.

Several studies have shown that there is a linear relationship in muscle and adipose tissue between the proportions of 18:1 trans-4 (transvaccenic acid) and the main CLA isomer, cis-9, trans-11 CLA, reflecting the synthesis of CLA from its precursor. The results of the study of Warren *et al.* (2007) confirm the linear relationship between 18:1 trans-11 and CLA and shows that both fatty acids are at higher proportions following the consumption of fresh grass compared with grass silage and concentrates. Wood *et al.* (2005) showed that there were three main CLA isomers in beef cattle muscle and the levels were affected by dietary oil source. The cis-9, trans-11 isomer

always exceeded 80% of the total, the other two isomers being trans-11, cis-13 and trans-11, trans-13. Other factors that affect grass fatty acid composition and there by meat fatty acid composition include the preservation process. Excessive drying of grass prior to producing hay or silage reduces PUFA proportion through the action of plant enzymes and fatty acid oxidation (Dewharst *et al.*, 2003)

Sheep

The effects of grass based diets compared with concentrate diet are similar in sheep to those in cattle. Fisher *et al.* (2000) conducted a study in which Suffolk cross sheep were grazed on low land pasture or fed with a standard concentrate diet for three months before slaughter and reported that all the long chain n-3 PUFA were higher in the grass fed lambs than in those fed concentrate, including DHA. Wachira *et al.* (2002) fed diets containing 6% oil from different sources to sheep between 24 to 44 Kg live wt. The results showed that the proportion of 18:3n-3 in the group fed linseed was higher than for the grazed lamb but the proportion of 20:5n-3, 22:5n-3 and 22:6n-3 was lower. The results confirmed the effect of grass diet in raising levels of long chain n-3 PUFA.

Chicken

Like that of most monogastrics, the fatty acid composition of chicken is influenced by the type of fat in the diet (Phetteplace and Watkins, 1989& 1990). Changes in fatty acid composition due to diet are seen in all tissues. The adipose fat of broilers fed a basal diet with no added fat contained 30.7% saturated, 34.7% monounsaturated and 34.5% poly unsaturated fatty acids. Adipose fat from chicken fed the basal diet plus cotton seed oil contained 29% less monounsaturated, 29% more poly unsaturated and about the same proportion of saturated fatty acids as chickens fed the basal diet. The

fatty acid changes were due to a decrease in the amount of palmitoleic and oleic acids and an increase in linoleic acids. n-3 fatty acid levels in poultry meat can be increased by inclusion of fish oil or fish meal in the diet. The increase in n-3 fatty acids in chicken fed with fish oil was eight folds more than the chicken fed with chicken fat. The increase in the n-3 fatty acid content was primarily due to increase in eicosapentaenoic, docosapentaenoic and docosahexanoic acids.

Conjugated linoleic acids (CLA) are another group of fatty acids receiving considerable attention due to potential health benefits. A number of studies (Cresp and Esteve-Garcia, 2001., Badinga *et al.*, 2003) have shown that content of CLA in broiler meat and fat can be increased by including CLA in chicken's diet. Most of these studies have also shown that dietary CLA changes fatty acids levels in tissues by increasing saturated fatty acids and decreasing monounsaturated fatty acids, while usually not altering PUFAS.

Fatty Acid Composition of different Muscles, Tissues and Meat Products

Muscles in the carcass differ in fiber type, with muscles involved in rapid movement having predominantly white glycolytic type-II fibers and those involved in posture retention having predominantly red oxidative type-I fibers. All muscles contain a mixed population of fiber types, including intermediate types between these two extremes.

Red oxidative fibers contain more mitochondria and a higher proportion of phospholipid than white glycolytic fibers and as a result contain a higher proportion of PUFA.

Liver is also a metabolically active tissue with a high proportion of phospholipid in total lipids. The phospholipid in liver was less unsaturated than that in muscle. For example,

18:2n-6 was 12.4% and 5.8% of phospholipid fatty acids in muscle and liver respectively. The presence of long-chain PUFA in pigs subcutaneous fat although at low levels, contributes to a high nutritional value of pig meat.

Fatty Acid Composition of Chicken Eggs

Two chicken eggs constitute a typical serving, with each egg providing roughly 50g of edible material. Thus the values in tables showing the fatty acid concentration in g/100g edible portion can be used to approximate the fatty acid content of a serving of two chicken eggs. The values can be halved to obtain the amounts of fatty acids from a serving of one egg.

The fatty acid composition of chicken egg is as shown below.

These values apply to raw eggs, fresh or frozen as well as to whole eggs hard cooked in the shell. A 100g edible portion of whole egg contains 3.35 g saturated fatty acids, 4.46 g monounsaturated and polyunsaturated fatty acids, respectively. Almost one-half of the total fatty acids are monounsaturated, whereas a little more than one-third are saturated.

The values for fresh yolk are for yolk with a small amount of albumen. The albumen is essentially fat free. Therefore, the effect of its inclusion is to dilute slightly the fatty acid concentration in the yolk. The proportions of the various fatty acids for the fresh yolk are similar to the ratios for the whole egg. The levels of fatty acids for the yolk are higher than those of the whole egg, as the entire lipid in the yolk fraction. The yolk of an average large egg (60 g) weighs approximately 17g. Thus, the amount of various fatty acids provided per yolk can be readily calculated. However, it should be noted that the percentage of yolk is

not constant for eggs of various weight classes. Smaller eggs tend to have a larger ratio of yolk to albumin, and they would therefore have relatively higher fatty acid levels.

Omega - 3 Enrichment of Eggs

In recent years, following recommendations for the increased intake of n-3 fatty acid in human diets, there had been considerable interest in providing consumers with eggs containing elevated levels of n-3 fatty acids. Most studies have shown that the egg composition could be altered within two weeks of dietary changes. A change in yolk fatty acid composition can be expected within weeks as much of the development of the egg yolk occurs within the 10 days before ovulation.

In some studies fish oil were fed to laying hens, and the long-chain n-3 fatty acids in these oils appeared in eggs. In a typical study, 5% fish oil increased the egg DHA content from 2.9% to 11.8% of total yolk fatty acids. Hargis *et al.* (1991) observed that the long chain n-3

Table:3 Fatty Acid Composition of Chicken Eggs

Fatty Acid	Whole Egg g/100g
12:0	0.03
16:0	2.26
18:0	0.78
Total Saturated	3.10
16:1	0.30
18:1	3.47
Total monounsaturated	3.81
18:2	1.15
18:3	0.03
20:4	0.14
Total polyunsaturated	1.36
Total fats	9.94

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19

fatty acid concentration increased from 35 to 210 mg/100 g yolk, whereas the concentration of n-6 fatty acids, especially arachidonic acid decreased from about 100 to approximately 30 mg/100 g yolk upon feeding fish oil. High levels of long chain n-3 fatty acids might have suppressed the hepatic production of arachidonic acid from dietary linolenic acid. Most studies have showed roughly a fivefold increase in the long chain n-3 percentages when fish oil was fed. Yalcyn *et al.* (2007) found feeding of fish oil and flaxseed reduced the total saturated fatty acids content in chicken eggs. Similarly Nanjapan *et al.* (2013) reported that inclusion of n3 fatty acids rich feed ingredients in diet which are available in local market like bajra, linseed, rapeseed and fishmeal would be able to produce desired ratio of n3: n6 fatty acid enriched eggs with lesser cholesterol content.

Impact of Animal Fatty Acids on Human Health

Some SFA, that is those with less than 18-carbon atoms chain length, raise blood levels of low density lipoprotein (LDL) cholesterol, which increases the risk of atherosclerosis leading to chronic vascular disease (CVD) in man (Williamson *et al.*, 2005). On the other hand, MUFA and PUFA lower blood levels of LDL cholesterol. According to the recommendation of the World Health Organisation (2003), the total fat should constitute not more than 15 to 30% of total energy in the diet., SFA around 10%, n-6 PUFA around 5 to 8% and n-3 PUFA 1 to 2%. The U.K Department of Health (2004) has recommended that ratios between these fatty acid groups should be greater than 0.4 for PUFA: SFA and less than 4.0 for n-6: n-3 PUFA. Several studies agree that n-3 PUFA are necessary for proper brain and visual development in the foetus and have a role in reducing various cancers (Enser, 2001).

Trans fatty acids have more potent effects on LDL- cholesterol than SFA. Although trans

fatty acids are generally low in meat, there is some evidence that the trans fatty acids in meat and milk are less damaging to human health than those in other processed foods (Williamson *et al.*, 2005). Trans-11 18:1 (transvaccenic acid) is the precursor in tissue of the major CLA isomer, cis-9, trans-11 CLA, which is recognised to have several positive health benefits including inhibition of carcinogenesis and atherosclerosis and enhancement of the immune response.

Future prospects

The fatty acid composition of pork can be readily modified by diet since fatty acids are deposited unchanged by digestion. In ruminants, the effect of grass diet in increasing proportions of n-3 PUFA and possibly CLA is an interesting area of current research, leading to more desirable meat products for the consumer. Poultry represents a muscle food in which diet can be used to increase the concentration of bioactive fatty acids in the final product. Increasing n-3 fatty acids in poultry products by dietary supplementation of marine lipid is possible. However, such process will change the physical and chemical properties of muscle. Muscles containing high concentration of PUFA have lipids with lower melting point leading to muscle with soft and even liquid fat, eventually leading to consumer rejection. The fatty acid composition of meat is important for human health reasons and also has crucial effects on meat quality. Incorporating bioactive fatty acids which are beneficial to human health *vis-a-vis* maintaining consumer appeal in animal products by different methods offer great research prospects in the present health conscious society.

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THE SUCCESS STORY OF RKVY- SLBP CALF FEED SUBSIDY SCHEME IMPLEMENTED IN AYYAPPANCOVIL GRAMA PANCHAYATH, IDUKKI DISTRICT

Arun Kurian* and Josephine Francis #

*Veterinary Surgeon, Veterinary Dispensary, Mattukatta, Ayyapancovil.P.O., Idukki.

Veterinary Surgeon, Turkey Farm, Kureepuzha, Kollam

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INTRODUCTION

More than 75% of the female calves are born to the weaker sections of the society in Kerala and majority of them reach production stage only by 3 to 4 years (Agriculture(AHG)Department, 2001). Inadequate feeding and unscientific management practices are the major constraints identified, for this delay in attaining puberty in dairy animals of Kerala (Agriculture(AHG) Department, 2001). Profit from dairy farming can also be increased, if milk production starts at an early age and thereby increasing the productive life of dairy animals (Kurian, *et al.*, 2012).

The Calf Feed Subsidy Scheme (CFSS) under Special Livestock Breeding Programme (SLBP) is the most prestigious scheme implemented through the Department of Animal Husbandry (AHD) of Kerala. The main objective of this scheme is the early attainment of age at first calving in dairy animals and thereby, increasing their productive life and improving the milk production status of the state, leading to self sufficiency (Agriculture(AHG) Department, 2001).

The CFSS aims at providing good quality feed to ensure health, proper management, scheduled vaccination against common diseases, insurance coverage for unforeseen

loses, and better monitoring of calves, in order to bring the age at puberty, down to 14 – 16 months from the current state's average.

This study aims at evaluating the effectiveness of CFSS under RKVY-Special Livestock Breeding Programme implemented in Ayyapancovil Grama Panchayath, Idukki District.

Implementation and Evaluation of the scheme

100 crossbred healthy female calves, of the age 4 to 6 months born through artificial insemination, were enrolled under the scheme in September, 2011. The calves selected were ensured to be healthy and free from physical abnormalities. The calves were ear-tagged for identification and monitoring.

The subsidy for the envisaged Scheme under RKVY was INR 11395 for individual animals. The feed was distributed at subsidized rates to the calves enrolled in this scheme on monthly basis. The prescribed cattle feed ration for this scheme is as given in Table. 1

The calves were regularly monitored every three months and also on and then whenever possible. These calves were de-wormed at regular intervals under standard protocols, vaccinated against common diseases, and the owners were advised of proper management.

Table. 1 Schedule of Ration for different age groups

Age (Months)	Quantity of Feed (Kg)
4	30
5	45
6	52.5
7 to 18	60
19 to 32 months	75

The reproductive parameters like age at first heat, age at first artificial insemination (A.I), age of first conception and age at first calving of the individual animals were periodically recorded and evaluated. The averages of these values were compared with average values for the same for Kerala state and the effectiveness was evaluated. Moreover, the recording of milk production was done for possible animals using purposive sampling method and the average milk production was assessed.

The reproductive parameters under consideration were evaluated based on the age in months and the results are shown in table. 2. The average milk production of these animals was 16 liters per day.

The average age at puberty, age at first AI, and age at first conception for the 100 animals in this study were 14.88, 16.05, and 17.01 respectively. The age at first calving for 8, 42, 31, 13 and 6 animals were 22 months, 24 months, 27 months, 30 months and 33 months, respectively. The average age at first calving for these 100 animals was 26.09 months. These

averages were compared to the averages for the state (Cattle Sterility Office Bulletin, 2010-11) and is graphically depicted in graph.1.

CONCLUSION AND DISCUSSION

Six animals which had age of conception ≥ 22 months were identified to have infertility issues like defective oestrous cycle or first degree endometritis, which were treated using standard protocols. The average age at first conception and age at first calving would have been lower, if there were no such infertility problems for the animals in this study.

The milk production in these animals was higher when compared with the animals not included in the scheme, in the same locality. In addition, the off springs produced by the animals in the scheme were healthier and the body weight of these animals were higher, to those born to animals not included in the scheme, but using the same batch of frozen semen for artificial insemination.

The results of this study revealed that better feeding and proper management of the dairy animals from the younger ages, can decrease the age at puberty, age at first AI, age at first conception and age at calving to considerably low values, thereby maximizing the productive potential of dairy animals in Kerala, under prevailing conditions. The outcome emphasizes the fact that, heritable characteristics of age of puberty, age at first conception and age at calving is low but are more influenced by environmental factors (Mukasa-Mugerwa,

Table. 2. The recorded reproductive parameters of the 100 animals in the scheme

Reproductive parameters evaluated	Age in months				
	9-12	13-15	16 -18	19-21	> 21
Age at first heat	28	52	16	4	0
Age at first AI	12	49	35	4	0
Age at first conception	8	48	26	12	6

1989). The scheme was a complete success, as it achieved all the objectives intended. The success of this programme, points out that, the genetic potential of the dairy animals in Kerala in the present scenario can be exploited to the maximum, by better feeding and management practices.

The authors recommend widespread implementation of the Calf feed Subsidy Scheme by enrolling all the female calves born in the state, thereby decreasing the age at first calving of dairy animals and maximizing the productive life of dairy animals. This eventually increases the milk production of the state leading to self sufficiency.

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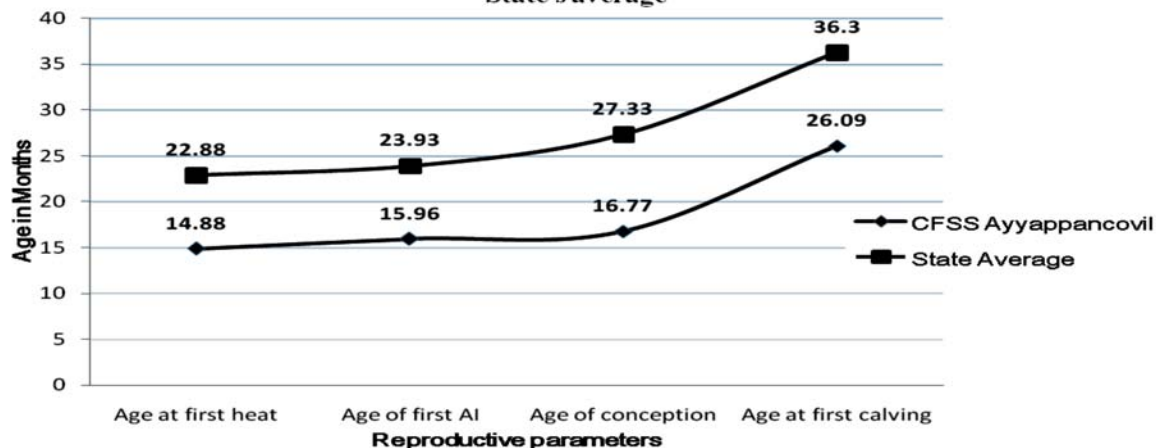
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Graph.1. Comparison of the reproductive parameters of the Scheme with State's average



LAMENESS IN DAIRY CATTLE: NUTRITIONAL APPROACHES FOR PREVENTION AND MANAGEMENT

Axsa P. Thomas¹ and M. T. Dipu²

¹MVSc Scholar, Department of Animal Nutrition,

²Assistant Professor, Department of Animal Nutrition,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur

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ABSTRACT

Role of nutritional factors on incidence and management of lameness in dairy cattle has been discussed. Excessive grain feeding and high ratio of non-fibre carbohydrate to neutral detergent fibre can affect the ruminal function resulting in lameness. Feedstuffs which contain more soluble carbohydrate will increase the production of more lactic acid in rumen resulting in release of vaso-active substances like histamines and endotoxins, which may alter the microvasculature of hoof and results in laminitis. Fat and body condition score have a positive role in improving hoof quality and thickness of the digital cushion, thus prevent sole ulcers and white line disease. Feeding management during transition period is very critical for management of lameness. Total mixed ration containing sufficient amount of fibre can be effectively used to reduce the incidence of lameness. Incidence of lameness can be lowered by adequate incorporation of biotin and organic trace minerals that helps in the production and maintenance of healthy keratinised tissues.

INTRODUCTION

Lameness is considered as one of the most significant welfare and productivity issue causing considerable economic loss for the producer and the dairy industry in general. Reduced milk production, lowered fertility and

involuntary culling of lactating dairy cattle can result from lameness. In India, the prevalence of clinical lameness in lactating cows and buffaloes is about 9 and 2 per cent respectively and 40 to 50 per cent cases have subclinical lesions (Randhawa, 2006). Lameness can have multiple causes including nutritional, managemental, environmental, genetic and infective factors. Early detection and proper treatment of the condition can minimize losses, improves the outcome, and reduces animal suffering.

Laminitis is a leading reason for lameness. Laminitis in dairy cattle has been closely associated with rumen function, with production of excessive organic acids in the rumen. Incidence of laminitis also reflects improper ration formulation or feeding management. Feeding of high concentrate diet to lactating cows in order to optimize milk production can contribute to the incidence of lameness. Moreover, rapid urbanization has limited the availability of cultivable land, which in turn leads to a rapid reduction in production and availability of fibre resources. Due to these reasons, dairy farmers in Kerala uses concentrate as the major feed resource and inclusion of fibre in the diet is limited, which can predispose the animal to lameness. Evidence exists on the effects of carbohydrates, protein, non-forage fiber sources and length of fiber particles, as well as other nutritional components such as

macro-minerals, trace minerals and vitamins on hoof epidermis and hoof horn quality. The present article focuses on the role of nutritional factors on the incidence and management of lameness in dairy cattle.

NUTRITIONAL FACTORS CONTRIBUTING TO LAMENESS

- High carbohydrate diet
- Low fibre
- High dietary protein
- Dietary anti-nutritional factors
- Vitamin disorders
- Mineral deficiencies

Effect of dietary carbohydrates

Inclusion of high levels of fermentable carbohydrates in the diet can cause an increase in the level of volatile fatty acids (VFA), a reduction in rumen pH and finally accumulation of lactic acid in rumen (Nordlund *et al.*, 2004). During acidosis, there will be production of vaso-active substance like histamine through ruminal conversion of amino acid histidine from the dietary protein by the bacteria *Allisonella histaminiformans*. The low ruminal pH also enhance the endotoxin release by gram negative bacteria (such as *E. coli*). These substances can cause vascular changes within the dermal capillary beds of corium. The pooling of blood in corium leads to ischemia, inflammation and necrosis of the corium-epidermal junction. Due to these events the functioning of keratin produced cells gets impaired resulting hoof lesions (Westwood *et al.*, 2003). In milder and prolonged cases of acidosis, the surviving animals suffer from laminitis. The pH stabilization in rumen can be achieved by use of dietary buffers, particularly when the diet contains a high proportion of cereal grains. Agents used as buffers include sodium bicarbonate, sodium sesquicarbonate, potassium bicarbonate, magnesium carbonate, calcium carbonate and bentonite. Microbial

feed additives (e.g. *Megasphaera elsdenii* and *Selenomonas ruminantium*) can also be used to control excessive accumulation of acids in the rumen.

EFFECT OF DIETARY FIBRE

An adequate intake of fibre is necessary for maintaining ruminal pH within the normal range. Roughage stimulates chewing and saliva secretions, which will neutralize the acids produced during rumen fermentation and maintain optimum pH. The concept of total mixed ration (balanced mixture of concentrate, roughage and micronutrients / feed additives) can be effectively used to incorporate fibre sources such as crop residues to animals in a completely balanced form. The total dietary dry matter (DM) of total mixed ration should contain a minimum of 25 per cent neutral detergent fibre (NDF), with 19 per cent NDF from coarse forage (NRC, 2001). Moreover, the cows should fed diets with adequate amount of physically effective NDF. i.e., feed particle containing NDF with size greater than 1.2 cm. This particle size stimulates rumination and salivation in cattle.

DIETARY PROTEIN

An inadequate supply of the sulphur containing amino acids (methionine and cysteine) may increase incidence of lameness as a result of the formation of soft horn. Adequate proportion of cysteine and methionine in claw horn of cows are necessary for the formation of disulphide bond during keratinization. High concentrations of rapidly degradable protein may produce high levels of rumen ammonia that may 'buffer' changes in rumen pH. A rapid association of ammonia with hydrogen ions removes hydrogen from solution and may neutralize upto 10–15 per cent of VFA produced. Further, microbial growth provides a quantitatively important sink for hydrogen. The toxic effects of high concentrations of

blood ammonia and (or) urea may compromise the sensitive germinal cells of the lamellae and corium. The release of toxic amine – histamine from the amino acid histidine can lead to coriosis and lameness. Use of bypass protein technology for animals producing more 10 litres of milk will be desirable for optimizing production and to reduce the incidence of lameness.

TRANSITION PERIOD AND LAMENESS

The transition period for a dairy cow is from 3 week pre-partum to 3 week post-partum. The transition period is a turning point in the productive cycle of the cow. The manner in which these changes occur and how they are managed are of great importance as they are closely linked to lactation performance, clinical and subclinical post-partum diseases, and reproductive performance that can significantly affect profitability. The transition from low energy – high fibre (dry cow ration) to high energy – low fibre (lactation ration) requires ruminal adaptation of the animals. Such rations also help in maintaining the normal body condition score (Bilcalho *et al.*, 2013). High yielding cows requires more nutrients and energy for milk production and are usually prone to lameness than low yielding cows (Green *et al.*, 2002). The decreased body condition score of the cow during calving to early lactation may predispose to fat mobilization and lameness. Cows mobilize fat from the digital cushion of hoof reaching its lowest point four months into lactation and the incidence of lameness was highest during first lactation (Hirst *et al.*, 2000; Livesey *et al.*, 2000).

ANTI-NUTRITIONAL FACTORS

Nitrate

Nitrate toxicity is seen in dairy cows grazing rapidly grown crops and pastures following dry or drought conditions. Forage

crops such as maize, oat hay, alfalfa hay and Johnson grass have tendency to accumulate nitrate. Under normal rumen pH there is rapid reduction of nitrate to nitrite. This nitrite in rumen reduced to ammonia and is either utilized by the rumen microbes or converted to urea and excreted through urine. Toxicity happens if the reduction of nitrate to nitrite exceeds the reduction of nitrite to ammonia. Excess nitrite in circulation converts haemoglobin to methaemoglobin, reducing the oxygen carrying ability of the blood. Nitrite is a potent vasodilator. Stagnation and pooling of blood in the peripheral circulation, including the vascular beds of the corium, may induce anoxia and the accumulation of tissue toxins, causing laminitic lesions.

Mycotoxins

Ergotism: Ergot poisoning results from ingestion of alkaloids produced by the fungus *Claviceps purpurea*, which infects the mature seed head of rye grass, wheat and barley. This can result in lameness, swelling and gangrene of foot and lower hindlimbs, and loss of extremities as a result of the vaso-constricting alkaloids, ergotamine, ergonovine (ergometrin) and ergocriptine.

Fescue foot and Perennial ryegrass endophyte: Lameness in cattle grazing standard (or wild type) high endophyte tall fescue (*Festuca arundinacea*) is associated with alkaloids produced by the endophyte *Neotyphodium coenophialum* and *Neotyphodium lolii* particularly the ergot peptine alkaloid, ergovaline, effects as a dopamine agonist and vaso-constrictor. It will compromise blood flow to the extremities of cattle leading to sloughing of the hoof.

Vitamins

Biotin

Biotin is required for the synthesis of long

chain fatty acids and helps in the production of complex lipid molecules in the intercellular cementing substance. Adult rumen may synthesize adequate amounts of biotin for various biological needs. Typical dairy diets containing more than 50% of DM as grain or concentrate may decrease ruminal synthesis of biotin. Supplementation of biotin (non-rumen protected) in feed at 20 mg/day can reduce the incidence of lameness (Hedges *et al.*, 2001). Ration with high forage can stimulate ruminal biotin synthesis.

Other vitamins

Vitamins A, D and E have integral role in the structure and quality of keratinized horn tissue. Vitamin A is required in the differentiation of keratinizing cells. Vitamin D is a regulator of calcium metabolism and has a positive effect on keratinization. Vitamin E is a lipid-soluble anti-oxidant and maintains lipid-rich, cellular membranes in the intercellular cementing substance of horn tissue.

Minerals

Copper: It activates thiol oxidase enzyme, responsible for formation of disulfide bonds between Cystein residues of keratin filaments. Cattle suffering from a subclinical Cu deficiency showed heel cracks, foot rot, and sole abscesses due to insufficient cytochrome C oxidase activity, resulting in deficient energy supplies for differentiating keratinocytes (Ballantine *et al.*, 2002).

Zinc: Zinc helps in the formation of the structural proteins during the keratinization process. It regulates calmodulin, protein kinase C, thyroid hormone binding, and inositol phosphate synthesis. Zinc is required for activation of the cytosolic enzyme Cu/Zn superoxide dismutase (SOD), which prevent peroxidation of lipid.

Manganese: Manganese helps in the

activation of galacto transferase and glycosyl transferase enzymes needed for the synthesis of chondroitin-sulfate side chains of proteoglycan molecules that are essential in the formation of cartilage and bone. Manganese activates pyruvate carboxylase and is responsible for gluconeogenesis.

Selenium: It is a constituent of the enzyme glutathione peroxidase, and protects both the intra- and extra-cellular lipid membranes against oxidative damage. Excessive supplementation of Se may be damaging to developing keratinocytes.

Calcium: Helps in the keratinization and cornification process. Calcium is needed for activation of epidermal transglutaminase (TG), which is active in cross-linkage of the cell envelope keratin fibers and involved in the terminal differentiation of the epidermal cells (Tomlinson *et al.*, 2004). Inadequate vascular supply or hypocalcemia may lead to depressed TG activity and formation of dyskeratotic horn.

NUTRITIONAL MANAGEMENT

Ensure optimum fibre in the diet and at least 15 per cent of fibre particles should exceed 1.5 inches in length. Forage NDF should exceed 19 to 21 per cent depending on forage digestibility and inclusion level of non-forage fibre.

Avoid excessive grain feeding and enhance use of slowly fermentable polysaccharides. Add buffers such as sodium bicarbonate (100 to 150 gm per day) to lactation diets. Use total mixed ration. Feed forages before grain or concentrates if total mixed ration is not used.

Follow transition ration. Use minimum of two rations (close-up dry cow and fresh cow rations) for rumen adaptation during transition period. Do not change the energy level between the transition rations to more than 10 per cent.

CONCLUSION

Attention to proper hoof health is an important part of day-to-day dairy management. By improving hoof quality and reducing the effects of hoof injuries and diseases, significant improvements can be made to dairy profitability. Proper nutrition is a key factor in this regard since it acts as a first line of defense in maintaining the hoof integrity. Hoof health management requires a team approach and involves the dairyman, veterinarian and nutritionist. Research should be focused to develop farmer friendly technologies for management of lameness and hoof health in cattle.

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MEAT FROM LABORATORY-NOT A FANTASY ANYMORE

Sreekumar.T.R¹, Mohd Matin Ansari²

¹M.V.Sc student, ² PhD Scholar,
Indian Veterinary Research Institute, Bareilly, Uttar Pradesh

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Research on stem cells have bestowed us with new opportunities and potential applications in the field of regenerative medicine, basic science research, gene targeting, cloning and transgenic animal production etc to name a few. Its potential application in livestock production include enhanced reproductive performance, improved growth rate and feed utilization, improved carcass composition and increased disease resistance, improvement in milk and meat production and their composition. Recently it has opened up an exciting new window of opportunity- production of meat in invitro conditions. i.e. from lab itself!.

AN ALTERNATIVE TO CONVENTIONAL MEAT PRODUCTION.

In recent years, the notion of finding an alternative to meat production from livestock is gaining strength. Conventional meat production is getting more undesirable because of concerns about environment degradation, sustainability, public health and animal welfare. With increasing human population and rising living standards, especially in developing economies the demand for meat is expected to double by the middle of this century. This necessitates further intensification of livestock rearing for meat purpose. This scenario could worsen the threats posed by impending climate change. Livestock meat production

accounts for considerable portion of green house gas emission, land usage, water and energy consumption. Of the three major green house gases specifically carbon dioxide, methane and nitrous oxide, the contribution of livestock to their total emission is 9%, 39% and 65% respectively (FAO 2006). Livestock is far less efficient in terms of converting feed to protein. Public concern about animal welfare is also on increase and it may affect the consumer behavior. Lastly public health concerns- diabetes, cardiovascular disease and colorectal cancer are associated with red meat consumption.

NOVELTY OF CULTURED MEAT.

One of the many alternatives under investigation is culturing meat based on stem cell technology. There is scope for novel products. Blends of meat from different sources could create hitherto unimaginable meat. Physio-biochemical composition of meat can be altered to make it healthier or specialized meat product. With cultured meat, meat production can be made more efficient because they can keep all the variables under control. There is no need to slaughter any livestock. Desirable factors (for eg- poly unsaturated fatty acids) could be increased in content. It might be appealing to vegetarians who are against slaughtering of animals for meat.

CURRENT STATUS OF CULTURED MEAT

Meat is nothing but skeletal muscles and associated mesenchymal tissues like bone, cartilage, fat and fibrous tissue. Stem cell technologies *viz-* isolation and characterization of stem cells, *ex vivo* culture of cells and tissue engineering, have enabled production of bio-artificial muscles (BAM) from skeletal muscle resident stem cells also called satellite cells. Currently they are primarily used as research tools and are far from convincing meat alternative. But they are found to be valuable protein source.

Stem cells of various types can be used for culture of meat. Most suitable is the Myoblast or satellite cells which are responsible for muscle regeneration after injury. Advantage with these cells is that once cultured into sufficient number of cells, these cells could readily differentiate into myotubules and myofibrils. Negative side with these cells is that it is difficult to maintain the cells in replicative state in cell culture until sufficient number of cells are obtained. To overcome this problem, embryonic stem cells or induced pluripotent stem cells could be used as alternatives. Other components of meat like fat tissue, bone and cartilage could be made from cell types like adult adipose derived stem cells, adult tissue derived stem cells which has shown propensity for differentiating into osteocytes, chondrocytes and mature adipocytes.

Advances in large scale culture of mammalian cells have become possible due to advances in cell media, incubators and serum production. Biggest challenge in skeletal muscle cell culture is optimizing various variables in cell culture and controlling the interaction between them. Large scale high throughput analysis should be set up to optimize culture conditions. As of now serum based media is considerably superior to synthetic media in cell culture but eventually

serum based media could be completely replaced with synthetic media which is devoid of any serum products. Serum free culture using surface bound substrate adsorbed signaling molecules like vitronectin and laminin was found to aid differentiation into myotubules. There are two phases in culture of muscle cells from satellite cells- the proliferative phase and the differentiation phase. Main aim is to achieve maximum possible doubling of satellite cells before differentiation phase. Currently with available technology 20 doublings can be achieved. Biologic modulators have been designed to optimize proliferation and delay differentiation.

After getting sufficient number of cells, next step is their differentiation into skeletal muscle and coerce them to produce protein. A combination of mechanical, biochemical and metabolic stimuli appears to be having effect on inducing hypertrophy.

Tissue engineering using collagen or matrigel scaffolds were found to help to anchor most mesenchymal cells and also skeletal muscle cells and organize them to develop tension within fiber. This static tension boosts protein production and in addition with specific coatings electric current was also found to be a good agent that induces maturation of cells.

Application of these techniques has made generation of small BAMs feasible. Biggest challenge is creation of bigger BAMs with built in blood vessels or channels which could create continuous flow of nutrients and oxygen. With the development of technologies like bioprinting which could deposit cells and biomaterials into spatial orientation that simulate physiologically relevant geometries, there is hope that these challenges can be successfully dealt with.

WILL THE CULTURED MEAT BE APPETIZING?

Most people who are not onboard with the future of cultured meat doubt about the appetizingness of cultured meat. Contractile proteins comprise the bulk of skeletal muscles but other proteins are also there which are important for texture, color and taste of muscle tissue. Myoglobin is one such particularly important protein. It is haeme carrying protein particularly responsible for the pink color of meat and likely determines taste as well. The transcriptional regulators and activators of myoglobin have been identified and well understood. Contractile activation of muscle in hypoxia setting will maximally stimulate myoglobin production. It seems feasible to increase myoglobin content using stimuli that are compatible with tissue engineering of products that eventually should be consumed. With extension of research to other proteins which have some say in taste factor, meat mimic with acceptable taste is likely to be produced.

World noticed with awe when an artificial burger made of lab grown meat was unveiled recently by team led by Professor Mark Prost, scientist in Maastricht University, Netherlands. It was the culmination of research project of 5 years. It was made from cow shoulder muscle stem cells and it did cost \$ 3,00000. So definitely production of cultured meat in commercial scale further warrants refinement and extension of current technology and improving scalability. There is a long way to go and issues like scalability, quality control, prevention of disease/ contamination etc needs to be sorted out. But the concept and potential of cultured meat is never in doubt and in future one may not be surprised to see cultured meat being offered along with conventional meat in a supermarket and there won't be two minds in selecting the one with an element of environmental friendliness associated with it.

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STUDIES ON THE INFLUENCE OF FAT PERCENTAGE OF MILK ON NISIN ACTIVITY

Radha, K. and Anna Anandh, M.

Assistant Professor, Department of Dairy science,
College of Veterinary and Animal sciences, Mannuthy

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ABSTRACT

Bioassay was performed to study the influence of fat percentage of milk on nisin activity. *Micrococcus luteus* (MTCC2848) was used as an indicator organism. Standardized milk, toned milk, double toned milk and skim milk added with 50, 100, 200 and 300 IU nisin/ml were studied for the nisin activity during a chiller ($4\pm 1^\circ\text{C}$) storage period of 24 days. The initial nisin concentrations in standardized milk samples were 41.4 ± 0.51 , 86.0 ± 0.71 , 175.0 ± 0.71 and 267.4 ± 1.78 respectively. The values decreased significantly during storage to 3.6 ± 0.51 , 12.8 ± 1.02 , 39.6 ± 3.33 and 97.8 ± 3.80 respectively on 24th day. Toned milk samples had initial nisin concentrations of 42.0 ± 0.71 , 86.0 ± 0.71 , 174.0 ± 0.70 and 266.2 ± 1.56 respectively. The values decreased significantly during storage to 9.20 ± 0.57 , 18.60 ± 1.50 , 48.0 ± 2.77 and 98.6 ± 4.30 respectively on 24th day. The initial nisin concentrations in double toned milk samples were 44.0 ± 0.71 , 86.6 ± 0.93 , 175.4 ± 1.08 and 266.4 ± 1.50 respectively. They decreased progressively during storage and the values on 24th day were 11.0 ± 0.55 , 30.2 ± 2.08 , 48.4 ± 3.76 and 105.4 ± 4.96 respectively. The skim milk samples had initial nisin concentrations of 43.6 ± 0.51 , 87.6 ± 0.75 , 175.0 ± 1.00 and 267.2 ± 1.77 respectively. The values at 24th day of storage were 11.2 ± 0.37 , 31.0 ± 2.88 , 51.4 ± 4.89 and 111.0 ± 5.34 respectively. The rate of decline in nisin activity was higher in

standardized milk followed by toned milk. In double toned and skim milk decrease in nisin activity was relatively slower. The results showed a clear negative relationship between fat percentage and nisin activity.

Keywords: Milk, Fat percentage, Nisin, Bio-Assay, Bacteriocins.

INTRODUCTION

Non-thermal and low thermal treatments are attracting interest of the food industry due to their capability of assuring the quality and safety of food. Among them, bacteriocins from lactic acid bacteria, such as Nisin, Pediocin PA-1, Lacticin 3147 and Enterocins may be potentially useful for the dairy industry. Utilization of Bacteriocins alone, or combined with other treatments, could represent a promising advance for the microbiological safety and maintenance of sensory properties in milk and milk products (Lopez and Belloso, 2008). Exploitation of bacteriocin such as nisin as a bio preservative is a newer approach to achieve extended shelf life in regions with inadequate refrigeration facilities. Nisin is produced by safe food grade bacteria and it is also having favourable properties such as good stability under conditions of processing and easy degradation in the human gastro-intestinal tract. This makes it an ideal bio-preservative for use in foods (Daeschel, 1989; Ray 1992). Nisin activity is reported to be influenced by the fat

content of the product (Jung *et al.*, 1992). Nisin has been recognized as a safe food preservative by the joint Food and Agriculture Organisation and World Health Organisation (FAO/WHO) expert committee which has allowed a level of 3.3×10^6 units/kg body weight and permitted an unconditional acceptable daily intake (ADI) to be set at 3.3×10^4 units/kg body weight (Thomas *et al.*, 2000). It is the only commercially produced bacteriocin and is used in more than 50 countries for the last 60 years (Danisco, 2002). Nisin was also approved by the FDA (1988) as GRAS (Generally recognized as safe).

MATERIALS AND METHODS

Fresh cow milk obtained from the cattle farm maintained at Indian Veterinary Research Institute, Izatnagar was used in all the experiments after suitable standardization. Nisaplin, a commercial preparation of nisin with an activity of 1000 IU/mg was obtained from Danisco International, Brabrand, Denmark. Nisin was added at the rate of 50, 100, 200 and 300 IU/ml at about one hour prior to pasteurization in order to facilitate thorough dispersion. After pasteurization these samples were stored at $4 \pm 1^\circ\text{C}$ and checked for the activity of nisin during storage for a period of 24 days in order to assess the influence of fat percentage on the nisin activity. Residual nisin concentrations were determined during storage period in standardized milk, toned milk, double toned and skim milk added with 50, 100, 200 and 300 IU nisin/ml. Bioassay was carried out as per the method described by Wolf and Gibbons (1996) with suitable modifications. *Micrococcus luteus* (MTCC2848) obtained from Institute of Microbial Technology, Chandigarh, was used as indicator organism. Bacto-agar medium (Bacto-peptone -10g; NaCl-3g; yeast extract-1.5g; glucose-1g; Bacto-agar-10g and Disodium hydrogen Phosphate (Na_2HPO_4) -10g) with 1% of Tween 20 was used for bioassay. The indicator organism at

1% concentration was inoculated into medium and then dispensed into sterile petridishes. On solidification, test wells were bored into the agar by using an 8mm diameter metal tube. Standard nisin solutions were dispensed into the wells and plates were incubated at 30°C for 48 hours. Similarly, milk samples with added nisin were also dispensed into the wells and plates were incubated at 30°C for 48 hours. After incubation, zones of inhibition were measured to the nearest 0.1mm. Regression analysis was applied to estimate the residual nisin concentration during storage (Snedecor and Cochran, 1994).

RESULTS & DISCUSSION

The nisin concentrations (IU/ml) in standardized, toned, double toned and skim milk during storage are presented in table 1.

The initial nisin concentrations in standardized milk samples added with 50 and 100 IU nisin/ml were 41.4 ± 0.51 and 86.0 ± 0.71 respectively. The values decreased during storage to 3.6 ± 0.51 and 12.8 ± 1.02 respectively on 24th day. Samples added with 200 and 300 IU nisin/ml had initial concentrations of 175.0 ± 0.71 and 267.4 ± 1.78 respectively and the values decreased to 39.6 ± 3.33 and 97.8 ± 3.80 respectively at 24 days of storage. Toned milk samples added with 50 and 100 IU nisin/ml had initial nisin concentrations of 42.0 ± 0.71 and 86.0 ± 0.71 respectively at zero day of storage. The values decreased during storage to 9.20 ± 0.57 and 18.60 ± 1.50 respectively on 24th day. In samples with 200 and 300 IU nisin/ml the initial concentrations were 174.0 ± 0.70 and 266.2 ± 1.56 respectively and the values significantly decreased to 48.0 ± 2.77 and 98.6 ± 4.30 respectively at 24 days of storage.

The initial nisin concentrations in double toned milk samples added with 50 and 100 IU nisin/ml were 44.0 ± 0.71 and

Table.1: Mean \pm SE Nisin concentration values (IU/ml) in different types of pasteurized milk during storage at 4 \pm 1 $^{\circ}$ C

Storage period (Days)	Nisin Concentration IU/ml				Days Mean
	50	100	200	300	
Standardized milk					
0	41.4 \pm 0.51 ^a	86.0 \pm 0.71 ^a	175.0 \pm 0.71 ^a	267.4 \pm 1.78 ^a	142.4 \pm 19.89 ^a
4	40.8 \pm 0.37 ^a	84.4 \pm 0.25 ^a	173.0 \pm 0.71 ^a	265.8 \pm 1.32 ^a	141.0 \pm 19.82 ^a
8	40.0 \pm 0.55 ^a	81.2 \pm 0.37 ^b	163.8 \pm 1.36 ^b	260.8 \pm 1.16 ^b	136.4 \pm 19.39 ^b
12	33.4 \pm 0.68 ^b	62.4 \pm 2.84 ^c	140.8 \pm 1.16 ^c	227.4 \pm 1.54 ^c	116.0 \pm 17.31 ^c
16	24.8 \pm 0.86 ^c	43.0 \pm 1.79 ^d	112.2 \pm 2.16 ^d	193.6 \pm 2.66 ^d	93.4 \pm 15.26 ^d
20	12.2 \pm 0.86 ^d	28.6 \pm 1.21 ^e	75.0 \pm 1.84 ^e	152.6 \pm 2.36 ^e	67.1 \pm 12.52 ^e
24	3.6 \pm 0.51 ^e	12.8 \pm 1.02 ^f	39.6 \pm 3.33 ^f	97.8 \pm 3.80 ^f	38.4 \pm 8.51 ^f
Toned milk					
0	42.0 \pm 0.71 ^a	86.0 \pm 0.71 ^a	174.0 \pm 0.70 ^a	266.2 \pm 1.56 ^a	142.1 \pm 19.74 ^a
4	41.8 \pm 0.58 ^a	85.4 \pm 0.51 ^a	172.0 \pm 0.55 ^a	262.0 \pm 0.84 ^a	140.3 \pm 19.38 ^a
8	41.2 \pm 0.59 ^a	81.8 \pm 0.66 ^b	165.4 \pm 1.63 ^b	253.8 \pm 1.16 ^b	135.5 \pm 18.74 ^b
12	34.8 \pm 1.02 ^b	69.4 \pm 1.03 ^c	143.8 \pm 1.85 ^c	224.4 \pm 1.72 ^c	118.1 \pm 16.73 ^c
16	24.4 \pm 0.87 ^c	53.0 \pm 0.71 ^d	117.0 \pm 2.75 ^d	188.8 \pm 2.67 ^d	95.8 \pm 14.55 ^d
20	16.8 \pm 0.58 ^d	36.0 \pm 0.95 ^e	76.4 \pm 2.73 ^e	145.4 \pm 3.41 ^e	68.6 \pm 11.3 ^e
24	9.2 \pm 0.57 ^e	18.6 \pm 1.50 ^f	48.0 \pm 2.77 ^f	98.6 \pm 4.30 ^f	43.6 \pm 8.08 ^f
Double toned milk					
0	44.0 \pm 0.71 ^a	86.6 \pm 0.93 ^a	175.4 \pm 1.08 ^a	266.4 \pm 1.50 ^a	143.1 \pm 19.63 ^a
4	43.0 \pm 0.70 ^a	84.4 \pm 0.68 ^{ab}	172.4 \pm 0.93 ^a	263.6 \pm 1.12 ^a	140.8 \pm 19.48 ^a
8	41.4 \pm 0.60 ^a	82.0 \pm 0.71 ^b	168.0 \pm 0.95 ^b	259.8 \pm 1.02 ^b	137.8 \pm 19.27 ^b
12	35.6 \pm 0.51 ^b	70.8 \pm 0.86 ^c	153.2 \pm 0.97 ^c	228.6 \pm 1.72 ^c	122.3 \pm 17.11 ^c
16	29.8 \pm 0.37 ^c	56.0 \pm 1.41 ^d	125.4 \pm 2.09 ^e	195.2 \pm 1.85 ^d	101.8 \pm 14.72 ^d
20	20.2 \pm 0.86 ^d	43.8 \pm 1.36 ^e	80.4 \pm 3.78 ^f	152.6 \pm 2.75 ^e	74.2 \pm 11.54 ^e
24	11.0 \pm 0.55 ^e	30.2 \pm 2.08 ^f	48.4 \pm 3.76 ^g	105.4 \pm 4.96 ^f	48.7 \pm 8.23 ^f
Skim milk					
0	43.6 \pm 0.51 ^a	87.6 \pm 0.75 ^a	175.0 \pm 1.00 ^a	267.2 \pm 1.77 ^a	143.4 \pm 19.67 ^a
4	42.8 \pm 0.58 ^a	87.4 \pm 0.68 ^a	173.6 \pm 0.93 ^a	266.8 \pm 1.93 ^a	142.6 \pm 19.62 ^a
8	42.6 \pm 0.51 ^a	83.6 \pm 0.93 ^b	166.0 \pm 1.58 ^b	263.9 \pm 2.20 ^b	139.0 \pm 19.43 ^b
12	37.8 \pm 0.37 ^b	72.4 \pm 0.92 ^c	149.0 \pm 2.68 ^c	232.7 \pm 0.86 ^c	123.0 \pm 17.24 ^c
16	30.8 \pm 0.36 ^c	59.4 \pm 1.08 ^d	121.0 \pm 4.30 ^d	201.2 \pm 1.77 ^d	103.1 \pm 15.03 ^d
24	11.2 \pm 0.37 ^e	31.0 \pm 2.88 ^f	51.4 \pm 4.89 ^f	111.0 \pm 5.34 ^f	51.2 \pm 8.76 ^f

· Means \pm SE are averages of five replications

· Means with common superscripts in a column (alphabets) do not differ significantly (P< 0.01)

86.6±0.93 respectively. Then the values decreased progressively during storage and the concentrations on 24th day were 11.0±0.55 and 30.2±2.08 respectively. In samples added with 200 and 300 IU nisin/ml the initial values were 175.4±1.08 and 266.4±1.50 respectively and declined to 48.4±3.76 and 105.4±4.96 respectively at 24 days of storage. The skim milk samples had initial nisin concentrations of 43.6±0.51 and 87.6±0.75 respectively in samples added with 50 and 100 IU nisin/ml. Nisin concentrations progressively decreased during storage and the values at 24th day of storage were 11.2±0.37 and 31.0±2.88 respectively. Samples added with 200 and 300 IU nisin/ml had initial concentrations of 175.0±1.00 and 267.2±1.77 respectively and the values declined to 51.4±4.89 and 111.0±5.34 respectively at 24 days of storage.

The rate of decline in nisin activity was higher in standardized milk followed by toned milk. In double toned and skim milk the decrease in nisin activity was relatively slower. The results showed a clear negative relationship between fat percentage and nisin activity.

Several studies have shown that nisin activity is diminished in foods that contain high fat. Jung *et al.* (1992) reported a 33% decrease in initial nisin activity of skim milk and more than 88 % decrease in nisin activity of milk containing 12.9% fat. Nisin was more effective in controlling *Staphylococcus aureus* in skim milk than in whole milk and this is attributed to the effect of milk fat content (Jones, 1974). Cytoplasmic membrane of the microbes was the major target of nisin action. Possibly this was disrupted by nisin's interaction with phospholipid components (Henning *et al.*, 1986).

It appears that binding or adsorption of the polypeptide structure of nisin occurs with certain food components, which makes it inactive or unavailable to inhibit microbes.

Exactly how lipids interact with nisin and affect its activity is not clearly understood, but this phenomenon warrants further investigation in order to optimize the effective use of nisin in food applications.

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HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *Boerrhaviadiffusa* L. AGAINST CARBON TETRACHLORIDE (CCl₄) INDUCED HEPATOTOXICITY IN RATS

Mini Bharathan* and Joy .A.D

Assistant Professor, Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences, Mannuthy

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ABSTRACT

Objective of the present study was to assess the hepatoprotective activity of the methanolic extract of *Boerrhavia diffusa* L. against CCl₄ induced hepatotoxicity in rats. CCl₄ was administered @2.5 mg/kg as 1:1 in olive oil. The methanolic extract of *B.diffusa* (whole plant) was administered in the dose rates of 250 and 500 mg/kg orally at 12h intervals and after 36h., blood was collected, serum separated and biochemical parameters like SGOT, SGPT, ALP, total bilirubin and direct bilirubin were estimated. The liver samples were subjected to histopathological studies. Treatment with the extract significantly reduced the liver damage induced by CCl₄ as indicated by a decrease in the elevated levels of all the biochemical parameters to near normal values in a dose-dependant manner. The histopathological findings were also complimentary to these results. The methanolic extract of *B. diffusa* possessed significant protective activity against CCl₄ induced liver toxicity in rats.

Keywords: *Boerrhaviadiffusa* L, hepato toxicity, carbon tetrachloride

INTRODUCTION

Boerrhavia diffusa L. commonly known as “punarnava” in Sanskrit and ‘spreading hogweed’ in English has been used in traditional Ayurvedic medicines alone and in

combination with other plants for the treatment of liver, gall bladder, renal and urinary disorders since ages. The earliest mention of this plant is seen in Charaka Samhita (Charaka, 1949). The plant was included as one of the extensively investigated medicinal plants in India by Vohora (1989). Abraham (1979) reported that the plant as a whole was effective in jaundice, oedema and blood pressure. Two known lignansureodendrin and syringarexual mono-β-D glucoside have been isolated from the methanolic extract of the roots of the plant (Lami *et al.*, 1991) and the former was found to exhibit Ca channel antagonistic effect. Singh *et al.* (1991) observed that the ethanolic extract of *B. diffusa* @ 250 mg/kg did not have any teratogenic effect. Rawar *et al.* (1997) investigated the effect of seasons and thickness of roots on the hepatoprotective effect of the plant against thioacetamide induced hepatotoxicity in rats. They found that an aqueous extract of roots of diameter 1-3 cm collected during summer (May) exhibited maximum protection of the serum enzymes SGOT, SGPT and ALP.

An earlier study by the same author has revealed that the methanolic extract @ 200 mg/kg body weight possess significant anti-inflammatory activity both acute and chronic inflammatory models in rats. The present study was conducted to find out the effect of methanolic extract of *B. diffusa* on CCl₄

induced hepatotoxicity in rats.

MATERIALS AND METHODS

Chemicals

CCl₄ was purchased from Merck India Ltd. The auto analyser kits for estimation of serum ALT, AST, AP, TB and DB were purchased from Merck India Ltd.

Plant material

The whole plant of *B. diffusa* L. were collected from the premises of Veterinary College, Mannuthy and authenticated at Medicinal Plants Division of Horticultural College of KAU.

Animals

Wistar albino rats weighing around 150-200 g of either sex procured from SABS, Mannuthy were used for the study. They were housed in well ventilated cages (temperature 30 ± 2°C, humidity 65-70% and 12 h light/dark cycle) and fed with standard rodent diet from SABS, Mannuthy and drinking water ad libitum. Animal studies were conducted according to the Ethics Committee regulations of COVAS, Mannuthy.

Extraction

The plant materials (whole plant including leaves, stem and roots) were dried under shade at 30°C, pulverized and extracted with methanol in a soxhlet apparatus. The extract was evaporated to dryness under reduced pressure.

CCl₄ induced hepatotoxicity

Rats were divided into 4 groups of 8 each. Group I (control) was administered a suspension of distilled water and olive oil (1:1 proportion). Group II served as toxic control and received 1:1 mixture of CCl₄ suspension in olive oil @ 2.5 ml/kg bodyweight. Group III and IV were treated with methanolic extract of *B. diffusa* @ 250 and 500 mg/kg po. All the doses were

repeated at 12 hours and 24 hours respectively. 36 hours after the initial dose, the animals were sacrificed under light ether anaesthesia. Blood was collected from the retro-orbital plexus of all the rats and allowed to clot for 30 minutes at 37°C. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 minutes and analysed for SGOT, SGPT, ALT, TB and DB (Bergmeyer, 1980 and Perry *et al.*, 1986).

Histopathological studies

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histopathological studies. Initially, the material was fixed on 10% buffered neutral formation for 48 hours. The sections were then dehydrated in gradual ethanol (50-100%) cleared in xylene and embedded in paraffin sections (4-5 µm thick) were prepared and stained with hematoxylin and eosin dye for photomicroscopic examination.

Statistical Analysis

All the data are expressed as mean ± SEM. One way analysis of variance was used for the statistical analysis of data. Students' t test (Woolson, 1987) was used to determine the significance. The probability value of P<0.05 was considered as significant.

RESULTS

The levels of all the serum parameters studied were increased significantly in the toxicant group i.e., Group II (Table 1). The levels of AST, ALT, total and direct bilirubin showed significant decrease in the group IV when compared with the toxicant Group i.e., Group II. The levels of AP and TB were also decreased, though not significantly. Similarly the levels of all the parameters except AP were decreased in the Group III also, though not significantly.

The results of histopathological study can be read from the Fig. 1, 2, 3 and 4. which correspond to the groups I, II, III and IV respectively.

Table 1. Assessing the hepatoprotective activity of the methanolic extract- results of biochemical study

Group No	Treatment	ALT (U/l) (Mean ± SE)	AST (U/l) (Mean ± SE)	AP (U/l) (Mean ± SE)	TB (mg/dl) (Mean ± SE)	DB (mg/dl) (Mean ± SE)
I	Vehicle	209.5 ± 21.85	73.25 ± 11.97	310 ± 46.99	0.34 ± 0.5	0.28 ± 0.5
II	Toxicant	1150.86 ± 281.68	3405.86 ± 739.72	1375.25 ± 281.0	1.81 ± 0.37	1.2 ± 0.4
III	Ext @ 200 mg/kg	696.375 ± 41.067	607.125 ± 198.827	1392.25 ± 226.53	1.8 ± 2.8	0.963 ± 0.18
IV	Ext @ 400 mg/kg	78.375 ± 10.48*	425.5 ± 70.22*	1025.85 ± 140.99	0.713 ± 1.2	0.5125 ± 0.16*

*Significant at 5% level



Fig. 1 The hepatic cells are radially placed and each cell has a large spherical nucleus with pronounced nucleolus and granular cytoplasm

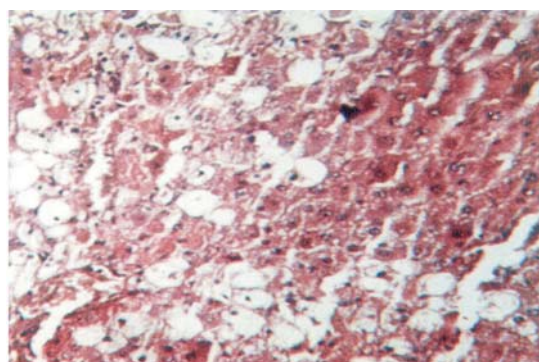


Fig. 3. Hepatic cells have become more distend with prominent nucleus and are arranged in the form of cords. Vacuolation also is lessened.

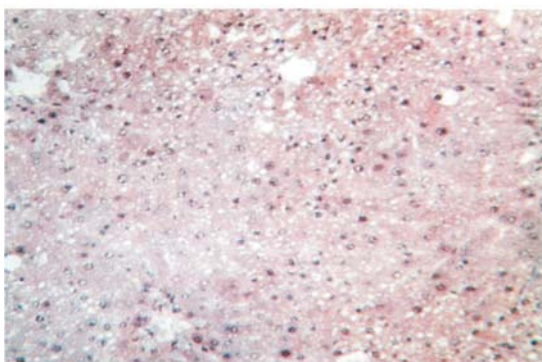


Fig.2 There is heavy destruction of the overall arrangement of liver cells because most of the cells are in a ruptured state and without cytoplasm. Space formation and high degree of vacuolation are also seen.

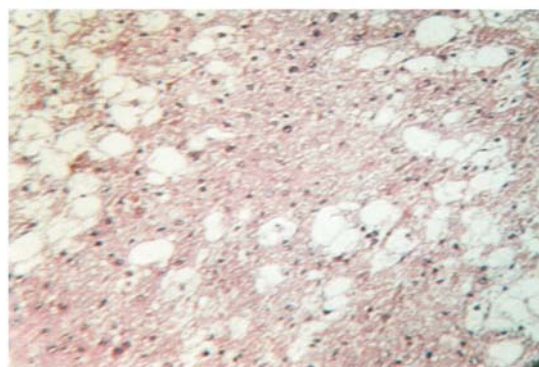


Fig.4. The liver section is almost normal, with minimum vacuolation and clear hepatic cells.

DISCUSSION

The results suggest that the methanolic extract of *B. diffusa* @ 400 mg/kg possess significant hepatoprotective activity. CCl_4 produces hepatotoxicity by metabolic oxidation. It is transformed by the cytochrome P450 system to produce trichloromethyl radical and trichloromethylperoxy radical which are responsible for the oxidative degradation in the adipose tissue resulting in fatty infiltration, destruction of Ca^{2+} homeostasis and cell death (Clawson, 1989). As a result, there will be leakage of marker enzymes like SGOT, SGPT and ALP in the serum and increase in serum TB and DB levels (Recnagel, 1989).

The plant *B. diffusa* has been found to contain a variety of phytochemicals i.e. flavonoids like 5-7 dehydroxy 3'-4' dimethoxy 6-8-dimethyl flavonoids, reducing sugars, triterpenes like B-sitosterol, alkaloids like punarnavine, tannins, amino acids like alanine, aspartic acid, methionine, threonine and histidine and lignins like lignodendrin (Asolkar *et al.*, 1992). According to Heinrich *et al.* (1998) flavonoids bind to enzymes and cell membranes and complex heavy metal ions, participate in the electron transfer of enzyme systems and exhibit free radical scavenging activity. Similarly, the hepatoprotective activity of many flavonoids have been reported by Rajnarayana *et al.* (2001). The results are also complementary to the findings of Rawal *et al.* (1997) who found that the roots of *B. diffusa* have significant protective activity against thioacetamide induced hepato toxicity in rats. The flavonoids and other constituents may be responsible for the hepatoprotective activity of the plant. The isolation and characterization of the flavonoids and other constituents and their pharmacological screening need detailed trials.

ACKNOWLEDGEMENT

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EFFECT OF PHYTASE SUPPLEMENTATION IN SWINE RATIONS ON BONE AND CARCASS CHARACTERISTICS

Shyama, K.,¹ Gangadevi, P.² and Syam Mohan, K. M³

^{1&3}Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy

²College of Veterinary and Animal Sciences, Pookode

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ABSTRACT

A feeding trial for a period of 114 days was conducted using 36 weaned Large White Yorkshire x Desi piglets (18 castrated males and 18 females, belonging to Centre for Pig Production and Research, Mannuthy) forming three groups with six replicates each, to assess the effect of phytase supplementation on bone and carcass parameters. The animals were divided into three groups (as uniformly as possible with regard to age, sex and weight and animals of each group were allotted randomly to six pens with two piglets in each pen) and were fed with three experimental rations, T1- Control ration containing 0.6 per cent calcium and 0.3 per cent phosphorus, T2 - Control ration without any mineral supplements and with 750 units of phytase/kg feed and T3-Control ration without phytase and mineral supplementation. At the end of the feeding and digestibility trials representative animals from each treatment group were slaughtered and carcass parameters were recorded. Dressing percentage was lower ($P<0.01$) for pigs fed rations T2 and T3 while loin eye area was lower ($P<0.05$) for T3 group than that of T1 and T2 and there was no significant differences ($P>0.05$) between the treatments with respect to their back fat thickness. X-ray examination of femur and mandible and histological examination of kidney samples also did not show any abnormalities among pigs of T1 and T2 and

T3 groups. However rickety beads were seen on ends of ribs on carcass evaluation, in pigs fed T3 ration. The bone ash content was also lower ($P<0.01$) for animals fed ration T3 than that of T1 and T2. Pigs fed ration containing no added minerals but supplemented with phytase was showing similar percentage of bone ash as that of pigs fed the control ration (T1). Feed cost /kg gain of pigs belonging to group T1 was higher than that of T2 and T3. It can be concluded that phytase supplementation of rations resulted in alleviation of the mineral deficiencies in animals fed without any mineral supplementation but with phytase alone without any gross abnormalities of kidney or bone in pigs.

Key words – phytase, mineral digestibility, bone mineralization, pigs

INTRODUCTION

India produces 481 thousand tones of pork per year which is 17.4 per cent of total meat production (FAO, 2010). Swine rearing is an enterprising livelihood of farm sector owing to the fast growth, high feed conversion and prolificacy of pigs and pork can fill up the large gap between the availability and requirement of meat in the country. Swine are fed mainly with cereal grains which are generally low in Ca while P is present mainly as phytate P with low availability and phytates also form complexes with Ca, Mg and other rations reducing their

availability in monogastric animals (Mc Donald *et.al.*, 2002). Under field condition, pigs are reared on kitchen/hotel wastes alone without any mineral or vitamin supplementation and feed additives such as phytase has been used, to increase the digestibility of phytate-P to monogastric animals, and also helping to decrease the need for P supplementation to diets (Brady *et al.*, 2003., Maguire *et al*, 2003). Leg weakness is an important problem in fast-growing pigs which affects the thriftiness of fattening pigs and also increases the culling rate in breeding animals. Calcium (Ca) and P play an important role in the skeletal development of pigs. Rearing of pigs without any mineral supplementation, which is the common practice among pig breeders of the State, will result in reduced growth performance and in bone abnormalities. Hence an investigation was undertaken to study whether phytase supplementation can alleviate negative effects of mineral deficiency by assessing the effect of phytase supplementation on bone and carcass parameters in cross bred pigs.

MATERIALS AND METHODS

Experimental Animals

Thirty six Large White Yorkshire x Desi weaned piglets (18 castrated males and 18 females) belonging to the Centre for Pig Production and Research, Mannuthy were randomly selected and were divided into three groups, as uniformly as possible with regard to age, sex and weight. Piglets of each group were allotted randomly into six pens with two piglets in each pen. They were randomly allotted to the three experimental treatments.

Housing And Management

All animals were dewormed before the start of the experiment. Each replicate was housed in separate pen in the same shed with concrete flooring and facilities for feeding and watering. The animals were washed every

day in the morning before 10 AM and stalls were cleaned twice daily before morning and afternoon feeding. All the animals were fed with the respective ration in mash form and restricted feeding was followed throughout the experimental period. They were allowed to consume as much feed as they could, within a period of one hour. Balance of feed was collected and weighed before the next feeding. Clean drinking water was provided in all the pens for twenty four hours throughout the experimental period.

Experimental Rations

The animals were fed with standard grower ration up to 50 kg body weight and finisher ration from 50 to 70 kg body weight formulated as per NRC (1998), to contain 18 percent CP and 3200 kcal of ME /kg of feed and 16 per cent CP and 3200 kcal of ME / kg of feed, respectively. The three experimental rations were T1-Control ration containing 0.6 per cent and 0.3 per cent supplemented calcium and phosphorus respectively, T2- Control ration without any mineral supplements and with 750 units of phytase / kg feed (Liu *et.al.*,1997), (Phytase was obtained as Maxiphos - Polchem Hygiene laboratories PVT.Ltd, Pune containing 2500 units of phytase per gram) and T3-Control ration without phytase and mineral supplementation. The ingredient and chemical composition (AOAC, 1990) of the starter and finisher rations are furnished in Tables 1 and 2. Piglets of the three groups were maintained on the three experimental rations T1, T2 and T3 from weaning till they attained slaughter weight of 70 kg. Daily feed intake was recorded.

The feed, faeces and bone samples were analyzed for proximate principles (AOAC, 1990) and minerals such as Ca, Mg, Mn, Cu and Zn were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer 3110) after wet ashing using nitric acid and perchloric acid (2:1). Phosphorus content of the feed and

faecal samples were analyzed by colorimetry (Vanado-molybdate method, AOAC, 1990) using Spectrophotometer (Spectronic 1001 plus, Milton Roy, USA).

Radiological Examination

X- ray of mandible and femur bones of two animals, selected randomly from each group was done at Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences Mannuthy, using Seimans, 300 MA to study the effect of the experimental

rations on bone development.

Slaughter Data

On attaining the slaughter weight of 70 kg, six male animals each from the three treatment groups were slaughtered at Meat Technology Unit, Mannuthy and data on carcass weight, back fat thickness, loin eye area were recorded. The back fat thickness was estimated as an average of the measurement of subcutaneous fat with skin at the level of 1st rib, last rib and last lumbar vertebrae (Sekher, 2003). Dressing

Table 1. Ingredient composition of experimental starter and finisher diets

Ingredients	Starter rations			Finisher rations		
	T1	T2	T3	T1	T2	T3
Yellow maize, kg	70	70	70	76	76	76
Soya bean meal, kg	29.4	29.4	29.4	23.5	23.5	23.5
Salt, kg	0.5	0.5	0.5	0.5	0.5	0.5
Lysine, kg	0.1	0.1	0.1			
Total	100	100	100	100	100	100
To 100 kg of the above mixture added						
Dicalcium phosphate, kg	1.7	-	-	1.7	-	-
Shell grit, kg	0.6	-	-	0.6	-	-
Zinc oxide, g	75			75		
Indomix AB ₂ D ₃ , g ¹	25	25	25	25	25	25
Rovi BE, g ²	25	25	25	25	25	25
Phytase, g ³	-	30	-	-	30	-

1 Indomix A, B2, D3, K (Nicholas Piramal India Ltd, Mumbai) containing VitaminA- 40,000 IU, VitaminB2-20mg, Vitamin D3-5000 IU and Vitamin K-50mg, per gram

2 Rovi BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B1-4mg, Vitamin B6-8mg, Vitamin B12-40mg, Niacin-60mg, Calcium pantothenate-40mg, Vitamin E- 40mg, per gram.

3 Maxiphos (Polchem Hygiene laboratories PVT.Ltd, Pune) containing 2500 units of phytase per gram

Table 2. Chemical composition of grower¹ and finisher diets, %

Parameter	Grower Rations Treatments			Finisher rations Treatments		
	T1	T2	T3	T1	T2	T3
Dry matter, %	92.80	92.30	92.24	88.85	88.53	87.83
Crude protein, %	18.43	18.48	18.37	16.15	16.32	16.63
Ether extract, %	2.8	2.54	2.58	2.58	2.79	2.64
Crude fibre, %	3.57	3.23	3.14	3.49	3.48	3.32
Total ash, %	6.35	5.19	4.82	5.7	3.42	3.30
Nitrogen free extract, %	68.85	70.56	71.09	71.68	73.99	74.11
Acid insoluble ash, %	1.74	1.22	0.97	0.76	0.66	0.57
Calcium, %	0.75	0.2	0.19	0.75	0.20	0.20
Phosphorus, % (total)	0.57	0.25	0.24	0.56	0.26	0.25
Magnesium, %	0.33	0.24	0.24	0.34	0.24	0.25
Zinc, ppm	262.03	36.39	37.81	336.47	44.18	42.90
Copper, ppm	9.59	9.52	9.03	9.40	9.05	9.80
Manganese, ppm	13.79	13.44	12.98	13.16	13.58	12.44

¹ On DM basis

Table 4. Bone mineral¹ composition of animals, %

Treatments	Bone ash	Calcium	Phosphorus
T1	49.62 ^a	19.45 ^(NS)	8.63 ^(NS)
T2	48.66 ^a	20.78 ^(NS)	8.81 ^(NS)
T3	43.69 ^b	17.01 ^(NS)	8.05 ^(NS)
Pooled SE	0.89	0.83	1.58

¹Mean of six values a, b Means with different superscripts within each column differ (P<0.01 NS Nonsignificant)

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COMPARATIVE MORPHO-HISTOLOGY OF MUZZLE IN DEER AND GOAT

Maya, S., Chungath, J.J., Ashok, N., Lucy, K.M., Sreeranjini, A.R., and Indu, V.R.

Department of Veterinary Anatomy and Histology,
College of Veterinary and Animal Sciences,
Mannuthy, Kerala – 680 651.

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ABSTRACT

Comparative morpho-histology of muzzle in deer and goat was investigated in sambar deer, spotted deer and crossbred Malabari goats of various ages. Muzzle showed irregular lines mapping out small polygonal projecting areas. Minute spots were observed on the surface of muzzle in all the three species under study, representing the openings of the ducts of the nasolabial glands. Hairs were not observed on the muzzle of sambar deer and goat. The size of the hexagonal areas doubled as the age advanced from day-old to the adult stage. In spotted deer, the muzzle presented distinct elevations, which were separated by wide grooves with small intermediate projections. The intermediate space presented small scattered hairs rarely. Size of the elevations was smaller in goat than in the deer species with narrow intermediate spaces and resembled the pattern in spotted deer. Histologically, the muzzle of deer and goat was similar. Epidermis was composed of stratified squamous keratinized epithelium, which rested on highly vascular dermis. Large, lobulated nasolabial glands were partially located in the deep portion of dermis and mainly in the hypodermis.

INTRODUCTION

Specific body regions of mammals present structural and functional adaptations

which make the animal suitable for the biological requirements of its environment. Thus in ruminants, the apex of the nose and the rostral portion of the maxilla are modified to form extensive moist glandular *planum nasolabiale* (nasolabial plate) in ox and *planum nasale* in small ruminants (sheep and goat) known as muzzle (Sarma *et al.*, 2001). The form and size of the muzzle and nature of the integument show considerable species differences (Konig and Liebig, 2009). Anatomically, the surface of the muzzle exhibits hexagonal shaped areas separated from each other by grooves and also present small rounded openings of the nasolabial glands (Farag, 2007). The present study was conducted to elucidate the comparative species differences in the morphological and histological features of the muzzle in sambar deer (*Cervus unicolor*), spotted deer (*Axis axis*) and crossbred Malabari goats (*Capra hircus*).

MATERIALS AND METHODS

The present study was conducted using skin samples collected from five sambar deer, two spotted deer and twelve crossbred Malabari goats of various ages. The muzzle region was collected from the deer brought for post mortem at the pathology department of the College from Thrissur zoo or from Forest Department and of goat freshly slaughtered at the Meat Technology Unit of the College. After recording the gross surface observations under

a stereo zoom microscope, the specimens were fixed in 10 per cent neutral buffered formalin for 48 hours. The fixed specimens were washed, dehydrated and embedded in high melting paraffin (MP 58-60°C). Serial sections of 5µm thickness were made and stained histologically using Gomori's one step trichrome method (Luna, 1968).

RESULTS AND DISCUSSION

Sambar deer and spotted deer presented dark brown muzzle *planum nasolabiale* (nasolabial plate), which corresponded to the bare middle part of the external surface of the upper lip and the surface between the nostrils. Muzzle region or *planum nasale* in goat was restricted to the area between the nostrils.

In sambar deer, even though the muzzle appeared smooth to the naked eye, under stereo zoom microscope it showed irregular lines mapping out small polygonal projecting areas (Fig. 1). Minute spots were observed on the surface of muzzle in all the three species under study, which represented openings of the ducts of the nasolabial glands. In the buffalo, the small rounded openings of the nasolabial glands were in the center of the hexagonal shaped area on the surface of the muzzle (Farg, 2007).

In all domestic mammals other than the horse, the integument around the nostrils is

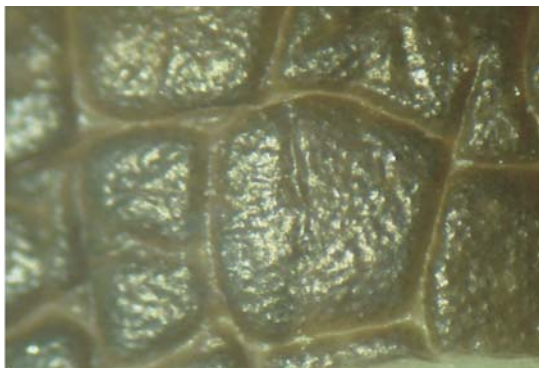


Fig.1 Muzzle from day-old sambar deer. Stereo zoom microscopy x 200x

hairless and sharply demarcated from the unmodified skin. In horses the unmodified skin with some tactile hairs surrounds the nostrils. In the ox, the integument of the rostral region is modified to form the smooth hairless muzzle (Konig and Liebig, 2009). In the present study, hairs were not observed on the muzzle of Sambar deer and it resembled in appearance that of the large ruminants, in accordance with the findings of Banks (1981), on the *planum nasolabiale* of the ox.

The size of the hexagonal areas doubled as the age advanced from day-old to the adult stage. But the gross morphological features remained the same (Fig. 2).

In spotted deer, the muzzle presented distinct elevations, which were separated by wide grooves with small intermediate projections. The intermediate space presented



Fig. 2 Muzzle from adult sambar deer. Stereo zoom microscopy x 100 x

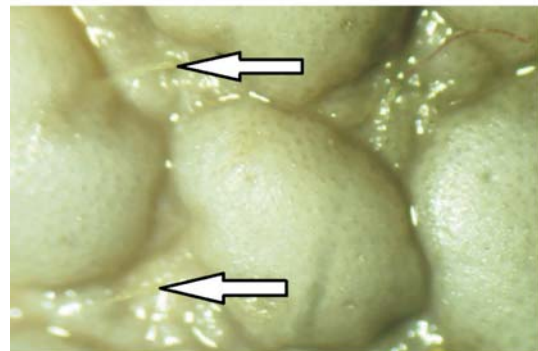


Fig.3 Muzzle from adult Female spotted Deer with hair (arrows) in the intermediate space. Stereo zoom microscopy x 200 x

small scattered hairs rarely (Fig.3).

The size of the elevations was smaller in goat (Fig.4) than in the deer species. These projections on the muzzle resembled the pattern in spotted deer. But the intermediate spaces were narrow and did not present any hair in goat. This finding confirmed the observations of Banks (1981) that the *planum nasale* of small ruminants (sheep and goat) are devoid of hairs. Towards the junction with the unmodified skin with the hair follicles reappeared and the thickness of the epidermis reduced (Figs.4, 5).

Histologically, the muzzle of deer and goat was found to consist of epidermis, dermis and hypodermis (Fig. 6). The epidermis was generally composed of stratified squamous keratinized epithelium, which rested on highly vascular dense irregular connective tissue (dermis) containing some bundles of skeletal muscle fibers and nerves. The large, lobulated nasolabial glands were partially located in the deep portion of dermis and mainly in the hypodermis. Each lobule was composed of secretory acini and the duct system. The secretory acini were lined by pyramidal shape cells with centrally located round nuclei resemble those of the serous end-pieces of the salivary glands. Each lobule was drained by intercalated duct which connects to highly acidophilic duct resemble the striated duct of salivary glands. These findings were in accordance with the observation made by



Fig.4 Muzzle from 5 months-old male Crossbred Malabary Goat. Stereo zoom microscopy 200x

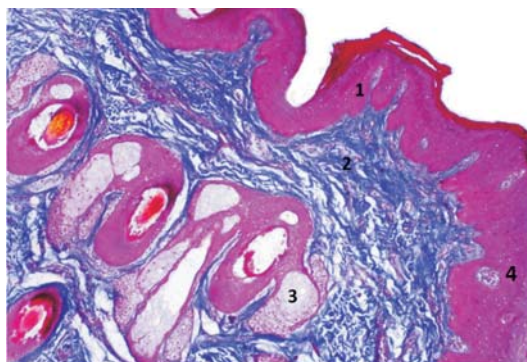


Fig.5 Junction of the muzzle with the unmodified skin in adult male Cross bred Malabary Goat. Gomori's One step trichrome x 100x

1.Epidermis of unmodified skin 2. Dermis 3. Gland 4.Thick epidermis of muzzle

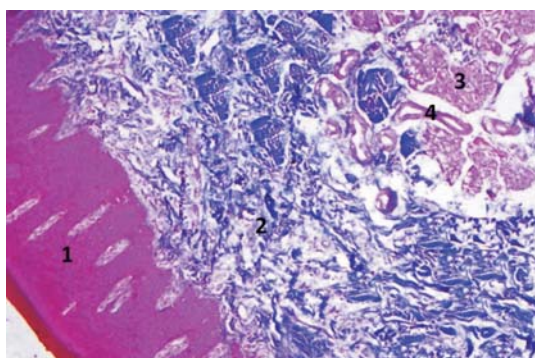


Fig.6 Muzzle region in adult sambar deer. Gomori's One step trichrome x 100x

1.Epidermis 2. Dermis 3. Glands 4. Duct

Kassab *et al.* (2008) in buffaloes. The serous glands within the mucosa keep cool and moisten the cornified epithelium overlying the skin of muzzle (Konig and Liebig, 2009).

The nature of the nasolabial glands is a matter of debate. Although, some investigators described these glands as eccrine glands resembling those found in foot pad of carnivores (Meyer and Tsukise, 1989) and carpus of the pig (Calhoun and Stinson, 1981), others considered them as a combined form of both eccrine and apocrine glands and refers to them as intermediate gland in pig (Montagna and Yun, 1964). Additionally, these glands were previously explained as specialized salivary glands. The secretion of these glands and other

glands on specific body regions have been shown to contain abundant glycol conjugates with various saccharides residues that have abroad biological significant to the skin function, such as, interspecies communication, the signalling of sexual activity, and water retention on the epidermal surface (Tsukise *et al.*, 1988).

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REPRODUCTIVE CHARACTERISTICS IN TRIPLE CROSS CATTLE

Ramesh, J. Padodara* and Arya, J.S**

Assistant Professor*, Professor & Head**

Department of Physiology & Biochemistry

Veterinary College, Junagadh Agricultural University, Gujarat

Veterinary College, Anand Agricultural University, Anand

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ABSTRACT

The study was conducted on 21 pregnant triple cross-bred (1/2 Kankrej×1/4 Jersey×1/4 Holstein Friesian) heifers and cows maintained at Livestock Research Station, Anand Agricultural University, Anand. The gestation length, placental weight, placental expulsion time and birth weight of calf differed non-significantly between primiparous and multiparous triple crossbred cows which delivered male or female calves. All the above traits were non-significantly correlated with each other.

Key words: Triple cross-bred, gestation length, placental weight, Placental expulsion time, birth weight

INTRODUCTION

Successful parturition is important in the economics of the livestock production and helps to judge the worth of an animal. The gestation length and placental characteristics of the dam throw light on the quality of the calf born. Hence, the present study was conducted to note the gestation length and placental characteristics **and birth weight** of calves in Triple Cross Cattle (1/2 Kankrej×1/4 Jersey×1/4 Holstein Friesian).

MATERIALS AND METHODS

The study was conducted on 21 pregnant

triple cross-bred (1/2 Kankrej×1/4 Jersey×1/4 Holstein Friesian) heifers and cows maintained at Livestock Research Station, Anand Agricultural University, Anand. Gestation length, placental weight and expulsion time were recorded. The birth weight of the calf was recorded within one hour of birth using standard weighing machine. Statistical analyses were done using Unequal Completely Randomization Design (CRD) and the correlation co-efficient between the characteristics were determined as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The Mean ± SE values of the traits studied and their coefficient correlation are presented in Table-1 and 2, respectively.

1. Gestation length (GL) (days)

No significant difference was observed in gestation period for male and female calves and also between primiparous and multiparous animals, but male calves were carried for a longer duration than female calves (Table-1). The calving sex ratio of male : female calves was 8:13 in the 21 calvings studied. Similar finding of male : female ratio was observed by Sattar *et al.* (2005). Rokonuzzaman *et al.* (2009) observed 275 ±3.95, 276±4.26, 275±4.41 and 277±3.31 days in Frisian cross, Sahiwal cross, Sindhi cross and indigenous cattle respectively which was non-significantly differed.

Table-1 Reproductive characteristics in triple cross cattle (Mean \pm SE)

Animal (n)	GL (days)	PW (kg)	PET (hr)	BW (kg)
Male calves (8)	---	2.82 \pm 0.07	5.80 \pm 0.84	23.88 \pm 1.87
Female calves (13)	279.38 \pm 1.64	2.86 \pm 0.09	6.62 \pm 1.44	22.46 \pm 0.84
Primiparous cows (8)	279.85 \pm 1.16	2.83 \pm 0.09	4.59 \pm 0.48	21.38 \pm 0.98
Multiparous cows (13)	272.90 \pm 6.12	2.92 \pm 0.07	7.28 \pm 1.56	24.00 \pm 1.21
Pooled (21)	276.83 \pm 2.76	2.75 \pm 0.10	6.13 \pm 0.94	23.00 \pm 0.87

GL- Gestation length, PW- Placental weight, PET- Placental expulsion time and BW- Birth weight

2. Placental weight (PW) (kg)

The average weight of placenta in Triple cross cows which gave birth to male calves and to female calves and between primiparous and multiparous cows showed non significant difference. However, primiparous animals had heavier placenta than multiparous animals. The current findings agreed with those of Bhageerathi *et al.* (2002) in HF X Deoni cows and to Sultan *et al.* (1987) in HF cows. Placental weight recorded in the present study are lower than those reported by Rao *et al.* (1966) on Ongole cows (3.34 kg) but higher than those recorded by Patel *et al.* (1983) in Kankarej cows (2.63 kg). Bhambure *et al.* (1984) found non significantly higher weight of placenta (3.14 \pm 0.28 kg) in HF X Kankarej than in Kankarej (2.86 \pm 0.18 kg) and Jersey X Kankarej cows (2.86 \pm 0.22 kg).

3. Placental expulsion time (PET) (hrs)

No significant difference was observed in

placental expulsion time between cows which delivered male or female calves. Time taken for expulsion of placenta by multiparous animals was non significantly higher than primiparous animals which may be attributed to the relatively young age of primiparous cows with resultant increased muscle tone strength to expel the placenta. Placental expulsion time in the present study was higher than those reported by Rao *et al.* (1966), Patel *et al.* (1983) and Bhambure *et al.* (1984) in various breeds of cattle.

4. Birth Weight of calves (BW) (kg)

The difference in birth weight among male and female calves and between calves born to primiparous and multiparous cows was non-significant. However, male calves had more birth weight than female calves and similarly multiparous animal gave birth to heavier calves than primiparous animals. The findings in the present study were similar to that of Bhambure *et al.* (1984) who reported the average birth weight of calves in Kankarej,

Table-2 Correlation Coefficients (r) amongst different reproductive characteristics in triple cross cattle

Parameters	BW	GL	PET	PW
BW	-	-	-	-
GL	0.35	-	-	-
PET	-0.22	0.22	-	-
PW	-0.03	0.20	-0.03	-

GL- Gestation length, PW- Placental weight, PET- Placental expulsion time and BW- Birth weight

Jersey × Kankarej and Holstein × Kankarej groups to be 25.08 ± 1.59 , 21.06 ± 1.42 and 28.60 ± 0.97 kg, respectively. Rao *et al.* (1966) who observed the male calf birth weight was 28.30 ± 0.5 kg and female calf birth weight as 24.77 ± 1.13 kg. However, Patel *et al.* (1983) noted that the female calves (24.31 ± 0.43 kg) were heavier than male calves (23.54 ± 0.44 kg) in Kankarej cross bred. Also Rokonzaman *et al.* (2009) observed 22.52 ± 0.33 , 22.19 ± 0.35 , 20.16 ± 0.86 and 17.0 ± 0.36 kg in Frisian cross, Sahiwal cross, Sindhi cross and indigenous cow calf respectively.

Coefficient correlation among the traits studied revealed non significant differences among them (Table-2). Placental expulsion time showed non significant negative correlation with placental weight and birth weight of calves. However, it was positively but non significantly correlated with gestation length. Birth weight of calves was negatively correlated to placental weight but was positively correlated to gestation length. The findings agreed with that of Mukasa and Mattoni (1990).

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VISCERAL GOUT IN POULTRY-A REPORT

Deepa Chirayath¹ and Rejitha, T.S.²

¹ Assistant Professor, Department of Clinical Veterinary Medicine,
College of Veterinary and Animal Sciences, Mannuthy

² Technical Assistant, District Animal Husbandry Office, Thrissur.

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ABSTRACT

Visceral gout is characterized by precipitation of urates in the kidneys and on the serous surfaces of the heart, liver, mesenteries, air sacs or peritoneum. Visceral urate deposition is generally due to a failure of urinary excretion which may be due to obstruction of ureters, renal damage or dehydration. Dehydration due to water deprivation is a common cause of visceral gout in domestic poultry. A case of visceral gout in 12 week old pullet during the month of March was reported and histopathological lesions were described.

Keywords: Visceral gout, Poultry

INTRODUCTION

Visceral gout, which has also been called visceral urate deposition is characterized by precipitation of urates in the kidneys and on the serous surfaces of the heart, liver, mesenteries, air sacs or peritoneum. The deposits on serosal surfaces appear grossly as a white chalky coating, and those within viscerae may be recognized only microscopically. Visceral urate deposition is generally due to a failure of urinary excretion which may be due to obstruction of ureters, renal damage or dehydration. Dehydration due to water deprivation is a common cause of visceral gout in domestic poultry. Outbreak of visceral gout in poultry have also been attributed to

vitamin A deficiency, secondary to urolithiasis, treatment with sodium bicarbonate, mycotoxins such as oosporin, renal cryptosporidiosis and treatment with sodium bicarbonate (Crespo and Shivaprasad, 2003, Sodhi, *et al.* 2008 and Eldaghayes *et al.* 2010). Dehydration due to water restriction leading to visceral gout cause increased mortality in poultry flocks (Takahashi *et al.* 2009).

CASE HISTORY AND OBSERVATION

A batch of pullets of 12 weeks old were presented in the middle of March 2011 to the College Veterinary Hospital with a complaint of anorexia and droopiness for the past 3 days. Faecal sample was positive for *Ascaridia galli* ova. One bird died on examination table and was sent for post mortem examination.

Gross pathology

At necropsy the bird appeared in good nutritional condition. There was white chalky material extensively distributed over the peritoneum and pericardium. Other findings were pale swollen kidneys and swollen spleen. No abnormalities were detected in other tissues including leg and foot articulations.

Histopathology

Section of kidney showed glomeruli of varying sizes, many of them showing oedema and necrosis of the glomerular tufts. Some of the glomeruli were hypocellular. The lining cells

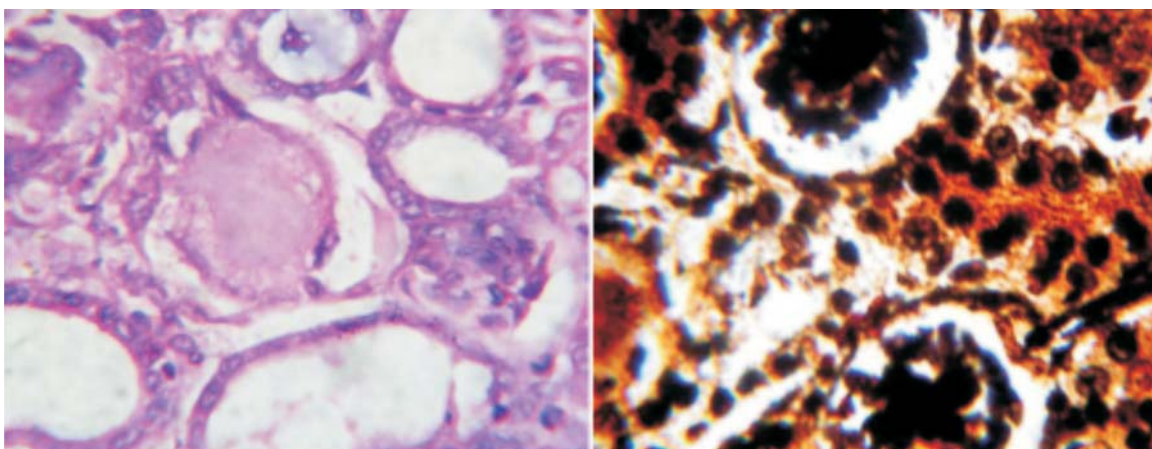


Fig.1. Section of kidney showing A) bluish purple stained needle like urate deposits in a renal tubule, H&E,X1000
B) Showing black coloured urate deposits in renal tubules, De-Galantha's staining X1000

of renal tubules appeared oedematous in some places. Interstitial tissue showed hemorrhage and congestion. Many of the tubules contained urate deposits which appear bluish purple needle like mass (Fig. 1A). On De Galantha's staining (Luna, 19 68) urate deposits inside the renal tubules appeared black in colour (Fig.1B).

RESULTS AND DISCUSSION

In the present case dehydration must have led to the kidney damage, as heat stroke is common in animals in Kerala during summer months. Sufficient drinking water supply should be ensured to birds during summer to prevent visceral gout.

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HYPERPLASTIC PROSTATITIS IN A JACK RUSSELL TERRIER- A CASE REPORT

Ambily, V.R^{1*}., Kanaran,P.P² and Usha Narayana Pillai³

¹Assistant Professor, ³Associate Professor, Department of Clinical Veterinary Medicine
College of Veterinary and Animal Sciences, Mannuthy, Trichur
Kerala Veterinary and Animal Sciences University, Pookode, Kerala

²Deputy Director, Animal Husbandry Department, Kerala

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ABSTRACT

Prostatic diseases usually occurs in male dogs above six years of age. The present report deals with hyperplastic prostatitis in a two year old Jack Russell terrier and its successful therapeutic management. Clinical signs encountered were anorexia, fever and constipation. Symmetrically enlarged prostate could be palpated on trans-rectal examination. Cytological examination of prostatic discharge revealed hyperplastic prostatic cells with inflammatory response. Ultrasonographic examination revealed normoechoic to hyperechoic symmetrically enlarged gland with a relatively smooth contour. The animal was treated with enrofloxacin @ 5 mg/kg body weight orally for 15 days which resulted in an uneventful recovery.

Key words: prostatitis, pollakiuria, prostate, tenesmus

INTRODUCTION

The canine prostate gland is a bilobed structure with a palpable median raphe, which completely surrounds the urethra just distal to the internal sphincter. Prostatic diseases usually occurs in male dogs above six years of age. Usually animals with prostatic diseases exhibit lower bowel signs like tenesmus, hematochezia and constipation in contrast to humans with prostatic diseases, where lower urinary tract signs like pollakiuria, dysuria and

hematuria will be prominent. Some dogs with prostatic disease may exhibit a wide-based gait in the hind limbs, called the 'prostatic shuffle', which is an attempt to ease discomfort while walking. Hemorrhagic and purulent urethral discharge is a common sign of prostatic disease in dogs. In breeding dogs, prostatic diseases result in decreased libido (due to discomfort), hemospermia and reduced fertility. The present report deals with hyperplastic prostatitis in a Jack Russell terrier and its successful therapeutic management.

CASE HISTORY AND OBSERVATION

A two year old male Jack Russel terrier was presented to University Veterinary Hospital, Mannuthy with a history of anorexia, fever and constipation for about one month. The dog was treated previously in a local veterinary hospital before the referral. The temperature, pulse and respiration rates were 103°F, 115/minute and 28/ minute, respectively. The visible mucous membranes were congested. Haematological analysis revealed leukocytosis (14000/cu.mm) with neutrophilia (84%), and mild anaemia with haemoglobin of 10.8g%, RBC of 4.85million/cu.mm and PCV of 30%. Serum biochemical analysis revealed mild hypoalbuminaemia, (2.3 g/dl) and hyperglobulinaemia (4.2g/dl). Serum creatinine and blood urea nitrogen were with in normal range. On trans-rectal examination, symmetrically enlarged prostate could be palpated and the animal exhibited pain on palpation of prostrate, which suggested prostatitis.

Prostatic fluid was collected by trans-rectal prostatic massage and the fluid obtained was subjected to cytological examination. Prostatic smears were prepared from the collected prostatic fluid and stained with Giemsa stain and observed under oil immersion objective of microscope. Microscopic examination of Giemsa stained prostatic smears revealed hyperplastic prostatic cells with large number of neutrophils (Fig.1). Catheterized urine sample was sterile upon culture and sensitivity. Ultrasonographic examination revealed normoechoic to hyperechoic symmetrically enlarged gland with a relatively smooth contour (Fig.2).

TREATMENT AND DISCUSSION

Based on clinical signs, trans-rectal examination, prostatic cytology and ultrasonography, the condition was diagnosed

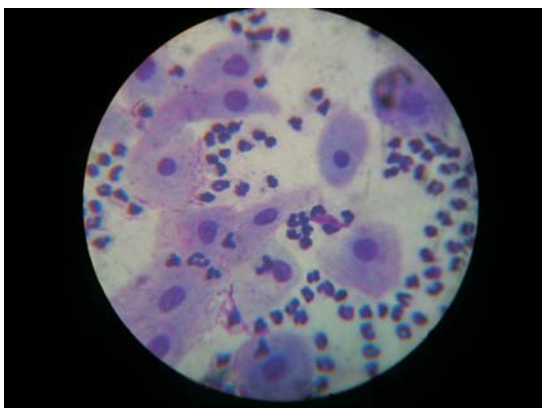


Fig.1. Hyperplastic prostatic cells with neutrophils indicating an inflammatory response



Fig.2. Ultrasonogram revealing normoechoic to hyperechoic symmetrically enlarged prostate gland.

as hyperplastic prostatitis. The animal was treated with enrofloxacin @ 5 mg/kg body weight orally for 15 days which resulted in an uneventful recovery.

Clinical signs such as straining to defaecate and constipation may result secondary to displacement and narrowing of large intestine due to prostatomegaly (Hoffer et al., 1977). Haematological analysis of animals with prostatitis revealed leukocytosis with neutrophilia (Smith, 2008). Barsanathi and Finco (1979) suggested that most of the prostatic infections were secondary to migration of bacteria from the urethra although spread through blood, semen and rectal flora was also possible. In this case, even though prostatic infection was there, urine sample was sterile. This may be due to the fact that the animal has undergone previous antibiotic therapy with an antibiotic sensitive to urinary tract infection, but not having any action on prostate. In prostatic diseases, antibiotics which can diffuse through blood prostate barrier could be selected. Enrofloxacin could diffuse through blood prostate barrier and attain a therapeutic concentration in prostate gland (Duque et al.(2009).

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PRE PARTUM CERVICO VAGINAL PROLAPSE IN A RABBIT- A CASE REPORT

Ambili John¹, Upasana Ratnakaran², M.P. Unnikrishnan³ and B. Bibin Becha⁴

^{1 & 2} PhD Scholar, ^{3 & 4} Assistant Professor,

Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala

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ABSTRACT

A pregnant non descript rabbit doe aged 2 years was presented with cervico vaginal prolapse. The prolapsed mass was cleaned using potassium permanganate lotion and reduced back by digital manipulation and retained *in situ* by applying Buhner's suture. Oral antibiotic therapy was given for five days. Suture was removed on sixth day. Anuneventfull recovery was recorded.

Key words: Buhner's suture, cervico vaginal prolapse

INTRODUCTION

Cervico vaginal prolapse is often encountered in all species of domestic animals, but most commonly in cows and ewes (Roberts, 1971). Excess antepartum relaxation of pelvic tissues and increased intra abdominal pressure has been reported as the main etiology for prepartum vaginal prolapse (Jackson, 2004). Treatment of the condition can be done by conservative, suturing or surgical procedures.

The present paper reports a case of prepartum cervico vaginal prolapse in a rabbit doe and its successful management.

CASE HISTORY AND OBSERVATIONS

A non descript rabbit doe aged 2 years was presented to University Veterinary Hospital, Mannuthy with a history of a mass protruding

from the vulva since that day morning. The animal was mated 25 days back. On abdominal palpation foetus could be detected. The animal was straining and conjunctival mucous membrane was observed pale. Rectal temperature and respiratory rate were noted to be 102.1^oF and 51/min respectively. Clinical examination revealed complete prolapse of both cervices along with the vagina, as a reddish mass of about 3 cm length protruding from the vulva (Fig. 1). The condition was diagnosed as prepartum cervico vaginal prolapse.

TREATMENT AND DISCUSSION

The prolapsed mass had become oedematous and lacerated. It was cleaned with potassium permanganate lotion (1:1000) and dipped in saturated magnesium sulfate solution for reducing the oedema. The prolapsed mass was lubricated with liquid paraffin and epidural anaesthesia was given at the lumbosacral junction using 0.5 ml of two percent lignocaine solution. After lifting the hind quarters and by applying gentle digital manipulation, the prolapsed mass was reduced back into the pelvic cavity (Fig. 2). It was retained *in situ* by adopting Bhuner's suturing technique using braided silk. Amoxicillin- clavulanic acid syrup was administered orally at the rate of 50mg/kg body weight for five days. After 5 days the suture was removed and subsequently after two days the animal kindled normally.

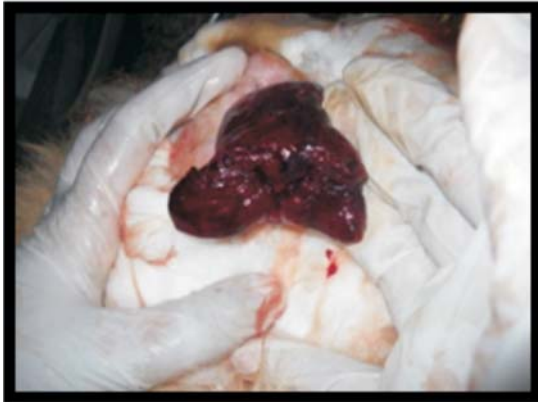


Fig. 1. Prolapsed mass showing both cervixes & vagina



Fig. 2 After reducing the prolapse

Though peripartum complications are rare in rabbits, dystocia and its management was reported by Islam *et al.* (2006). Successful treatment of cervico vaginal prolapse in a rabbit doe was reported by George *et al.* (1993). Becha *et al.* (2011) reported a case of chronic vaginal prolapse in a rabbit and its correction by reefing operation.

SUMMARY

A case of prepartum cervico vaginal prolapse in a rabbit doe and its successful correction is reported.

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SURGICAL MANAGEMENT OF BILATERAL MANDIBULAR FRACTURE IN A DROMEDARY CAMEL

Jayamohan.T.V.,¹ Rafeek. A.K.,² Anilkumar.V.T.³ and Baby.P.G.⁴

^{1,2} Animal Health Division, Abudhabi Food Control Authority, UAE.

^{3,4}Al Sebaq Veterinary Clinic, Sharjah, UAE

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INTRODUCTION

Mandible in camel is an elongated bone consisting of two halves which fuse together during the first few months of life. Bilateral or very rarely unilateral mandibular fractures are common in male camel especially during the rutting season and occur across the first premolars or quite cranial or caudal to this point in inter-dental space. Presence of mental canal and alveoli of the first premolars render this part of the bone quite weak and prone to easy fracture. Standard interdental wiring technique using 1.0 mm diameter stainless steel, silver or copper wire is the method of choice to repair mandibular fractures in camels (Gahlot *et al.*, 1984). Successful management of bilateral mandibular fracture in a male dromedary camel by interdental wiring using 1mm Kirschner wire is reported.

CASE HISTORY AND TREATMENT

A seven year old male dromedary camel weighing about 600 kg suffering from bilateral mandibular fracture by hitting on a water tank accidentally was presented to Madinat Zayed Veterinary Hospital, Abudhabi, UAE. Physical examination revealed open, bilateral, oblique, mandibular fractures caudal to the canine teeth (Fig.1).

The wounds showed severe bleeding and the animal was showing severe pain, salivation and distress. Body temperature was



Fig.1

37.4° C. Tachycardia (80 beats per minute) and tachypnoea (17 breaths per minute) were noticed.

The camel was sedated with 100mg Xylazine hydrochloride intravenously and was restrained in sitting posture by keeping the fore limbs and hind limbs tied separately under the body. The oral cavity was kept open using a mouth gag and was flushed using warm saline solution. Using an electric drill, 1mm diameter holes were drilled across the gums of first cheek teeth (second premolar and first molar) on both the sides. Two 1mm K-wires were passed through the predrilled holes on either side. The medial end of each wire was passed through the space between the central incisors.

By careful manipulation, the lower jaw was pulled forwards and upwards to achieve proper reduction. The medial and lateral ends of the wires on each side were twisted carefully using a wire twister to effect proper alignment

of the fractured ends. The twisted ends were cut using a wire cutter about 1cm away from the base and the cut ends were bent downwards between the gap of the incisors to avoid injury to the gum and the lower lip (Fig 2).

Long acting Amoxicillin trihydrate(15mg/kg body weight) was administered intramuscularly to prevent infection and Ketoprofen hydrochloride (2.2mg/kg body weight) was administered intramuscularly to control pain and inflammation. The oral cavity was washed twice daily with warm saline solution. The camel was allowed to drink water on the first day and advised to eat small quantity of tender grass from the second post-operative day. Normal feeding was advised only after 10 days. Examination after one month revealed good clinical union of the fractured fragments. A small sub-mandibular abscess found developed on the right side below the fracture site which was drained and treated as per standard protocols. The wires were removed using a wire cutter by careful manipulation on the 50th post-operative day under sedation using 100mg xylazine hydrochloride intravenously. The animal made an uneventful recovery (Fig3)

RESULTS AND DISCUSSION

Trauma was found to be the most important cause of fracture in camels of above 6months

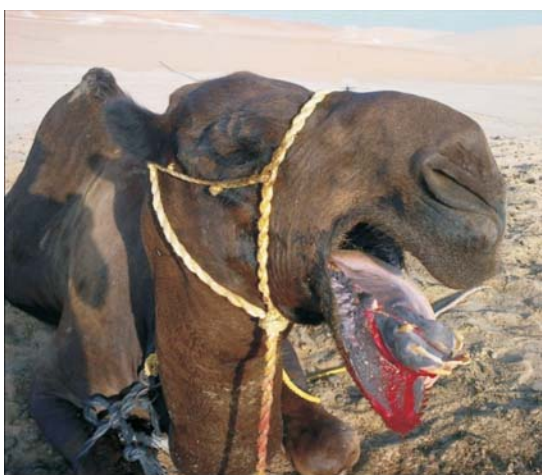


Fig.2



Fig.3

of age (Ahmed and Fahd, 2012 and Kumar *et al.*, 1979). In the postoperative period, the lateral limbs of the wires slip downward in line with the normal slope of the incisive part of the mandible, a complication commonly observed in the old ages as the incisor teeth take an outward slope with the advancing age (Hanuman and Gahlot, 2001). Embedment of the lateral limb of the wire in the gums also results in loosening of the wire with consequent ventral deviation of the cranial fracture fragment (Henninger and Warren, 1997 and Siddiqui and Telfah, 2010). Development of sub-mandibular abscesses is a very common postoperative complication of these fractures and can lead to osteomyelitis if not drained and treated in time (Gahlot *et al.*, 1984). In the present case the only complication noticed was a small sub-mandibular abscess which was successfully managed.

SUMMARY

A case of bilateral mandibular fracture in a dromedary camel and its successful surgical management by interdental wiring has been reported.

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OCCURRENCE OF FOOT DISORDERS IN ELEPHANTS

Giridas, P.B., Usha Narayana Pillai and Alex, P.C.

Department of Clinical Veterinary Medicine
College of Veterinary and Animal Sciences
Mannuthy, Thrissur, Kerala

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INTRODUCTION

Foot disorders are seen in more than 50 per cent of Captive Asian Elephants at some time or other in their lives and constitute the single most important ailment (Fowler, 2001). Studies on occurrence of foot problems of Indian elephants are limited and poorly documented. Hence the present study was undertaken to have a comprehensive view on foot disorders of Captive Asian Elephants.

CASE HISTORY AND OBSERVATION

One hundred and sixty three animals belonging to seven different states of India with considerable captive elephant population were examined for the occurrence of foot disorders. Eighty seven cases were identified and studied in detail. The occurrence of foot disorders in relation to age, sex and limbs affected were recorded.

RESULTS AND DISCUSSION

A total number of 87 (33.37%) animals were found positive for one or other types of foot disorders. Among these 72.4 per cent were males and 27.6 per cent were females. The hind foot of male elephants usually comes in contact with urine during micturition and could be one of the reasons for higher occurrence of foot disorders in male elephants (Csutiet *al.*, 2001).

Age wise occurrence of foot disorders

revealed that captive Asian Elephants of India suffered more from the foot disease in their middle age 42.5 (21-40 years) when compared to less than 20 years (9.2 per cent).

Major foot disorders recorded in the present study were over grown nail (16.3%) (Fig1.), over grown cuticle (14.9%) (Fig2),



Fig 1. Overgrown nail



Fig 2. Overgrown cuticle



Fig 3. Pitted sole



Fig 4. Pitted sole

pitted sole (14.9%) (Fig 3), cracked sole (9.9%) (Fig4), split nail (8.3%), over worn sole (7.5%), cracked heel (4.8%), injury (4.8%), over grown sole (4.5%), pododermatitis (4.3%), ingrown nails (2.7%), abscess (2.7%), laminitis (2.4%), arthritis (1.9%).

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From Editor's Desk

Swine flu tightens its grip over India

Swine flu has tightened its grip over India, with the death toll reaching close to 1000. Fresh cases have been reported from across the country, including Delhi, Rajasthan, Gujarat, Uttar Pradesh, Jammu and Kashmir, West Bengal, Nagaland and Bihar.

As per the latest figures released by Health Ministry, the fatal disease has claimed 965 lives so far, while total number of people tested positive till February 25, 2015 has also increased to 16,235. Rajasthan and Gujarat have been the worst-hit states so far with 234 and 231 deaths, respectively.

According to Meteorology Division in India, unusual weather conditions prevailing across the country have aggravated the situation and with the rainy days expected ahead, there is a possibility of steep rise in the numbers. This has raised serious concerns over H1N1 influenza, as weather has played major role in intensifying the flu this season.

Gujarat state government has also imposed a ban on most public gatherings of five or more people in Ahmedabad in a bid to halt the spread of swine flu. Officials have also asked people to wear mask as precautionary measure.

In 2014, 937 cases were reported in total, including 218 deaths.

Weather is a key factor in letting the virus sustain and spread. The virus survives comfortably in the winter season and even during the spring, since the temperature does not shoot up much. Low temperatures and high humidity is making the environment conducive for the H1N1 virus to proliferate.

Back to back weather systems this season have kept the humidity levels high and have also influenced the wind pattern across the plains of northwest India and adjoining areas, resulting in swine flu virus to sustain for a longer period.

Moreover, a spell of rain between February 28 and March 02 will cover larger parts of the country including Madhya Pradesh, Maharashtra, Chhattisgarh, Uttar Pradesh, Bihar and Jharkhand, which might lead to further spreading of the swine flu virus.

Doctors are hoping for some respite, only once hot and dry days set in with mercury rising. Till then, increased awareness and proper precautionary measures are the only recourse. Government has also directed states to provide protective kits to health workers.

WHAT IS SWINE FLU?

◆ Swine influenza, also called pig influenza, swine flu, hog flu, pig flu or H1N1 virus, is an infection caused by any one of several types of swine influenza viruses.

◆ H1N1 is also known as swine flu because the virus was similar to influenza viruses that cause illness in pigs.

◆ It is highly contagious, spreading quickly from person to person.

◆ In 2009, H1N1, or swine flu was called a pandemic by World Health Organization (WHO)

From Editor's Desk

because of its massive spread across the world.

How does it spread?

- ◆ Swine flu is contagious.
- ◆ Influenza viruses infect the cells lining your nose, throat and lungs. It spreads in the same way as the seasonal flu.
- ◆ The virus spreads when you touch an infected surface or breathe cough and sneeze droplets in the air.

The Symptoms of swine flu

- ◆ Swine flu symptoms are similar to most influenza infections.
- ◆ They can include - fever, cough, headache, sore throat, fatigue, body aches, diarrhoea, vomiting, difficulty in breathing or shortness of breath.
- ◆ Small children may refuse to take feeds.

The related complications

- ◆ Swine flu can lead to more serious complications, including pneumonia and respiratory failure.
- ◆ It can also make conditions like diabetes or asthma worse.
- ◆ It can also lead to neurological conditions ranging from confusion to seizures.

Who are at higher risk?

- ◆ The children younger than five years of age, people 65 years and older, pregnant women and people of any age with certain medical conditions such as cancer, heart or lung disease, diabetes, and those with weakened immune system.

How do you know it's swine flu?

- ◆ Most of the symptoms of swine flu are same as that of seasonal flu.
- ◆ People with swine flu may be more likely to feel nauseous and throw up than people who have seasonal flu.
- ◆ Only a lab test is the only way to know for sure.

What's the treatment for swine flu?

- ◆ The person suffering from swine flu should take proper rest, keep warm and drink plenty of water to avoid dehydration.
- ◆ Person suffering from this can also take antiviral medicines and antibiotics.

How can you avoid getting swine flu?

- ◆ The best protection is to get a flu vaccine every year.
- ◆ Washing hands and in keeping a good hygiene are the most effective way of slowing the spread of flu.

Dos and Don'ts

- ◆ Cover mouth and nose when you cough or sneeze
- ◆ Wash your hands with soap or use sanitiser
- ◆ Avoid touching your nose, eyes and mouth
- ◆ Drink plenty of water and have nutritious food
- ◆ Sleep for 8 hours every night
- ◆ Avoid handshakes/contact greetings
- ◆ Avoid self-medication

Home remedies to avoid swine flu

- ◆ Eat five Tulsi (Basil) leaves every morning. The therapeutic properties of Tulsi cure throat infections and keeps lungs clear.
- ◆ One can have two pods of raw garlic early in the morning with lukewarm water.
- ◆ Aloe vera (gwarpatha) also boost immunity.
- ◆ Have a glass of hot or lukewarm milk every night with some amount of haldi (turmeric).
- ◆ A small piece of camphor (kapoor) approximately the size of a tablet should be taken once or twice a month.