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Review article

Full length articles

Short Communications

Review article

Full Length Article

Household Livelihood Security Index (HLSI) of livestock farmers vis-à-vis other occupational groups in rural Tamil Nadu

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ABSTRACT

An attempt was made to compare the Household Livelihood Security Index (HLSI) of Livestock farmers vis-à-vis other occupational groups in rural Tamil Nadu using randomly selected 540 sample households from six poverty prone sample districts. HLSI was constructed for each sample household based on the previous literature and variations among different occupational groups and districts were compared. The HLSI was found to be the highest among crop + livestock farmers (0.4887), followed by fishermen (0.4821), non-farm (0.4701), livestock farmer (0.4583), crop cultivators (0.4347) and agricultural labourers (0.3860) with the overall HLSI of 0.4435. Among different districts, HLSI was found to be the lowest in Pudukottai district (0.3815), followed by Ariyalur (0.4324) and Ramanathapuram (0.4455). Overall, it could be concluded that health and infrastructure indices among different occupations and locality was calculated to be higher when compared to other domains. The study revealed that there is an urgent need to improve the empowerment index irrespective of the locality and occupations.

Key words : Household Livelihood Security Index, Livestock farmers, Occupation

INTRODUCTION

A livelihood comprises the capabilities, assets (stores, resources, claims and access) and activities required for a mean of living; a livelihood is sustainable which can cope with and recover from stress and shocks, maintain and enhance its capabilities and assets and provide sustainable livelihood opportunities for the next generation (Chambers and Conway, 1992). Every individual has varying livelihood status depending upon their existing assets value, pursuing occupation, managerial capacity and consumption / expenditure behaviour. The superior livelihood status of the households in a society reflects the sign of development. As the livelihood component is a complex element, its method of

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measurement also varies. Among various factors influencing the standard of living, the source of income namely occupation plays a major role. Hence, the present study attempts to assess the Household Livelihood Security Index among various occupational groups in poverty prone districts of rural India, which will help in policy making.

MATERIALS AND METHODS

A Composite Index (CI) was constructed through factor analysis based on secondary data related to livelihood in order to select six sample districts of Tamil Nadu *viz.,* Thiruvannamalai, Villupuram, Dharmapuri, Pudukottai, Ariyalur and Ramanathapuram located in southern part of India. From each selected district, 90 respondents comprising different occupational groups were selected through multi-stage random sampling leading to the sample size of 540 sample households. The data pertaining to the objectives of the study were collected through personal interview using a structured pre-tested interview schedule. The period of data collection was from August 2013 to October 2014.

Lindenberg (2002) analysed livelihood security areas under five broad dimensions: economic security, food security, health security, educational security and empowerment. Hahn *et al.* (2009), CARE (2004) and Akter (2012) developed a set of multiple indicators to assess each of the household security dimensions. From this recommended set, a suite of indicators was selected depending upon their importance. Some of the important variables like average per capita calorie intake, protein intake, fat intake, landholdings, livestock holdings, *etc.* which were considered to determine the livelihood of the households were included in the present study. Infrastructure had a major contribution to human development and livelihood security (Gopalakrishnan and Leelavathi, 2011; Kusharjanto and Kim, 2011). Hence, indicators of infrastructure facility were also included in the present study for constructing the Household Livelihood Security Index (HLSI). The indicators considered for the present study is listed in Table 1.

Following Akter (2012), HLSI was constructed based on balanced weighted average approach, where each indicator contributes equally to the overall index. Since each indicator was measured on a different scale, indicators were standardized similar to standardization technique adopted for measuring Human Development Indices (also adopted by Hahn *et al.,* 2009 and Akter, 2012). If the indicator $zind_j$ is positively associated with the livelihood, a standardized indicator *'j'* was calculated as

$$zind_j\,(+) = \frac{Indicator_j - Min.\,j}{Max.\,j - Min.\,j}$$

where, maximum and minimum values of indicators were from the same occupational group / district within which the household belonged.

Among various variables, the liabilities, dependency ratio, health domain indicators / sickness and all infrastructure domain indicators except access to communication equipment had negative association with livelihood improvement. Hence, as per the generalization of relative approach underlying the Human Development Index

reported by UNDP (1990) and Anand and Sen (1994), such negatively associated indicators variable were indexed as follows:

$$zind_j\,(-) = \frac{Max.\,j - Indicator_j}{Max.\,j - Min.\,j}$$

Once each indicator ($zind_j$) representing a particular livelihood security domain was standardized, relevant household livelihood security index for the particular domain was constructed by averaging the standardized indicators;

$$HLS_i = \frac{\sum_{j=1}^{J} zindj}{J}$$

where, J was the number of indicators used to construct the index. Once HLS index was constructed, then composite overall Livelihood Security (LS) index for the household was constructed by using the formula

$$HLSI_i = \frac{\sum_{i=1}^{n} wi\ HLSi}{\sum_{i=1}^{n} wi}$$

where, w was the weight determined by the number of indicators used to construct each HLS index. Weights vary between households because of household level variation in the number of indicators.

RESULTS AND DISCUSSION

The distribution of sample respondents based on location of living and primary occupation is displayed in Table 2. It is evident from the table that out of 540 sample respondents, 90 respondents each were from Thiruvannamalai, Villupuram, Dharmapuri, Pudukottai, Ariyalur and Ramanathapuram districts representing four agro-climatic zones *viz*., North-Eastern, North-Western, Cauvery Delta and Southern zones. Nearly 40 per cent of the respondents practised livestock farming and 13 per cent practised cropping as their primary occupation. Crop and livestock farming together were practised by 28.89 per cent of the sample respondents. Agricultural labourers and non-farm workers represented 6.30 per cent and 6.48 per cent of the total sample respondents, respectively. About 5 per cent of the respondents belonged to fishermen category. Majority of livestock farmers belonged to cattle farming (37.56 per cent) and goat farming (36.62 per cent) category. About one-sixth and one-tenth of the sample of livestock farmers belonged to sheep farming and buffalo farming, respectively. The proportion of crop cultivators, livestock farmers, crop p'um livestock farmers, agricultural labourers and non-farm workers were 14.44 per cent, 28.89 per cent, 38.89 per cent, 6.67 per cent and 11.11 per cent, respectively in Thiruvannamalai district (41.11 per cent). The livestock farmers constituted major proportion (38.89 per cent) of sample respondents in Villupuram district, followed by crop + livestock farmers (36.67 per cent) and crop cultivators (12.22 per cent). Agricultural labourers and non-farm workers together comprised about one-tenth of sample respondents each in Villupuram and Thiruvannamalai districts. Regarding the primary occupation, the pattern of distribution of sample respondents of Dharmapuri district was similar to that of Villupuram district. About one-half

of the respondents from Pudukottai and Ramanathapuram districts belonged to livestock farmers' category.

The sample respondents were post stratified into four categories based on the land holdings as shown in Table 3. From the table, it is evident that majority of sample respondents in Ramanathapuram district (72.22 per cent) and Pudukottai district (64.45 per cent) belonged to landless category. About one-half of the respondents from Villupuram and Dharmapuri districts and one-third from Thiruvannamalai and Ariyalur districts fell under the landless category. Out of the total respondents, marginal farmers belonged to about 45 per cent in Thiruvannamalai and Ariyalur districts, about 35 per cent in Villupuram and Dharmapuri districts and about 24 per cent in Pudukottai district and about 18 per cent in Ramanathapuram district. The occupation-wise distribution of sample respondents based on landholdings is presented in Table 3. The table clearly indicated that all the fishermen and agricultural labourers included in the present study were landless. Out of crop cultivators, marginal farmers and small farmers constituted 52.78 per cent and 27.78 per cent, respectively. Rest of the crop cultivators (19.44 per cent) had more than two hectares of land. Majority of livestock farmers (82.63 per cent) and non-farm workers (85.71 per cent) belonged to landless category. The findings were in line with Ali (2007). As they had no land, they might have oriented towards livestock farming and non-farm occupation as their prime source of income. A very meagre proportion of livestock farmers (0.94 per cent) and none of the non-farm workers had more than two hectares of landholding. It is evident from the table that about 15 per cent of livestock farmers belonged to marginal farmer category having less than one hectare of land and as they reared livestock to earn primary source of income and they might have cultivated crop as a subsidiary occupation. As a result, crop residues could be utilized for their primary (livestock) occupation and farmyard manure could be utilized for their secondary occupation (cropping) thereby a kind of integrated farming was practised in the study area. To summarize, one-half of total respondents were landless followed by marginal farmers (33.70 per cent), small farmers (10.56 per cent). Only a meagre proportion (5.74 per cent) had more than two hectares of land.

Perusal of the Table 4 revealed that women-headed households were noticed among 44.44 per cent of sample respondents in Villupuram district, followed by Pudukottai (32.22 per cent), Ariyalur (30.00 per cent), Thiruvannamalai (23.33 per cent), Ramanathapuram (15.56 per cent) and Dharmapuri (6.67 per cent) districts. Among different occupations, women headed households were more prevalent among agricultural labourer category (67.65 per cent), followed by livestock farming category (31.92 per cent), crop + livestock farming (21.15 per cent) and non-farm workers (17.14 per cent). Out of total 137 women sample respondents, about 50 per cent were of livestock farmers and 24 per cent were of crop + livestock farmers, which clearly that indicated women empowerment has occured through livestock farming activity. As a whole, the gender distribution of head of the households was in the ratio of about 3 men : 1 woman.

The table 4 vividly indicated that out of 540 sample respondents, 41.11 per cent were found to be illiterates, 36.11 per cent were found to have primary level of education and about 21.11 per cent of them had secondary level of education. Only a meagre proportion (1.67 per cent) of the sample respondents was graduates. The proportion of illiterate headed households were maximum in Dharmapuri district (48.89 per cent), followed by Pudukottai (47.78 per cent), Ramanathapuram (43.33 per cent) and Villupuram (42.22 per cent) districts. Among the nine graduates in the sample respondents, three were from Thiruvannamalai district, two from Dharmapuri district and one each from the rest of the sample districts. As shown in Table 5, it is evident that out of 72 crop cultivators, 42 per cent were illiterates, about 35 per cent were educated up to primary level and rest were educated up to secondary level. None of the crop cultivators, agricultural labourers and non-farm workers was graduates. Among the livestock farmers about one-half of the respondents were illiterates and rest were educated from primary to collegiate level. Majority of agricultural labourers were illiterates (52.94 per cent) and primary level educated (41.18 per cent). About one-half of the sample fishermen had primary level of education and about one-fourth had above secondary level of education. It is peculiar to note that out of nine graduate respondents, seven had crop + livestock farming occupation and one each practised livestock farming and fishing activity as their main occupation.

Perusal of Table 6 showed that majority of the total sample respondents belonged to Hindu religion (88.15 per cent), followed by Christians (8.52 per cent) and Muslims (3.33 per cent). The distribution pattern of three religions was more or less similar in all the selected districts except in Ramanathapuram, where the proportion of Christians (23.33 per cent) and Muslims (8.89 per cent) was higher. The distribution of various occupational groups based on religion is portrayed in Table 7. The results implied that the about 95 per cent each of crop cultivator and crop + livestock farmer belonged to the Hindu religion and the rest were equally shared by Muslims and Christians. Majority of livestock farmers belonged to the Hindu religion (89.67 per cent), followed by Christians (6.57 per cent) and Muslims (3.76 per cent). The same pattern was noticed among agricultural labourers. It is peculiar to note that about two-thirds of the fishermen belonged to Christian religion and rest of them belonged to Hindu religion. The community-wise classification of sample respondents as shown in Table 6 implied that Scheduled Tribe (ST) community constituted about one-third of the sample respondents in Thiruvannamalai district and about one-fourth of them each in Villupuram and Dharmapuri districts, respectively. About 58 per cent, 51 per cent and 37 per cent of the sample respondents belonged to Scheduled Castes (SC) in Pudukottai, Ramanathapuram and Ariyalur districts, respectively. The SC proportion was comparatively less in Thiruvannamalai (11.11 per cent) and Dharmapuri (14.44 per cent) districts. The representation of Other Communities (OC) was about one to two per cent of the sample respondents in various selected districts of Tamil Nadu. The occupation-wise distribution of

community is presented in Table 7 and it could be inferred that about one-half of the crop cultivators belonged to the Backward Caste (BC) and about one-fifth belonged to the Most Backward Caste (MBC). Majority of the selected agricultural labourers (61.76 per cent) and livestock farmers (54.93 per cent) belonged to SC community. About one-fourth each of the fishermen and non-farm workers belonged to SC community. Overall, it could be concluded that the pattern of representation of different communities were in the order of BC (37.04 per cent), SC (32.78 per cent), MBC (15.74 per cent), ST (12.96 per cent) and OC (1.48 per cent).

The mean value and standard deviation of household security indicators, which were grouped into different domains representing security areas such as economic, food / nutrition, health, education, empowerment and infrastructure were calculated and is shown in Table 8. Finally, the Household Livelihood Security Index (HLSI) was computed for the sample respondents comprising different occupational groups from the selected districts of Tamil Nadu and are presented in Table 9. The HLSI was found to be the highest among crop + livestock farmers (0.4887), followed by fishermen (0.4821), non-farm (0.4701), livestock farmer (0.4583), crop cultivators (0.4347) and agricultural labourer (0.3860) with the overall HLSI of 0.4435. Among different districts, HLSI was found to be the lowest in Pudukottai district (0.3815), followed by Ariyalur (0.4324) and Ramanathapuram (0.4455). The districts from North-Eastern and North-Western zone had higher HLSI (0.4706 to 0.4908), when compared to Cauvery Delta and Southern zones (0.3815 to 0.4455) as shown in Table 10.

Among various domains of HLSI, health security index was found to have higher value (0.8163), followed by infrastructure index (0.6919). The index values of economic, food and education were found to be at the moderate level (0.3025 to 0.3737). Among different domains, empowerment index was found to be the least (0.1477). The results clearly indicated the need for improvement of empowerment. The selected livestock farmers had the maximum index value for health security (0.7968), followed by infrastructure security (0.6733), economic security (0.4016), food security (0.3200) and empowerment (0.1682). The food security indices were higher among the fishermen, livestock farmers and crop cultivators, when compared to non-farm workers and agricultural labourer. This might be due to the reason of food availability and accessibility in their own households / farms.

Overall, it could be concluded that health and infrastructure indices among different occupations and locality was calculated to be higher when compared to other domains. There is an urgent need to improve the empowerment index irrespective of the locality and occupations. Among agricultural labourers, economic, food, health, education and empowerment should be addressed immediately. Attention should also be given to improve the economic security, food security and educational security among different occupational groups and locality. Special focus is warranted for improvement of

different security domains in Ariyalur and Pudukottai districts.

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Table 1. Household Livelihood Security Indicators

S.No.	Indicators
A.	**Economic domain**
1	Per capita income in rupees
2	Asset value per person in rupees
3	Current savings per person in rupees
4	Employment level of family members (in mandays / year)
5	Dependency rate (Number of dependents / Number of earning members in a household)
6	Current liabilities per person in rupees
B	**Food / nutritional domain**
1	Household food grain stock for a month in rupees
2	Number of main meals taken by a women in a household
3	Per capita calorie intake per day (in kcal)
4	Per capita protein intake per day (in grams)
5	Per capita fat intake per day (in grams)
C	**Health domain**
1	Incidence of diarrhoea (days/month)
2	Incidence of other sickness (days/month)
3	Number of days, an active person unable to work due to sickness
D.	**Education domain**
1	Adult male literacy (number of years of education)
2	Adult female literacy (number of years of education)
3	Number of children enrolled (6 – 15 years of age) (Number of children enrolled / number of children in a family)
E.	**Empowerment domain**
1	Participation in a development organization / social activity
2	Access to services / organization that offer services (no./month)
3	Household participation in planning and decision making (number of persons involved in a household)
F.	**Infrastructure domain**
1	Distance to nearest town in kms.
2	Distance to nearest school in kms.
3	Distance to hospital in kms.
4	Distance to access to safe drinking water in kms.
5	Distance to sanitation facility in kms.

Table 2 Distribution of sample respondents based on location and primary occupation

(numbers)

S.No.	Primary occupation	Agro-climatic zones / Districts						Overall
		North-Eastern zone		North-Western zone	Cauvery Delta zone		Southern zone	
		Thiruvannamalai	Villupuram	Dharmapuri	Pudukottai	Ariyalur	Ramanathapuram	
1	Cropping	13 (14.44)[a]	11 (12.22)[a]	15 (16.67)[a]	10 (11.11)[a]	21 (23.33)[a]	2 (2.22)[a]	**72 (13.33)[a]**
2	Livestock farming	26 (28.89)[a] (100.00)[b]	37 (41.11)[a] (100.00)[b]	37 (41.11)[a] (100.00)[b]	48 (53.33)[a] (100.00)[b]	23 (25.56)[a] (100.00)[b]	42 (46.68)[a] (100.00)[b]	**213 (39.44)[a] (100.00)[b]**
	a. Cattle	22 (84.62)[b]	30 (81.08)[b]	28 (75.68)[b]	-	-	-	**80 (37.56)[b]**
	b. Buffalo	4 (15.38)[b]	7 (18.92)[b]	9 (24.32)[b]	-	-	-	**20 (9.39)[b]**
	c. Sheep	-	-	-	9 (18.75)[b]	6 (26.09)[b]	20 (47.62)[b]	**35 (16.43)[b]**
	d. Goat	-	-	-	39 (81.25)[b]	17 (73.91)[b]	22 (52.38)[b]	**78 (36.62)[b]**
3	Crop + Livestock farming	35 (38.89)[a]	33 (36.67)[a]	28 (31.11)[a]	17 (18.89)[a]	35 (38.89)[a]	8 (8.89)[a]	**156 (28.89)[a]**
4	Fishing	-	-	-	-	-	30 (33.33)[a]	**30 (5.56)[a]**
5	Agricultural Labourer	6 (6.67)[a]	4 (4.44)[a]	4 (4.44)[a]	10 (11.11)[a]	6 (6.67)[a]	4 (4.44)[a]	**34 (6.30)[a]**
6	Non-farm occupation	10 (11.11)[a]	5 (5.56)[a]	6 (6.67)[a]	5 (5.56)[a]	5 (5.55)[a]	4 (4.44)[a]	**35 (6.48)[a]**
Total		**90 (100.00)[a]**	**90 (100.00)[a]**	**90 (100.00)[a]**	**90 (100.00)[a]**	**90 (100.00)[a]**	**90 (100.00)[a]**	**540 (100.00)[a]**

a – Figures in the parentheses indicate per cent to total and b – Figures in the parentheses indicate per cent to total livestock farmers

Table 3 Location-wise distribution of sample respondents based on landholdings

(numbers)

S.No.	Landholdings	Agro-climatic zones / Districts						Overall
		North-Eastern zone		North-Western zone	Cauvery Delta zone		Southern zone	
		Thiruvannamalai	Villupuram	Dharmapuri	Pudukottai	Ariyalur	Ramanathapuram	
1	Landless	29 (32.22)	42 (46.67)	44 (48.89)	58 (64.45)	32 (35.56)	65 (72.22)	**270 (50.00)**
2	Marginal	40 (44.45)	33 (36.67)	31 (34.44)	22 (24.44)	40 (44.44)	16 (17.78)	**182 (33.70)**
3	Small	12 (13.33)	9 (10.00)	6 (6.67)	7 (7.78)	16 (17.78)	7 (7.78)	**57 (10.56)**
4	Semi-medium, Medium and Large	9 (10.00)	6 (6.66)	9 (10.00)	3 (3.33)	2 (2.22)	2 (2.22)	**31 (5.74)**
Total		**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**540 (100.00)**

S.No.	Landholdings	Primary occupation						Overall
		Cropping	Livestock farming	Crop + Livestock farming	Fishing	Agricultural labour	Non-farm occupation	
1	Landless	-	176 (82.63)	-	30 (100.00)	34 (100.00)	30 (85.71)	**270 (50.00)**
2	Marginal	38 (52.78)	32 (15.02)	108 (69.23)	-	-	4 (11.43)	**182 (33.70)**
3	Small	20 (27.78)	3 (1.41)	33 (21.15)	-	-	1 (2.86)	**57 (10.56)**
4	Semi-medium, Medium and Large	14 (19.44)	2 (0.94)	15 (9.62)	-	-	-	**31 (5.74)**
Total		**72 (100.00)**	**213 (100.00)**	**156 (100.00)**	**30 (100.00)**	**34 (100.00)**	**35 (100.00)**	**540 (100.00)**

Figures in the parentheses indicate per cent to total

Table 4 Location-wise distribution of respondents based on gender and educational level of head of households

(numbers)

S.No.	Particulars	Agro-climatic zones / Districts						Overall
		North-Eastern zone		North-Western zone	Cauvery Delta zone		Southern zone	
		Thiruvannamalai	Villupuram	Dharmapuri	Pudukottai	Ariyalur	Ramanathapuram	
A	**Gender**							
1	Male	69 (76.67)	50 (55.56)	84 (93.33)	61 (67.78)	63 (70.00)	76 (84.44)	**403 (74.63)**
2	Female	21 (23.33)	40 (44.44)	6 (6.67)	29 (32.22)	27 (30.00)	14 (15.56)	**137 (25.37)**
B	**Educational level**							
1	Illiterates	23 (25.56)	38 (42.22)	44 (48.89)	43 (47.78)	35 (38.89)	39 (43.33)	**222 (41.11)**
2	Primary	43 (47.78)	17 (18.89)	27 (30.00)	41 (45.56)	31 (34.44)	36 (40.00)	**195 (36.11)**
3	Secondary	21 (23.33)	34 (37.78)	17 (18.89)	5 (5.55)	23 (25.56)	14 (15.56)	**114 (21.11)**
4	Collegiate	3 (3.33)	1 (1.11)	2 (2.22)	1 (1.11)	1 (1.11)	1 (1.11)	**9 (1.67)**
Total		**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**540 (100.00)**

Figures in the parentheses indicate per cent to total

Table 5 Occupation-wise distribution of respondents based on gender and educational level of head of households

(numbers)

S.No.	Particulars	Primary occupation						Overall
		Cropping	Livestock farming	Crop + Livestock farming	Fishing	Agricultural labour	Non-farm occupation	
A	**Gender**							
1	Male	68 (94.44)	145 (68.08)	123 (78.85)	27 (90.00)	11 (32.35)	29 (82.86)	**403 (74.63)**
2	Female	4 (5.56)	68 (31.92)	33 (21.15)	3 (10.00)	23 (67.65)	6 (17.14)	**137 (25.37)**
B	**Educational level**							
1	Illiterates	30 (41.67)	113 (53.05)	47 (30.13)	8 (26.67)	18 (52.94)	6 (17.14)	**222 (41.11)**
2	Primary	25 (34.72)	72 (33.80)	53 (33.97)	14 (46.67)	14 (41.18)	17 (48.57)	**195 (36.11)**
3	Secondary	17 (23.61)	27 (12.68)	49 (31.41)	7 (23.33)	2 (5.88)	12 (34.29)	**114 (21.11)**
4	Collegiate	-	1 (0.47)	7 (4.49)	1 (3.33)	-	-	**9 (1.67)**
Total		**72 (100.00)**	**213 (100.00)**	**156 (100.00)**	**30 (100.00)**	**34 (100.00)**	**35 (100.00)**	**540 (100.00)**

Figures in the parentheses indicate per cent to total

Table 6 Location-wise distribution of respondents based on religion and community of sample respondents

(numbers)

S.No.	Particulars	Agro-climatic zones / Districts						Overall
		North-Eastern		North-Western	Cauvery Delta		Southern	
		Thiruvannamalai	Villupuram	Dharmapuri	Pudukottai	Ariyalur	Ramanathapuram	
A	**Religion**							
1	Hindus	83 (92.22)	83 (92.22)	82 (91.11)	85 (94.45)	82 (91.11)	61 (67.78)	**476 (88.15)**
2	Muslims	2 (2.22)	2 (2.22)	2 (2.22)	2 (2.22)	2 (2.22)	8 (8.89)	**18 (3.33)**
3	Christians	5 (5.56)	5 (5.56)	6 (6.67)	3 (3.33)	6 (6.67)	21 (23.33)	**46 (8.52)**
B	**Community**							
1	Other Community	2 (2.22)	1 (1.11)	1 (1.11)	1 (1.11)	2 (2.22)	1 (1.11)	**8 (1.48)**
2	Backward Community	28 (31.11)	11 (12.22)	33 (36.67)	34 (37.78)	54 (60.00)	40 (44.45)	**200 (37.04)**
3	Most Backward Community	20 (22.22)	35 (38.89)	23 (25.56)	3 (3.33)	1 (1.11)	3 (3.33)	**85 (15.74)**
4	Scheduled Castes	10 (11.11)	23 (25.56)	13 (14.44)	52 (57.78)	33 (36.67)	46 (51.11)	**177 (32.78)**
5	Scheduled Tribes	30 (33.34)	20 (22.22)	20 (22.22)	-	-	-	**70 (12.96)**
Total		**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**90 (100.00)**	**540 (100.00)**

Figures in the parentheses indicate per cent to total

Table 7 Occupation-wise distribution of respondents based on religion and community of sample respondents

(numbers)

S.No.	Particulars	Primary occupation						Overall
		Cropping	Livestock farming	Crop + Livestock farming	Fishing	Agricultural labour	Non-farm occupation	
A	**Religion**							
1	Hindus	68 (94.44)	191 (89.67)	148 (94.88)	10 (33.33)	28 (82.35)	31 (88.57)	**476 (88.15)**
2	Muslims	2 (2.78)	8 (3.76)	4 (2.56)	-	1 (2.94)	3 (8.57)	**18 (3.33)**
3	Christians	2 (2.78)	14 (6.57)	4 (2.56)	20 (66.67)	5 (14.71)	1 (2.86)	**46 (8.52)**
B	**Community**							
1	Other Community	5 (6.95)	1 (0.47)	1 (0.64)	-	1 (2.94)	-	**8 (1.48)**
2	Backward Community	35 (48.61)	50 (23.47)	77 (49.36)	22 (73.33)	4 (11.77)	12 (34.29)	**200 (37.04)**
3	Most Backward Community	14 (19.44)	38 (17.84)	26 (16.67)	-	2 (5.88)	5 (14.29)	**85 (15.74)**
4	Scheduled Castes	7 (9.72)	117 (54.93)	15 (9.61)	8 (26.67)	21 (61.76)	9 (25.71)	**177 (32.78)**
5	Scheduled Tribes	11 (15.28)	7 (3.29)	37 (23.72)	-	6 (17.65)	9 (25.71)	**70 (12.96)**
Total		**72 (100.00)**	**213 (100.00)**	**156 (100.00)**	**30 (100.00)**	**34 (100.00)**	**35 (100.00)**	**540 (100.00)**

Figures in the parentheses indicate per cent to total

Table 8 Household Livelihood Security Indicators

S.No.	Indicators	Mean	Standard Deviation
A.	**Economic domain**		
1	Per capita income in rupees	1091.73	891.14
2	Asset value per person in rupees	39896.65	45089.24
3	Current savings per person in rupees	13.05	114.21
4	Employment level of family members (in mandays / year)	233.51	122.54
5	Dependency rate (Number of dependents / Number of earning members in a household)	1.89	1.45
6	Current liabilities per person in rupees	1186.99	9828.48
B	**Food / nutritional domain**		
1	Household food grain stock for a month in rupees	702.13	525.79
2	Number of main meals taken by a women in a household	2.69	0.46
3	Per capita calorie intake per day (in kcal)	1846.20	603.09
4	Per capita protein intake per day (in grams)	43.34	15.46
5	Per capita fat intake per day (in grams)	16.54	11.77
C	**Health domain**		
1	Incidence of diarrhoea (days/month)	0.26	0.77
2	Incidence of other sickness (days/month)	1.30	1.17
3	Number of days, an active person unable to work due to sickness	1.35	1.10
D.	**Education domain**		
1	Adult male literacy (number of years of education)	4.59	4.72
2	Adult female literacy (number of years of education)	3.70	3.76
3	Number of children enrolled (6 – 15 years of age) (Number of children enrolled / number of children in a family)	0.82	0.43
E.	**Empowerment domain**		
1	Participation in a development organization / social activity	0.20	0.49
2	Access to services / organization that offer services (no./ month)	0.59	0.84
3	Household participation in planning and decision making (number of persons involved in a household)	1.78	0.77
F.	**Infrastructure domain**		
1	Distance to nearest town in kms.	10.47	6.40
2	Distance to nearest school in kms.	5.25	3.41
3	Distance to hospital in kms.	6.40	3.86
4	Distance to access to safe drinking water in kms.	0.85	0.65
5	Distance to sanitation facility in kms.	2.04	0.97

Table 9 Household Livelihood Security Indices among various occupational groups in Tamil Nadu, India

S.No.	Occupation	Economic security index	Food security index	Health security index	Education index	Empowerment index	Infrastructure index	Household Livelihood Security Index (HLSI)
1	Cropping	0.4454 (0.0094)	0.3228 (0.0181)	0.5713 (0.0231)	0.4317 (0.0341)	0.1354 (0.0231)	0.6334 (0.0171)	**0.4347 (0.0086)**
2	Livestock farming	0.4016 (0.0057)	0.3200 (0.0115)	0.7968 (0.0106)	0.3953 (0.0153)	0.1682 (0.0083)	0.6733 (0.0083)	**0.4583 (0.0049)**
3	Crop + Livestock farming	0.4672 (0.0081)	0.3647 (0.0102)	0.8370 (0.0125)	0.5034 (0.0181)	0.1191 (0.0076)	0.6425 (0.0112)	**0.4887 (0.0049)**
4	Fishing	0.3759 (0.0238)	0.5032 (0.0292)	0.5778 (0.0389)	0.3983 (0.0446)	0.3389 (0.0314)	0.6674 (0.0279)	**0.4821 (0.0150)**
5	Agricultural Labourer	0.4086 (0.1088)	0.2590 (0.0868)	0.6765 (0.1286)	0.2410 (0.0715)	0.1225 (0.0286)	0.5567 (0.0852)	**0.3860 (0.0879)**
6	Non-farm occupation	0.4203 (0.0898)	0.3173 (0.0619)	0.7745 (0.1429)	0.4126 (0.1410)	0.3480 (0.0857)	0.6078 (0.1262)	**0.4701 (0.1035)**
Overall		**0.3737 (0.0025)**	**0.3173 (0.0065)**	**0.8163 (0.0061)**	**0.3025 (0.0082)**	**0.1477 (0.0053)**	**0.6919 (0.0049)**	**0.4435 (0.0027)**

Figures in parentheses indicate standard error

Table 10 Household Livelihood Security Indices among various regions of Tamil Nadu, India

S.No.	Districts	Economic security index	Food security index	Health security index	Education index	Empowerment index	Infrastructure index	Household Livelihood Security Index (HLSI)
1	Thiruvannamalai	0.4901 (0.0118)	0.3920 (0.0180)	0.8847 (0.0144)	0.3672 (0.0215)	0.1889 (0.0175)	0.5835 (0.0169)	**0.4856 (0.0080)**
2	Villupuram	0.4717 (0.0108)	0.3645 (0.0130)	0.7565 (0.0190)	0.3952 (0.0242)	0.1602 (0.0136)	0.6354 (0.0176)	**0.4706 (0.0075)**
3	Dharmapuri	0.4713 (0.0110)	0.4533 (0.0158)	0.7924 (0.0231)	0.3146 (0.0239)	0.2278 (0.0172)	0.6341 (0.0124)	**0.4908 (0.0081)**
4	Pudukottai	0.3827 (0.0091)	0.2042 (0.0154)	0.6481 (0.0179)	0.2698 (0.0161)	0.1120 (0.0123)	0.6259 (0.0153)	**0.3815 (0.0071)**
5	Ariyalur	0.3664 (0.0084)	0.3328 (0.0180)	0.7250 (0.0197)	0.3117 (0.0222)	0.2167 (0.0187)	0.6376 (0.0157)	**0.4324 (0.0078)**
6	Ramanathapuram	0.4041 (0.0090)	0.3661 (0.0160)	0.7139 (0.0202)	0.2736 (0.0195)	0.1917 (0.0138)	0.6691 (0.0147)	**0.4455 (0.0067)**
Overall		**0.3737 (0.0025)**	**0.3173 (0.0065)**	**0.8163 (0.0061)**	**0.3025 (0.0082)**	**0.1477 (0.0053)**	**0.6919 (0.0049)**	**0.4435 (0.0027)**

Figures in parentheses indicate standard error

Effect of dietary vitamin E and organic selenium supplementation on reproductive performance of Japanese quail breeders

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ABSTRACT

This study was conducted to evaluate the effect of different dietary level of vitamin E and organic selenium on the reproductive performance of Japanese quail breeders. A total of 252 Japanese quail birds aged 20 week with an average of 80 per cent egg production were used in this study. Trial birds were randomly divided into seven groups having male: female mating ratio of 1:3. Experimental birds were randomly divided into seven groups viz. T1- Control: Basal diet (standard Japanese quail ration), T2 - Basal diet with vitamin E 150mg/kg, T3 - Basal diet with vitamin E 300 mg/kg, T4 - Basal diet with organic selenium 0.3mg/kg, T5 - Basal diet with organic selenium 0.6mg/kg, T6 - Basal diet with combination of vitamin E 150mg/kg and organic selenium 0.3mg/kg, T7 - Basal diet with combination of vitamin E 300mg/kg and organic selenium 0.6mg/kg. The highest fertility (91 percent) and hatchability (Total eggs 80 percent; fertile eggs 89 percent) percentage were observed in experimental group which received vitamin E 150 mg per kg and selenium 0.3 mg per kg while the least fertility (78 percent) and hatchability (Total eggs 62 percent; fertile eggs 80 percent) percentage were recorded in the control group. From this study it can be concluded that dietary supplementation of vitamin E and organic selenium had highly significant ($P<0.01$) improvement infertility and hatchability percentage of Japanese quail.

Key words: Japanese quail breeder, vitamin E, organic selenium, fertility, hatchability

INTRODUCTION

Vitamin E is recognized as an essential nutrient for all species of animals as well as humans. Vitamin E is a generic term for a group of tocopherols and tocotrienols. Among the four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ) identified α-tocopherol is the most biologically active form and available in high quantities from vegetable oils, unprocessed cereal grains, and nuts. Vitamin E is an excellent biological chain breaking antioxidant in biological membranes, which prevents free radical, induced oxidative damage by trapping reactive oxyradicals (Packer, 1991).

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Selenium a trace element is frequently added to animal diet as a supplement for the maintenance of reproductive functions and a deficiency in dietary selenium causes decrease in sperm concentration, sperm motility, and sperm capacity in humans, lab animals and farm animals, including poultry. Selenium is an essential component of selenium dependent glutathione peroxidase (GSH-Px) which protects sperms against oxidative damage. GSH-Px plays a central role in antioxidant defense in the cell by removing hydrogen peroxide, and lipid hydroperoxide is formed during metabolism and superoxide radical dismutation.

Selenium and vitamin E are interrelated. Both are needed by animals and both have metabolic roles in the body in addition to an antioxidant effect. In some instances, vitamin E would substitute in varying degrees for selenium or vice versa. However, there are deficiency symptoms that respond only to selenium or vitamin E. Although selenium cannot replace vitamin E in nutrition, it reduces the amount of vitamin E required and delays the onset of vitamin E deficiency symptoms.

Japanese quail (*Coturnix coturnix japonica*), considered a tiny avian species has a high market value for its table delicacy. Japanese quail was first domesticated in Japan and occupy a significant position in poultry production activities. Efficient Japanese quail production is based on the feeding of a well-balanced diet to highly productive line of birds. In this respect, antioxidants play an important role in maintaining bird health, productive and reproductive performance (Surai, 2002) of Japanese Quails.

MATERIALS AND METHODS

Experimental Design

The experimental study was conducted at the Department of Poultry Science, Veterinary College and Research Institute, Namakkal, TamilNadu. A total of 252 Japanese quail birds of 20 weeks age with an average of 80 per cent egg production were selected for the trial. Feed ingredients used for formulation of ration were analysed for vitamin E and selenium content in addition to proximate composition. Vitamin E analysed by HPLC system procedure as described by Surai (2000) and selenium analysed by flourometric method procedure as described by AOAC (2000).

The Japanese quail breeder diets were formulated as per the standard prescribed by Shrivastav and Panda (1999), Central Avian Research Institute, Izatnagar except the vitamin E and selenium level in basal diet. Vitamin E in the form of dl-α-tocopheryl acetate, 50 per cent (Promix E, Addisseo company) and organic selenium in the form of Sel-Plex (Alltec Inc.) containing mainly as selenomethionine were incorporated into the basal diet either independently or simultaneously in the basal quail diet to form seven experimental groups. The ingredients and nutrient composition of Japanese Quails breeder Female and breeder male diets are presented in Table 1.

Experimental birds were randomly divided into seven groups with three replicates as follows

Treatment groups	Experimental diet	No. of birds	
		Male	Female
T_1	Basal diet (control)	9	27
T_2	Basal diet + vitamin E 150 mg/kg	9	27
T_3	Basal diet + vitamin E 300 mg/kg	9	27
T_4	Basal diet + selenium 0.3 mg/kg	9	27
T_5	Basal diet + selenium 0.6 mg/kg	9	27
T_6	Basal diet + vitamin E 150 mg/kg + selenium 0.3 mg/kg	9	27
T_7	Basal diet + vitamin E 300 mg/kg + selenium 0.6 mg/kg	9	27

The quails were reared in cages under standard managemental practices throughout the experimental period of eight weeks with male to female ratio of 1:3. They were provided with a photoperiod of 16 hours per day. The experiment was approved by the Institutional Animal Ethical Committee, of TANUVAS.

Eggs Collection

Eggs from all the experimental groups were collected twice daily at 9 PM and 7 AM. Soon after collection, eggs were examined for shell intactness, weight and size. Eggs unfit for hatching were discarded and the remaining eggs fit for setting were fumigated with formaldehyde gas for 20 minutes at "2X" concentration. They were stored at 12±1°C with 75±5 per cent relative humidity. Eggs with standard weight and shell quality were selected for setting in incubator after collection of 100 eggs per group.

Incubation and Hatching

Eggs were incubated in automatic incubator with a temperature of 99.5° F and relative humidity of 60 per cent. Egg turning was stopped three days before hatching and eggs were transferred to the Hatcher with a temperature of 98° F and 70 per cent humidity.

Fertility and Hatchability of Eggs

Hundred eggs from each replicate were placed in incubator. The usual recommended temperature and humidity were followed by turning during incubation period. The fertility assessment was performed by breaking all the unhatched eggs at the end of experimental period. Fertility and total Hatchability of eggs were calculated

Statistical Analysis

Statistical analysis was done by Completely Randomized Block Design as per Snedecor and Cochran, (1994).

RESULTS AND DISCUSSION

The mean fertility and hatchability percentage (both total eggs and fertile eggs) early and late embryonic mortality of Japanese quail eggs as influenced by dietary

supplementation of vitamin E and organic selenium are presented in Table 2.

Effect of vitamin E and selenium on fertility of Japanese quail breeder

The different levels of vitamin E and organic selenium supplementation showed highly significant ($P<0.01$) improvement in fertility percentage among treatment groups. The combination of vitamin E (150 mg/kg) and organic selenium (0.3 mg/kg) supplemented group (T_6) had significantly ($P<0.01$) increased fertility percentage compared to all other treatment groups. The control group had significantly ($P<0.01$) poor fertility percentages.

Kling and Soares (1980) observed that vitamin E deficiency in Japanese quail diet significantly decreased fertility percentage. El-Latif (1999) reported that combined supplementation of vitamin E at the rate of 25 and 50 mg per kg and selenium and 1 and 2 mg per kg diet significantly ($P<0.01$) increased fertility percentage. Franchini *et al*. (2001) reported that vitamin E supplementation at the rate of 100 and 200 ppm in the basal diet of male broiler breeder resulted in higher sperm fertility. Shinkareva and Trifonov (2003) indicated that hens fed with supplemented selenium at the rate of 0.1, 0.2 and 0.4 mg per kg diet had positively increased fertility.

On the contrary, Cantor *et al*. (1978) observed that supplementation of selenium to the breeder diet of both turkey hens and toms had no effect on fertility percentage. Hassan (1990) also observed no significant difference in fertility percentage of White Leghorn hens supplemented with selenium in the basal diet. No literature was traceable bonding a contrary report on supplementation on Japanese quail breeders.

The increase in fertility percentage of Japanese quail might be associated with higher dietary vitamin E and organic selenium supplementation. The spermatozoa of Japanese quail contain high proportion of long chain polyunsaturated fatty acids (PUFA). These extremely high concentrations of PUFA in spermatozoa are necessary to maintain fluidity and flexibility, physical properties needed for maintenance of sperm motility as well as sperm fusion during fertilization. Such high proportions of PUFA in avian spermatozoa render them more vulnerable to lipid peroxidation, which is considered to be an important factor in male subfertility. Vitamin E and selenium containing enzyme glutathione peroxidase which play an important role in protecting spermatozoa membrane lipids from peroxidation, thus maintaining the structural integrity of the spermatozoa (Surai, 1999).

Effect of vitamin E and selenium on hatchability of Japanese quail breeder

Japanese quail fed with different levels of vitamin E and organic selenium had highly significant ($P<0.01$) improvement in hatchability percentage of total eggs and significant improvement ($P<0.05$) in hatchability percentage of fertile eggs. Dietary vitamin E and selenium significantly increased hatchability percentage, with proportionate reduction in late embryonic mortality. The Japanese quail birds in T_6 group which received combination

of vitamin E (150 mg/kg) and organic selenium (0.3 kg/mg) had significantly ($P<0.01$) increased hatchability percentage. The values of fertile hatchability for T_6 were comparable statistically with other supplemented groups and numerical reduction in early and late embryonic mortality was also observed.

Japanese quail birds fed with low vitamin E soybean meal diet severely decreased hatchability percentage (Kling and Soares, 1980). Castrovilli *et al*. (2001) reported that breeder male ration containing two per cent linseed oil with vitamin E (50 and 250 mg/kg) and vitamin C (200 mg/kg) had increased the hatchability percentage.

Supplementation of selenium at different levels significantly increased hatchability percentage as observed by Cantor and Scott (1974), Latshaw *et al*. (1977), Cantor *et al.* (1978) in turkey and Shinkareva and Trifonov (2003) in breeder hens.

Combined supplementation of vitamin E (25 and 50 mg/kg) with selenium (1 and 2 mg/kg) significantly increased the hatchability. Ganpule and Manjunatha (2003) reported that inclusion of 0.1 ppm organic selenium in combination with 75 ppm vitamin E significantly ($P<0.01$) increased hatchability percentage.

Vitamin E and selenium prevent lipid peroxidation during embryonic development and this will reduce the percentage of late embryonic mortality. Increase in hatchability percentage of Japanese quail might be associated with the higher dietary vitamin E and selenium supplementation. Developing chick embryos contain high amount of PUFA in lipid fraction increase the susceptibility to peroxidative degradation. Antioxidants protect embryos from deterimental effect of the free radicals and lipid peroxidation.

During later stage of embryonic development, the embryonic tissues contain high amount of PUFA and this will lead to lipid peroxidation. A low oxygen pressure in the environment surrounding embryos damage developing embryonic tissue. The additional levels of vitamin E and selenium to prevent lipid peroxidation and oxygen tension in embryonic tissues at hatching. The results of this study indicate that it is possible to improve reproductive characteristics of Japanese quail breeders by supplementing dietary vitamin E and selenium (selenomethione).

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Table 1 Ingredients and nutrient composition of Japanese quail diet (on drymatter basis)

Ingredient (per cent)	Age (in weeks)	
	Breeder Female	**Breeder male**
Maize	53.75	21.25
Pearl millet	---	40.50
Soybean meal	26.14	22.75
Deoiled groundnut cake	11.00	11.89
Dicalcium phosphate	1.66	1.66
Shell grit	7.00	1.50
Salt	0.45	0.45
	100	**100**
Supplements (g/100 kg)		
Vitamin AB_2D_3K[1]	10	10
Choline chloride 60 %[2]	100	100
Trace mineral mixture[3]	100	100
Biocare[4]	30	30
Methionine[5]	30	30

Ingredient (per cent)	Age (in weeks)	
	Breeder Female	**Breeder male**
Nutrients (per cent)		
Drymatter	90.72	90.25
Crude protein	19.03	19.00
ME (kcal/kg)*	2700	2700
Crude fibre	4.85	5.36
Ether extract	3.89	3.21
Calcium	3.01	1.03
Available phosphorus	0.45	0.46

Lysine*	1.02	0.96
Methionine*	0.33	0.33
Vitamin E (mg/Kg)	14.76	12.46
Selenium (mg/Kg)	0.094	0.056

Calculated values

- Manufactured by M/s. Glaxo Smithkline, Mumbai. One gram of vitamin AB2D3K supplement contained 82500 IU of vitamin A, 50 mg of vitamin B2, 12000 IU of vitamin D3 and 10 mg of vitamin K.
- Manufactured by M/s. Jubilant Organosys Ltd. Gujarat, India.
- Manufactured by M/s. Neospark Drugs and Chemicals Private Ltd., Hydrabad. One gram of trace mineral mixture contained 54 mg of manganese, 52 mg of zinc, 20 mg of iron, 2 mg of iodine and 1 mg of cobalt.
- Manufactured by M/s. Tetragon Chemie Pvt. Ltd., Bangalore. One gram biocare contained 0.4 mg of biotin.
- Manufacture by M/s. Sumitomo Chemical Company Ltd., Tokya, Japan.

Table 2 Mean (±S.E) fertility and hatchability (%) of Japanese quail eggs as influenced by Dietary supplementation of vitamin E and organic selenium

Treatment groups	Fertility (%)	Hatchability (%)		Embryonic Mortality (%)	
		Total eggs	Fertile eggs	Early (<7d)	Late (>7d)
T_1 – Control	78.01±1.10^{A}	62.62±1.14 ^{A}	80.28±1.14^{a}	9.77±1.07	7.56±0.96
T_2 –Vitamin E150 mg/kg	85.46±0.83^{B}	72.60±1.74^{B}	84.90±1.30^{b}	9.52±1.14	5.58±0.64
T_3 – Vitamin E 300 mg/kg	86.02±1.50^{B}	74.81±0.79^{B}	87.07±1.50^{b}	7.96±0.97	4.97±0.66
T_4 – Selenium 0.3 mg/kg	85.10±1.33^{B}	73.94±1.13^{B}	86.93±1.24^{b}	8.22±0.61	4.85±0.75
T_5 – Selenium 0.6 mg/kg	86.11±1.23^{B}	75.43±1.44^{B}	87.64±1.67^{b}	8.36±1.08	4.00±0.84
T_6 – Vit-E 150 mg/kg + Se 0.3 mg/kg	90.59±1.35^{C}	80.35±2.00^{C}	88.71±1.88^{b}	7.26±1.74	3.70±0.93
T_7 – Vit-E 300 mg/kg + Se 0.6 mg/kg	86.12±0.71^{B}	75.38±1.40^{B}	87.52±1.40^{b}	7.91±1.08	4.57±0.44

The value given in each cell is the mean of six observations

$^{A-D}$ Means within a column with no common superscript differ significantly ($P<0.01$)

$^{a-c}$ Means within a column with no common superscript differ significantly ($P<0.05$)

Development and quality evaluation of functional shrikhand

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ABSTRACT

Functional shrikhand varieties such as low calorie and low fat shrikhand were developed by incorporation of stevia as low calorie sweetener and WPC as a fat replacer respectively. Low calorie shrikhand was prepared by incorporating stevia at different levels [0.025% (T1), 0.05% (T2) and 0.1% (T3)]. Shrikhand samples were evaluated based on physicochemical, microbiological and sensory qualities. There was no significant difference in titratable acidity and ash content but the total solids and protein content were significantly lower in low calorie shrikhand. The yeast and mould count increased and coliform count decreased during storage. The sensory quality of low calorie shrikhand was comparable to control. Low fat shrikhand samples were prepared by using WPC at three different levels [1(T1), 1.5(T2) and 2(T3) per cent]. Control shrikhand was prepared from skim milk without the addition of WPC. There was no significant difference in ash content. But there was a significant difference in acidity, total solids, protein and fat content between control and treatment samples. The yeast and mould count increased during storage but coliform count decreased during storage. The sensory quality of low fat shrikhand was similar to that of control.

Key words: Shrikhand,whey protein concentrate, fat replacer, stevia

INTRODUCTION

Shrikhand is a popular indigenous fermented milk product. It is popular in Gujarat, Maharashtra and certain parts of Karnataka and Madhya pradesh. It is prepared by blending chakka, a semi-solid mass obtained after draining whey from dahi with sugar, cream and other ingredients like fruit pulp, nuts, flavour and colour. Traditional shrikhand is high in fat and sugar content. Demand for functional dairy products such as low calorie and free fat dairy products are consistently increasing due to the growing awareness among the consumers. Fat replacers have facilitated the development of reduced fat and fat free food that have the taste and texture of high fat food with less fat and fewer calories. The commonly used fat replacers are of either carbohydrate or protein based. The use of whey protein concentrate (WPC) in low fat dairy products helps to maintain the sensory

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properties and make the product more nutritive. WPC has been considered as an interesting fat replacer due to its functional and technological properties. Increasing demand for low calorie food products has urged the food industry to find out natural low- calorie sweeteners. An ideal sweetener should be colorless, odorless, stable and easily soluble in food system. Stevia is such a natural low calorie sweetener extracted from the leaves of the plant *Stevia rebaudiana*. It is a functional sweetener and widely used as a nutraceutical. It prevents diabetes, tooth decay, decreases weight and improves digestion.

MATERIALS AND METHODS

Preparation of shrikhand

Shrikhand was prepared as per the method suggested by Aneja *et. al.*(2002). Fresh whole milk required for the study was collected from the University Dairy Plant, College of Veterinary and Animal Sciences, Mannuthy. Freeze dried mixed dahi culture (NCDC-352) was procured from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal. Whey protein concentrate (80 per cent) was procured from OMG LABS, Chennai-34, Tamil Nadu. Stevia was obtained from One cure herbs (India) Pvt. Ltd, Thiruvananthapuram.

The whole milk was preheated to 35^0C and skim milk was obtained by cream separation. The control skim milk shrikhand was prepared from skim milk (0.4% fat). Skim milk was heated to 71^0C for 10 min and then cooled to 30^0C. It was then inoculated with curd culture (NCDC 352) at 1 per cent level and incubated at 30^0C for 8 hours. It is then stirred and hung in a muslin cloth for six hours, to drain off whey to obtain chakka. Chakka was mixed with food grade cane sugar (20%) to get shrikhand. Treatment groups of skim milk shrikhand were prepared with the incorporation of WPC (80 per cent) at 1, 1.5 and 2 per cent level and 6 per cent sugar.

Treatment group of low calorie shrikhand samples were prepared from cow milk by using stevia at three different levels 0.025 (T_1), 0.05 (T_2) and 0.1 (T_3) per cent along with 2 per cent sugar.

Analysis of shrikhand

Chemical analysis

Control and treatment groups of shrikhand were analyzed for titratable acidity, fat, total solids, ash and protein contents according to the procedure described by the FSSAI (2016).

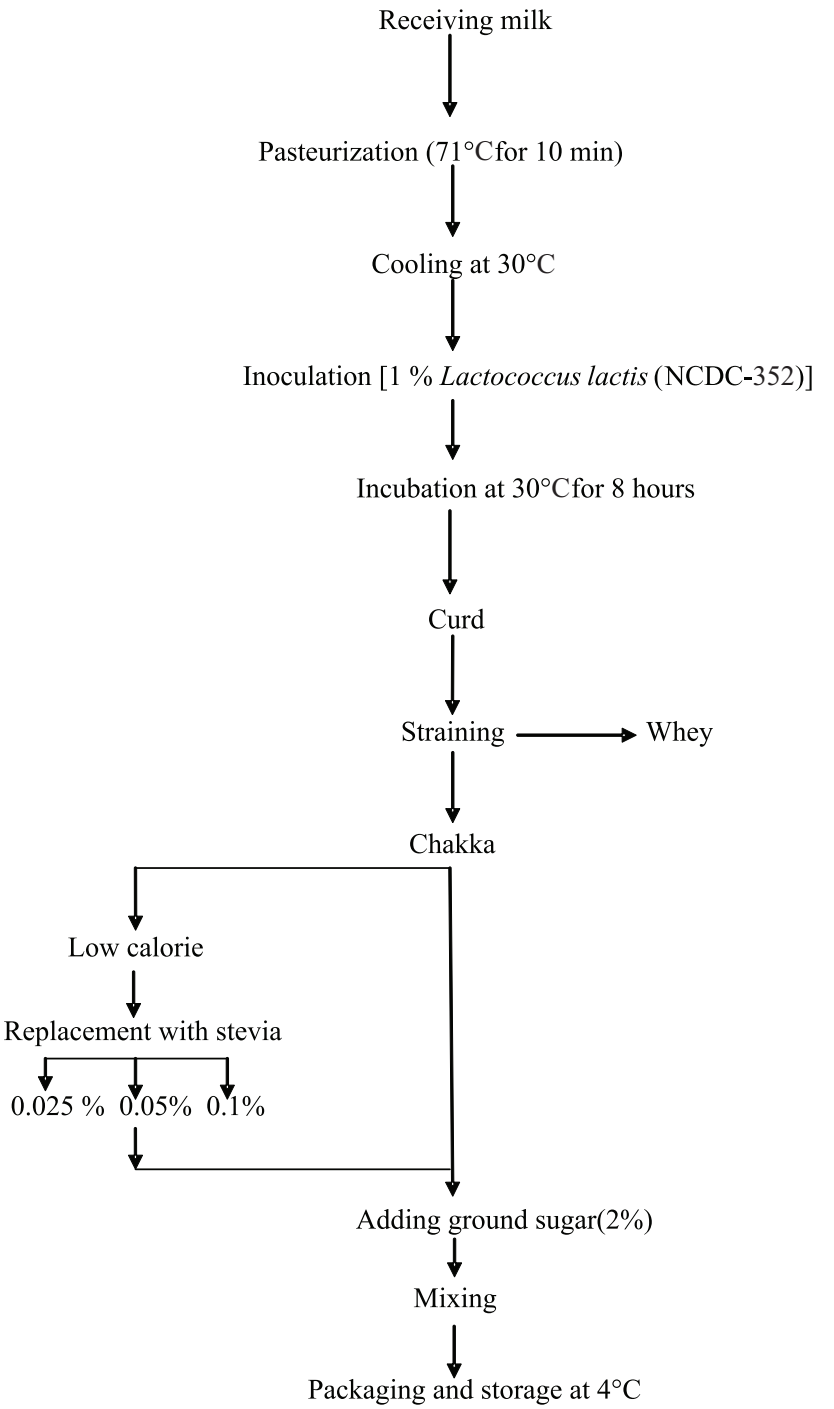
Microbiological quality

The coliform count and yeast and mould count of shrikhand samples were determined according to the procedure described by BIS (1981).

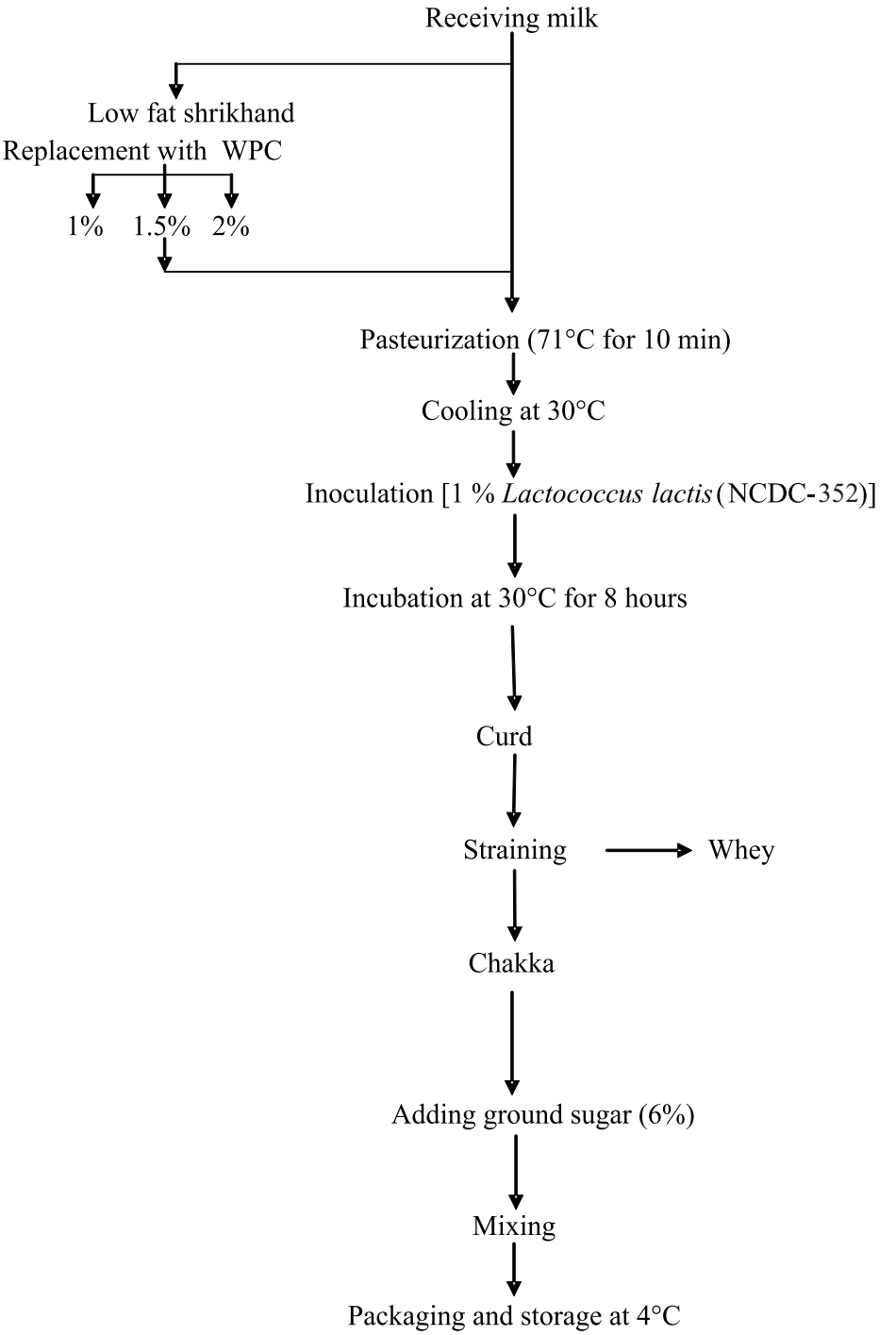
Sensory evaluation

The fresh shrikhand samples were evaluated for their sensory characteristics such as color and appearance, flavor, body texture and overall acceptability as per the method recommended by BIS (2003).

FLOW CHART FOR PREPARING LOW CALORIE SHRIKHAND



FLOW CHART FOR PREPARING LOW FAT SHRIKHAND



RESULTS AND DISCUSSION

Low calorie shrikhand

Physico-chemical qualities of low calorie shrikhand are presented in Table 1.

Titratable acidity

The mean titratable acidity of control shrikhand (C) was 1.38±0.06 per cent lactic acid. The mean titratable acidity of shrikhand samples incorporated with 0.025 per cent (T_1), 0.05 per cent (T_2) and 0.1 per cent (T_3) stevia were 1.41±0.04, 1.41±0.04 and 1.43±0.05 per cent lactic acid respectively. There was no significant difference in titratable acidity between control and stevia added shrikhand samples. Ozdemir *et. al.* (2015) had prepared low calorie ice cream with sucrose and stevia. They have also reported no significant difference in acidity between control and stevia incorporated samples.

Fat

The mean fat per cent of control shrikhand (C) and shrikhand added with stevia at 0.025 (T_1), 0.05 (T_2) and 0.1 per cent (T_3) were 9.03±0.03, 9.24±0.09, 9.26±0.07 and 9.41±0.07 respectively. Fat per cent of shrikhand showed a highly significant ($p \leq 0.01$) difference between control and stevia added shrikhand samples. There was no significant difference in fat per cent within treatment groups.Addition of stevia had increased the fat content of shrikhand. Singh *et al.*(2017) have also reported similar findings in stevia added kulfi samples.

Total solids

The mean total solids per cent of control shrikh and (C) and shrikhand incorporated with stevia at 0.025 (T_1), 0.05 (T_2) and 0.1 per cent (T_3) were 42.69±2.31, 26.49±1.83, 26.49±1.83 and 27.81±2.42 respectively. The total solids content was significantly lower in shrikhand samples incorporated with stevia. This might be due to the low level of incorporation of sugar. There was no significant difference in total solids percentage of between the treatment groups. Similar observations were made by Singh (2000).

Ash

The mean ash per cent of control (C) and shrikhand added with stevia at 0.025 per cent (T_1), 0.05 per cent (T_2) and 0.1 per cent (T_3) were 0.92±0.02, 0.91±0.02, 0.89±0.01 and 0.91±0.01 respectively. According to FSSAI (2017), total ash content in shrikhandshould not be more than 0.9 per cent on dry matter basis. The prepared samples met with the legal standards prescribed by FSSAI. Addition of stevia had not significantly altered the ash content.

Protein

The mean protein per cent of control shrikhand (C) and shrikhand incorporated with stevia at 0.025 per cent (T_1), 0.05 per cent (T_2) and 0.1 per cent (T_3) were 9.07±0.02, 8.69±0.08, 7.63±0.09 and 7.42±0.02 respectively. Protein per cent of shrikhand showed a highly significant ($p \leq 0.01$) difference between control and stevia added samples. There was also a significant difference in protein content

within the treatment groups. However, Deshmukhan *et al.* (2014) had found that there was no marked effect of stevia on protein content of ice cream.

Microbiological quality

Table 2 represents the microbiological quality of low calorie shrikhand.

Coliform count

Coliforms were present on the 1st and 5th day of storage. The mean coliform count of control shrikhand (C) were 1.05±0.05, 0.33±0.21 log cfu/g on 1st and 5th day of storage respectively. The corresponding values for T_1, $T_2$and T_3 groups shrikhand were 0.98±0.21, 0.50±0.22, 1.18±0.08, 0.33±0.21 and 1.10±0.06, 0.33±0.21 log cfu/g respectively for the same period. Coliforms were absent on 10th and 15th day of storage in all shrikhand samples. There was a significant difference in coliform count between control and T_1 but there was no significant difference between control, T_2 and T_3.

According to FSSAI (2015), coliform count of shrikhand should not be more than 10 cfu/g. All shrikhand samples met with the legal standards prescribed by FSSAI. Coliform count decreased during storage in all groups of shrikhand samples. This could be attributed to the development of acidity due to the production of lactic acid by starter cultures.

Nimashaji *et al.*(2018) also reported a progressive decrease in coliform count of shrikhand during storage.

Yeast and mould count

The mean yeast and mould count of control shrikhand (C) were 1.05±0.05, 1.36±0.04, 1.52±0.04 and 1.58±0.04 log cfu/g for 1st, 5th, 10th and 15th days of storage respectively. The corresponding values for 0.025 per cent stevia added shrikhand (T_1) were 1.25±0.05, 1.39±0.04, 1.47±0.04 and 1.68±0.03 log cfu/g. The yeast and mould count of 0.05 per cent stevia added shrikhand (T_2) were 1.06±0.17, 1.42±0.04, 1.47±0.04 and 1.65±0.02 log cfu/g for the same storage intervals.The values for 0.1 per cent stevia added shrikhand (T_3) were 1.15±0.07, 1.42±0.04, 1.56±0.03 and 1.65±0.02 log cfu/g respectively during 1, 5,10 and 15 days of storage. There was a progressive increase in yeast and mould counts in both control and treatment shrikhand samples during storage. However, the counts were within the legally permitted limit.

Sensory evaluation

Sensory scores of low calorie shrikhand are represented in Table 3.The mean flavour, colour and appearance, body and texture, container and overall scores of control shrikhand were 47.17±0.87, 27.83±1.25, 14.00±0.52, 4.33±0.21 and 93.33±2.50 respectively. The corresponding scores for shrikhand added with 0.025 per cent stevia (T_1) were 40.75±1.65, 26.00±1.63, 13.17±0.60, 4.17±0.31 and 84.08±3.68 respectively. The scores of 0.05 per cent stevia added shrikhand (T_2) were 43.33±1.41, 26.83±1.56, 13.33±0.80, 4.17±0.31 and 87.67±3.74 respectively. The scores for 0.1 per cent stevia added sample (T_3) were 45.17±1.49, 24.33±1.36, 13.00± 0.73, 4.17±0.31 and 86.67±3.45

respectively. The sensory scores for colour and appearance, body and texture and container had shown no significant difference between the control and stevia added shrikhand samples. However, sensory scores for flavour were significantly lower in stevia added shrikhand samples than control. Shrikhand prepared with 0.05 per cent stevia and 2 per cent sugar had obtained maximum over all acceptability scores among the treatment groups. Giri *et al.* (2014) had reported that kulfi prepared by replacing half the sugar content with stevia was similar to control in sensory characteristics.

Low Fat shrikhand

The results of physico-chemical analysis of low fat shrikhand are represented in Table 4

Titratable acidity

The mean titratable acidity of skim milk control shrikhand (C) was0.94±0.003 per cent lactic acid. The titratable acidity of skim milk shrikhand incorporated with 1 (T_1), 1.5 (T_2) and 2 per cent (T_3) WPC were 0.95±0.004, 0.97±0.002 and 0.97±0.002 per cent lactic acid respectively. Titratable acidity of shrikhand showed a highly significant difference ($p \leq 0.01$) between control and WPC added skim milk shrikhand samples. There was no significant difference in titratable acidity between T_1 and T_2 but there was a significant difference between T_1 and other two treatment groups. The incorporation of WPC into shrikhand resulted in an increase in acidity. Even though, there was an increase in titratable acidity due to the incorporation of WPC, the acidity has not exceeded the legal limit of 1.4 per cent lactic acid. The results are in agreement with the results reported by Venkatesh (2014).

Fat

The mean fat per cent of skim milk control shrikhand (C) was 2.91±.024. The fat per cent of shrikhand incorporated with WPC at 1(T_1), 1.5(T_2) and 2(T_3) per cent were 0.96±.010, 0.86±.011 and 0.56±.020 respectively. Fat per cent of shrikhand showed a highly significant ($p \leq 0.01$) difference between control skim milk shrikhand and WPC added skim milk shrikhand. Incorporation of WPC has resulted in reduction of fat content. The reduction was proportional to the level of incorporation.

According to FSSAI (2017), milk fat content in shrikhand should be minimum 8.5 per cent on dry matter basis. However, no specification was prescribed for fat content in low fat shrikhand.

Total solids

The mean total solids per cent of skim milk control shrikhand (C) was 41.30±0.257. The total solids per cent of skim milk shrikhand incorporated with WPC at 1(T_1), 1.5 (T_2) and 2 (T_3) per cent were 26.45±0.388, 25.60±0.235 and 26.25±0.203 respectively. Total solids per cent of shrikhand showed a highly significant ($P \leq 0.01$) difference between control and WPC added skim milk shrikhand but there was no significant difference between the treatment groups. Reduction of sugar content in WPC added shrikhand might have resulted in low total solids content.

Ash

The mean ash per cent of skim milk control shrikhand (C) was 0.94±0.015. The ash per cent of skim milk shrikhand incorporated with WPC at 1 (T_1), 1.5 (T_2) and 2 (T_3) per cent were 0.95±0.012, 0.93±0.014 and 0.92±0.013 respectively. There was no significant difference in ash content between control and WPC added skim milk shrikhand samples. Nimashaji *et al.,* (2018) have also reported similar findings in low fat shrikhand incorporated with whey protein concentrate at 2 per cent level.

Protein

The mean protein per cent of skim milk control shrikhand (C) was 8.99±0.026. Protein content of skim milk shrikhand samples incorporated with WPC at 1 (T_1), 1.5 (T_2) and 2(T_3) per cent were 10.37±0.094, 11.57±0.058 and 12.76±0.125 respectively. Protein per cent of shrikhand showed a highly significant difference between control and WPC added skim milk shrikhand samples.The addition of WPC had significantly increased the protein content of shrikhand samples. Similar findings were reported by Nimashaji *et al.,* (2018).

Microbiological quality

Table 5 represents the microbiological quality of low fat shrikhand.

Coliform count

The mean coliform count of skim milk control shrikhand (C) were 1.05±0.05 and 0.50±0.22log cfu/g respectively on 1st and 5th day of storage. In $T_{1,}$ the mean coliform counts were 1.10±0.06 and 0.33±0.21log cfu/g respectively. The corresponding values for T_2 were 1.18±0.08 and 0.38±0.25 log cfu/g respectively. In T_3 the coliform count were 1.20±0.06 and 0.50±0.22 log cfu/g respectively on 1st and 5th day of storage. Coliforms were absent on 10th and 15th day of storage. There was no significant difference in coliform count between control and treatment groups of shrikhand. According to FSSAI (2015), coliform count of shrikhand should not be more than 10 cfu/g. The prepared products met with the legal standards. The decrease in coliform count might be attributed to the development of acidity during storage.

Yeast and mould count

The mean yeast and mould counts of skim milk control shrikhand (C) were 1.10±0.06, 1.41±0.05, 1.52±0.03 and 1.60±0.03 log cfu/g for the 1st, 5th, 10th and 15th days of storage respectively. The values for 1 per cent WPC added skim milk shrikhand (T_1) were 1.25±0.05, 1.39±0.04, 1.47±0.04 and 1.63±0.04 log cfu/g respectively for the same period. The yeast and mould count of 1.5 per cent WPC added skim milk shrikhand (T_2) were 1.20±0.06, 1.42±0.04, 1.49±0.04 and 1.61±0.03 log cfu/g respectively. The values for 2 per cent WPC added skim milk shrikhand (T_3) were 1.28±0.06, 1.42±0.04, 1.56±0.03 and 1.65±0.02 log cfu/g respectively for the same storage intervals. There was a progressive increase in yeast and mould count in both control and treatment shrikhand samples during storage.

According to FSSAI (2015), yeast and mould count of shrikhand should not be more than 50 cfu/g.The prepared samples

met with the legal limit until 15 days of refrigerated storage. The findings were in close resemblance with the findings of Shridharrao (2012).

Sensory evaluation

The sensory scores of low fat shrikhand are presented in table 6.The mean flavour, colour and appearance, body and texture, container and overall scores of control skim milk shrikhand (C_2) were 48.00±0.26, 29.00±0.26, 13.83±0.31, 4.67±0.21 and 92.50±0.62 respectively. The scores for 1 per cent WPC added skim milk shrikhand (T_1) were 45.83±0.87, 28.67±0.33, 13.83±0.31, 4.50±0.22 and 92.83±1.54 respectively. The sensory scores for 1.5 per cent WPC added skim milk shrikhand (T_2) were 46.00±1.09, 28.83±0.31, 13.33±0.42, 4.50±0.22 and 92.67±1.80 respectively. The scores for 2 per cent WPC added skim milk shrikhand sample (T_3) were 45.00±1.29, 28.67±0.33, 13.67±0.33, 4.50±0.22 and 91.83±1.78 respectively. The sensory scores showed no significant difference between control and WPC added skim milk shrikhand samples.

Skim mik shrikhand incorporated with one per cent WPC had obtained better overall acceptability scores among the treatment groups. Berber (2011) had also reported better flavorin WPC incorporated yogurt.

Conclusion

From the above results, it could be concluded that, low calorie shrikhand could be prepared by partly replacing sugar with stevia. There was a significant reduction in total solids content due to the reduction of sugar content in stevia added shrikhand. A decrease in protein content and increase in fat content was observed in shrikhand samples incorporated with stevia. The sensory quality of low calorie shrikhand prepared by using 0.05 per cent stevia was comparable to control shrikhand. Low fat shrikhand could be prepared by using WPC as fat replacer. A significant increase in protein content and decrease in fat content was observed in WPC added skim milk shrikhand samples. A significant increase in titratable acidity was also noticed in WPC added skim milk shrikhand. The sensory quality of low fat shrikhand incorporated with 1 per cent WPC was comparable to control. Hence WPC can be effectively used as fat replacer for the preparation of low fat shrikhand.

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Table.1 Physico-Chemical qualities(Mean ± S.E) of low calorie shrikhand

Parameters	Control	Low calorie shrikhand		
		T_1(0.025 per cent stevia)	T_2 (0.05 per cent stevia)	T_3 (0.1 per cent stevia)
Acidity (% LA)	1.38±0.06[a]	1.41±0.04 [a]	1.41±0.04 [a]	1.43±0.05[a]
Fat (%)	9.03±0.02[a]	9.24±0.09[b]	9.26±0.07 [b]	9.41±0.07 [b]
Total solids (%)	42.69±2.13[a]	26.49±1.83[b]	26.49±1.83[b]	27.81±2.42[b]
Ash (%)	0.92±0.01 [a]	0.91±0.02 [a]	0.89±0.01[a]	0.91±0.01[a]
Protein (%)	9.07±0.02 [a]	8.69±0.08[b]	7.63±0.09[c]	7.42±0.02[d]

Mean within a row bearing different superscripts differ significantly (P≤ 0.01)
Mean within a row bearing same superscripts are homogenous

Table .2 Microbiological quality of (Mean ± S.E) low calorie shrikhand

Parameter	Samples	1st day	5th day	10th day	15th day
Coliform count log cfu/g	C (Control)	1.05±0.05[b]	0.33±0.21[a]	0	0
	T_1(0.025 % stevia)	0.98±0.21[c]	0.50±0.22[b]	0	0
	T_2(0.05 % stevia)	1.18±0.08[b]	0.33±0.21[a]	0	0
	T_3(0.1 % stevia)	1.10±0.06 [b]	0.33±0.21[a]	0	0
Yeast and Mold count log cfu/g	C(Control)	1.05±0.05[a]	1.36±0.04[b]	1.52±0.04 [b]	1.58±0.04[c]
	T_1(0.025 % stevia)	1.25±0.05[a]	1.39±0.04[b]	1.47±0.04 [b]	1.68±0.03[c]
	T_2(0.05 % stevia)	1.06±0.17[a]	1.42±0.04[b]	1.47±0.04 [b]	1.65±0.02[c]
	T_3(0.1 % stevia)	1.15±0.07[a]	1.42±0.04[b]	1.56±0.03 [b]	1.65±0.02[c]

Mean within a column bearing different superscripts differ significantly (P≤ 0.01)
Mean within a column bearing same superscripts are homogenous

Table.3 Sensory scores of (Mean ± S.E) low calorie shrikhand

Sample	Flavour	Colour and appearance	Body and texture	Container	Overall score
C	47.17±0.87[a]	27.83±1.25[a]	14.00±0.52[a]	4.33±0.21[a]	93.33±2.50[a]
T_1	40.75±1.65[b]	26.00±1.63[a]	13.17±0.60[a]	4.17±0.31[a]	84.08±3.68[b]
T_2	43.33±1.41[b]	26.83±1.56[a]	13.33±0.80[a]	4.17±0.31[a]	87.67±3.74[b]
T_3	45.17±1.49 [b]	24.33±1.36[a]	13.00± 0.73[a]	4.17±0.31[a]	86.67±3.45[b]

Mean within a column bearing different superscripts differ significantly (P≤ 0.01)
Mean within a column bearing same superscripts are homogenous

Table.4 Physico-Chemical qualities(Mean ± S.E) of low fat shrikhand

Parameters	Skim milk Control (C)	Low fat shrikhand incorporated with WPC at different levels		
		T_1 (1 per cent WPC)	T_2 (1.5 per cent WPC)	T_3 (2 per cent WPC)
Acidity (% LA)	0.94±.003[a]	0.95±.004[b]	0.97±.002[c]	0.97±.002[c]
Fat(%)	2.91±.024[a]	0.96±.010[b]	0.86±.011[c]	0.56±.020[d]
Total solids(%)	41.30±0.25[a]	26.45±0.38[b]	25.60±0.23[b]	26.25±0.203[b]
Ash(%)	0.94±0.015[a]	0.95±0.012[a]	0.93±0.014[a]	0.92±0.013[a]
Protein(%)	8.99±0.026[a]	10.37±0.094[b]	11.57±0.058[c]	12.76±0.125[d]

Mean within a row bearing different superscripts differ significantly (P≤ 0.01)
Mean within a row bearing same superscripts are homogenous

Table.5 Microbiological quality (Mean ± S.E) of low fat shrikhand

Parameter	samples	1st day	5th day	10th day	15th day
	C	1.05±0.05[a]	0.50±0.22[a]	0	0
	T_1	1.10±0.06[a]	0.33±0.21[a]	0	0
	T_2	1.18±0.08[a]	0.38±0.25[a]	0	0
	T_3	1.20±0.06[a]	0.50±0.22[a]	0	0
	C	1.10±0.06[a]	1.41±0.05[b]	1.52±0.03[b]	1.60±0.03[c]
	T_1	1.25±0.05[a]	1.39±0.04[b]	1.47±0.04[b]	1.63±0.04[c]
	T_2	1.20±0.06[a]	1.42±0.04[b]	1.49±0.04[b]	1.61±0.03[c]
	T_3	1.28±0.06[a]	1.42±0.04[b]	1.56±0.03[b]	1.65±0.02[c]

Mean within a column bearing different superscripts differ significantly (P≤ 0.01)
Mean within a column bearing same superscripts are homogenous

Table. 6 Sensory scores (Mean ± S.E) of low fat shrikhand

Sample	Flavour	Colour and appearance	Body and texture	Container	Overall score
C	48.00±0.26[a]	29.00±0.26[a]	13.83±0.31[a]	4.67±0.21[a]	92.50±0.62[a]
T_1	45.83±0.87[a]	28.67±0.33[a]	13.83±0.31[a]	4.50±0.22[a]	92.83±1.54[a]
T_2	46.00±1.09[a]	28.83±0.31[a]	13.33±0.42[a]	4.50±0.22[a]	92.67±1.80[a]
T_3	45.00±1.29[a]	28.67±0.33[a]	13.67±0.33[a]	4.50±0.22[a]	91.83±1.78[a]

Mean within a column bearing same superscripts are homogenous

Evaluation of methane reduction potential of sheanut cake based concentrate rations by *in vitro* gas production studies

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ABSTRACT

A study was conducted to evaluate the methane reduction potential of sheanut cake in ruminants. The nutrient composition *viz*., CP, NDF and ADF contents (%) were 15.2, 42.24 and 30.85 respectively. The total tannin phenol content was 7.08 % and predominantly in the form of hydrolysable tannins (5.85 %). Six isonitrogenous concentrate rations were prepared by including sheanut cake at levels of 0 (control), 15 % (T_1), 17.5 % (T_2), 20 % (T_3), 22.5 % (T_4) and 25 % (T_5) and were evaluated by *in vitro* gas production technique (IVGPT). *In vitro* gas production profiles indicated that there was significant ($P<0.01$) reduction in total gas production, methane, methane for 100 mg of truly digested substrate and NH_3 – N in sheanut cake based rations compared to control. Methane for 100 mg of truly digested substrate was significantly ($P<0.01$) reduced by 15.39 %, 20.37 %, 28.23 %, 27.28 % and 28.21 % in T_1, T_2, T_3, T_4 and T_5 respectively. The maximum methane reduction was observed at 20 % level thereafter no further reduction even after increasing sheanut cake. It can be concluded that sheanut cake could be used medium energy and protein source and also a potential feed ingredient in suppressing the methane production in ruminants.

Key words: Methane, sheanut cake, tannins, protozoa

INTRODUCTION

Greenhouse gas emission from ruminant production system is of particular importance because of their consequences on global climate. Enteric methane (CH_4) is one of the most potent greenhouse gas emitted by ruminants and accounts for 2 to 12 % loss of gross energy of feeds depending on the diet (Johnson and Johnson, 1995). Globally about 80 million tonnes of CH_4 is produced annually, agriculture contributing 47 % of these emissions with ruminants accounting for 39% (Gerber *et al.,* 2013). During past decade, intensive research has been conducted to develop effective and practical means to decrease the CH_4 emission from ruminants (Hristov *et al.,* 2013). Among them, use of unconventional

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feeds rich in tannins is one of the promising approach (Bhatta *et al.,* 2012). Sheanut cake (obtained from processing of the sheanut for shea butter) is one such potential alternative feed resource in ruminant diets (Kumar *et al.,* 2015). However, use of sheanut cake, level of inclusion and its antimethanogenic properties were not fully explored. Hence, the present *in vitro* study had been undertaken to evaluate the sheanut cake based concentrate ration and its level of inclusion on methane reduction potential and rumen fermentation characteristics.

MATERIALS AND METHODS

Sample Collection and Preparation

Sheanut cake was procured from the industry at Tadepalligudem, West Godavari District of Andhra Pradesh while other ingredients (maize, deoiled rice bran and soybean meal) were procured locally and were ground in a Willey mill to pass through 1 mm sieve and used for further analysis. Six isonitrogenous concentrate rations were formulated by including sheanut cake @ 0 % (control), 15 % (T_1), 17.5 % (T_2), 20 % (T_3), 22.5 % (T_4) and 25 % (T_5) in the ration. Ingredient composition is presented in table 1. Sheanut cake and concentrate rations were analyzed for proximate principles (AOAC, 2007), fibre fractions (Vansoest *et al.,* 1991) and tannins (Makkar, 2003).

In vitro gas production studies

The *in vitro* gas production studies were carried out using Hohenheim gas production technique (Menke and Steingass, 1988). Rumen liquor samples were collected from three crossbred cattle maintained on paddy straw, Napier hybrid grass (Co4) and concentrate, under anaerobic conditions. About 200 mg of samples incubated with 30 ml of buffered rumen inoculum in 100 ml calibrated glass syringes and were kept in water bath shaking incubator set at 39°C. At the end of incubation period (24 h), the total gas produced was measured and the gas samples were collected in vaccutainer for estimation of methane using gas chromatography (Perkin Elmer, Clarus 500 model) equipped with Flame Ionization Detector (FID). Samples of fermented fluid were collected for estimation of ammonia nitrogen (micro-diffusion method of Conway, 1957), total volatile fatty acids (Barnett and Reid, 1957) and total protozoa (Kamra *et al.* 1991). The pH of the rumen liquor was measured immediately after the end of incubation using digital pH meter. *In vitro* true dry matter digestibility was estimated as per the procedures of Van Soest and Robertson (1988). Metabolisable energy (MJ / kg DM) and Microbial biomass production (MBP) were calculated as per equations suggested by Menke *et al.* (1979) and Blummel *et al.* (1997) respectively.

$$\text{ME (MJ / kg DM)} = 1.06 + 0.157 \times \text{gas (ml / 200 mg DM)} + 0.0084 \times \text{CP (g/kg DM)} + 0.022 \times \text{EE (g/kg DM)} - 0.0081 \times \text{Ash (g/kg DM)}$$

$$\text{MBP (mg)} = [\text{TD (mg)}] - [(2.20 \times \text{net gas volume in ml})]$$

Where, TD = True digestible dry matter (Substrate incubated – Neutral detergent fibre)

Statistical analysis of the data was carried out by analysis of variance (ANOVA) as per the procedures suggested by Snedecor and Cochran (1994) using SPSS version 20.0. Treatment means were compared by using Duncan's multiple range test.

RESULTS AND DISCUSSION

Chemical composition of sheanut cake and sheanut cake based concentrate ratios were presented in table 1. Chemical composition revealed that sheanut cake contains 15.2 % crude protein, 8.26 % crude fibre, 7.29 % total ash and 64.47 % nitrogen free extract. The nutrient composition was comparable to that of palm kernel meal (Bhatta *et al.,* 2012). NDF and ADF contents were 42.24 % and 30.85 %, respectively and these values were corroborated with earlier findings (Kumar *et al.,* 2007 and oddoye *et al.,* 2012). However, higher NDF and ADF content were observed by Pousga *et al.* (2007) and Pullaiah (2013) than that reported in the present study.

The total tannin content was 7.08 % and most of them are hydrolysable tannins (5.85%) while condensed tannins were only 1.23 %. Tannins content was similar to that reported by Bhatta *et al*. (2012) in various sheanut by-products. Variation in the chemical composition might be attributed to the method of extraction of shea butter, maturity of nuts, handling of the nuts prior to processing, cultivation practices and agronomic factors (Oddoye *et al*., 2012).

Chemical composition of concentrates indicates that all the rations were isonitrogenous (20 % crude protein). NDF and ADF content of concentrate rations increased gradually as level of sheanut cake increased in rations due to presence of higher NDF and ADF content in sheanut cake compared to other conventional ingredients used in the ration.

In vitro gas production studies:

Effect of sheanut cake based concentrate rations on total gas (ml), methane (ml), *in vitro* true dry matter digestibility and methane (ml) per 100 mg of truly digested substrate was presented in table 2. The total gas production had significantly ($P<0.01$) decreased as the level of sheanut cake increased in concentrate rations. The total gas was lowered by 11.82 %, 13.12 %, 15.48 %, 16.99 % and 19.14% in T_1, T_2, T_3, T_4 and T_5, respectively than control. Similar trend in gas production was observed by Kumar *et al*. (2015) by replacing wheat bran and deoiled rice bran with sheanut cake in concentrate mixture at 0 to 9 % of the ration.

As the level of sheanut cake increased in rations, methane (ml) production was reduced significantly ($P<0.01$). The minimum level of sheanut cake that reduced the maximum methane was 20%. Similar trend was observed in methane (%) on total gas production. Reduction in the methane emission observed in the present study is in conformity with that of Bhatta *et al*. (2012) and who concluded that reduction of CH_4 was due to presence of active tannins. Kumar *et al*. (2015) also reported significant reduction of methane in feeds containing sheanut cake.

The methane (ml) per 100mg of truly digested substrate was also reduced significantly ($P<0.01$) with increasing levels of sheanut cake in concentrate rations. The reduction was 15.39, 20.37, 28.33, 27.28 and 28.21 per cent in T_1, T_2, T_3, T_4 and T_5, respectively compared with control.

Highest reduction was observed in T_4 but there was no significant difference between T_3, T_4 and T_5 therefore the minimum level of sheanut cake in rations with maximum methane reduction is 20% inclusion level. Reduction in total gas production and methane production with increasing sheanut cake level could be linked to role of sheanut tannins in reducing protozoa and/ or methanogenic archea population (Bhatta *et al*., 2012), reducing the digestibility of dry matter and organic matter and the release of H_2 (Jayanegara *et al.,* 2010; Patra and Yu, 2014). Jayanegara *et al.* (2010) reported that there is significant negative relation between total tannins and methane production.

In vitro true dry matter digestibility ranges from 82.78 (control) to 77.50 (T_5) and decreased significantly ($P < 0.01$) with increasing level of sheanut cake in the concentrates rations compared to control. Decline in digestibility might be attributed to tannins present in sheanut cake forming complexes with proteins and carbohydrates under ruminal pH conditions (McSweeney *et al*., 2001). Pullaiah (2013) reported that *in vitro* dry matter digestibility (IVDMD) was reduced significantly when sheanut cake is included at 10, 20 % level in complete rations. However Kumar *et al.* (2007) reported that when sheanut cake extract is included at 20 % and 40 % levels there was no significant difference in IVDMD using buffalo rumen liquor. The possible reason for difference in IVDMD may be due to inclusion of different ingredients used in the experimental rations and tannins content in sheanut cake.

***In vitro* fermentation characteristics:**

Effect of sheanut cake and sheanut cake based concentrate rations on pH, ammonia nitrogen, total volatile fatty acids (TVFA) concentration, microbial biomass, ME and protozoal count were presented in table 3. There is no significant change in pH among the treatments. Similar to these results, Travendale *et al.* (2005) also reported no change in pH by addition of tannin rich feeds like *Lotus pendunculatus* and *Medicago sativa.* Ammonia nitrogen content in treatment groups were significantly ($P<0.01$) reduced compared to control. Results in the present study were in agreement with the findings of Bhatta *et al.* (2012) who reported that when sheanut cake is incubated *in vitro* with and without poly ethylene glycol (PEG), there was significant reduction of ammonia nitrogen in the absence of PEG confirming that tannins present in sheanut cake binds with proteins and reduces their degradation. Proteins that escape being degraded in the rumen would enhance flow of proteins to intestines (Tan *et al.,* 2011), Hence sheanut cake can be incorporated in ruminant diets to protect degradable proteins.

The TVFA concentration decreased numerically when sheanut cake was included in concentrate rations, but there is no significant ($P>0.05$) difference among the treatment groups. Slight decrease in TVFA in sheanut based rations might be due to presence of tannins. This finding was in consistence with the findings of Getachew *et al.* (2008) with tannic acid and quebracho tannin. Bhatta *et al.* (2012) reported that incubation of sheanut cake with PEG resulted in substantial increase in TVFA.

Microbial biomass (mg/200 mg DM) was significantly (P<0.01) higher in all sheanut cake based rations (86.07 to 87.55) compared to control ration (80.31). However, there is no significant difference among the sheanut cake based rations. Banakar *et al.* (2017) reported similar microbial biomass in different unconventional feeds under *in vitro* conditions. Higher microbial biomass in treatment groups indicate that substrate incubated *in vitro* was well utilized by microbes for their growth and development.

Metabolisable energy (MJ/kg DM) was significantly (P<0.01) higher in control (8.45) and lower in T_5 (7.62). As the level of sheanut cake increased in rations, ME values decreased gradually. ME values reported in the present study was within range (7.39 to 12.02) of values reported by Kumar *et al.* (2015) for different concentrate feed ingredients. The difference in the ME values of feeds is reflective of difference in available carbohydrate, nitrogen content and fibre content of feeds.

There was significant (P<0.05) reduction in the total protozoa count in sheanut cake based ration compared to control. This finding was consistent with the findings of other researchers (Bhatta *et al.,* 2012 and Bharathidasan, 2018). Galindo *et al.* (2008) found that 39.42% reduction in total protozoa population when tannin containing feed *L.leucocephala* was included at 30 % level under *in vitro* conditions. Bhatta *et al.* (2012) also observed higher susceptibility of Entodinia to phenolics present in the sheanut byproducts resulting in decreased methane production by removing the methanogen populations attached to protozoa.

CONCLUSION

It could be concluded that sheanut cake is a potential unconventional feed resource with medium protein and energy content which can be used to replace conventional feed ingredients in concentrate rations for ruminants. At 20% level of inclusion, sheanut cake is having maximum methane reduction potential without much effect on rumen fermentation. Hence, sheanut cake could be used as energy and protein source as well as to reduce enteric methane emission. However, further biological trials need to be carried out to assess the effect of sheanut cake in ruminant rations.

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Table 1: Ingredient and chemical composition of sheanut cake and sheanut cake based concentrate rations

Ingredient	Control	T_1	T_2	T_3	T_4	T_5	Sheanut Cake
Maize	27	26	25	25	25	24.5	-
DORB	42	29.5	28.5	26	24	22	-
Soybean meal	28	26.5	26	26	25.5	25.5	-
Sheanut Cake	0	15	17.5	20	22.5	25	-
Mineral Mixture	2	2	2	2	2	2	-
Salt	1	1	1	1	1	1	-
Chemical composition of feed stuffs (% DM basis)							
OM	89.08	90.45	90.52	90.80	91.03	91.49	92.71
CP	20.07	20.16	19.92	20.23	19.98	20.18	15.20
EE	2.22	2.70	2.76	2.85	2.93	2.91	4.78
CF	10.37	9.20	9.17	8.92	8.73	8.30	8.26
NFE	56.42	58.39	58.67	58.80	59.39	60.10	64.47
NDF	25.46	26.59	27.02	27.12	27.32	27.53	42.24
ADF	8.41	11.38	11.95	12.43	12.92	13.43	30.85
TDN*	73.29	71.24	70.78	70.55	70.27	69.96	58.01

*Calculated values

Table 2: Effect of sheanut cake based concentrate rations on total gas (ml), methane (ml), *in vitro* true dry matter digestibility and methane (ml) per 100 mg of truly digested substrate.

Parameter	Control	T_1	T_2	T_3	T_4	T_5	SEM	P Value
Total gas production (ml)	38.75^{a}	34.17^{b}	33.67^{bc}	32.75^{bcd}	32.17^{cd}	31.33^{d}	0.47	0.01
Methane (ml)	8.01^{a}	6.60^{b}	6.18^{b}	5.54^{c}	5.53^{c}	5.38^{c}	0.17	0.01
% Methane on total gas production	20.67^{a}	19.32^{b}	18.38^{bc}	16.90^{d}	17.15^{cd}	17.17^{cd}	0.28	0.01
Methane (ml) for 100 mg of truly digested substrate	4.84^{a}	4.09^{b}	3.86^{bc}	3.46^{d}	3.52^{cd}	3.47^{d}	0.09	0.01
In vitro true dry matter digestibility (%)	82.78^{a}	80.67^{b}	80.22^{b}	79.80^{b}	78.53^{c}	77.50^{d}	0.42	0.01
Methane reduction (%)	-	15.39^{a}	20.37^{a}	28.23^{b}	27.28^{b}	28.21^{b}	1.31	0.01

Each value is mean of six observations

Means bearing different superscripts in the same row differ significantly ($P < 0.01$)

Table 3: Effect of sheanut cake based concentrate rations on pH, Ammonia Nitrogen, total volatile fatty acids (TVFA) concentration, microbial biomass, ME and protozoal count

Parameter	Control	T_1	T_2	T_3	T_4	T_5	SEM	P Value
pH	6.67	6.73	6.77	6.75	6.75	6.78	0.12	0.08
Ammonia –N (mg/dl)**	16.68^{a}	15.05^{b}	14.82^{b}	14.58^{b}	14.35^{b}	14.47^{b}	0.18	0.01
Total Volatile fatty acids (mM/l)	86.37	82.90	80.97	79.40	82.55	80.63	1.01	0.46
Microbial bio mass (mg)**	80.31^{b}	86.17^{a}	86.37^{a}	87.55^{a}	86.29^{a}	86.07^{a}	0.64	0.01
Metabolisable energy (MJ/ kg DM)**	8.43^{a}	7.95^{b}	7.86^{bc}	7.79^{bc}	7.71^{bc}	7.63^{c}	0.06	0.01
Total protozoa ($\times 10^5$/ml)*	5.08^{a}	4.25^{b}	4.17^{b}	4.08^{b}	3.83^{b}	3.67^{b}	0.13	0.02

Each value is mean of six observations

Means bearing different superscripts in the same row differ significantly * $P < 0.05$ **$P < 0.01$

Correlation of heart rate and body weight with various electrocardiographic and echocardiographic parameters in indigenous breeds of dogs

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ABSTRACT

The current study was aimed to study the correlation of heart rate and body weight with various electrocardiographic and echocardiographic parameters in indigenous breeds of dogs of Tamil Nadu such as Rajapalayam and Chippiparai. The dogs are known for their skill, companionship and their adaptation to the country's tropical climate. Study population consisted of 24 overtly healthy Rajapalayam (n=12) and Chippiparai (n=12) dogs, sight-hound breeds of Tamil Nadu. After recording the relevant parameters, the values were correlated statistically and results obtained.

Key words: Electrocardiography, Echocardiography, Indigenous dog breeds

INTRODUCTION

The goal in the diagnosis of diseases was to make a complete diagnosis with the fewest diagnostic procedures that are non-invasive or minimally invasive. Numerous modalities had been developed for diagnosis of cardiac disorders, which included angiography, cardiac catheterization, endomyocardial biopsy, nuclear cardiology, pneumoperi cardiography, electrocardiography, thoracic radiography, echocardiography and haemato-biochemical parameters (Gugjoo, 2011).

India has a rich canine genetic resource, besides the vast wealth of livestock germplasm. Indigenous canine breeds like Rajapalayam, Chippiparai, Mudhol hound, Rampur hound, Caravan hound, Banjara hound and Jonangi are well known. Of these, Rajapalayam and Chippiparai are the indigenous breeds of Tamil Nadu. But due to inflow of exotic canine breeds, the indigenous dogs had not received any attention from scientists (Karthickeyan *et al.,* 2015).

Electrocardiography (ECG), a non-invasive and relatively inexpensive technique was found to be the useful tool in diagnosing cardiac arrhythmias and provided

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information about the status of myocardium (Tilley, 1992). Echocardiography has evolved as the most promising tool in the study of anatomy, physiology and diagnosis of disease related to heart. It is considered as a gold standard in diagnosis of cardiac diseases (Dudas-Gyorki *et al.,* 2011).

Although variation in heart rate and body weight could explain differences in dog breeds, the effect of heart rate on various electrocardiographic parameters and body weight on echocardiographic parameters was observed in few exotic breeds of dogs by Bayon *et al.* (1994), Hanton *et al.* (1998) and Lonsdale *et al.* (1998).

Hence this study was aimed to study the correlation of heart rate and body weight with various electrocardiographic and echocardiographic parameters in indigenous breeds of dogs.

MATERIALS AND METHODS

The study was carried out on 24 overtly healthy Rajapalayam and Chippiparai breeds of dogs brought to Madras Veterinary College teaching hospital with the consent of the owner. Those dogs with vital parameters within the established reference range were considered to be clinically healthy and subjected for further evaluation.

Electrocardiography was carried out using RMS VESTA 301i Electrocardiograph using Lead II of standard bipolar limb lead system and subjective assessment of the Electrocardiogram (ECG) reading was done to ensure that the parameters were within the normal reference range for dogs (Tilley, 1992).

Esoate MyLab 20 ultrasound machine with a cardiac probe of 6MHz was used for recording the echocardiographic studies. Two dimensional echocardiographic images were recorded and stored for further evaluation. 2D echocardiography was done at right and left parasternal long axis view at fourth / fifth intercostal space ventrally between the sternum and costochondral junction (Thomas *et al,* 1993).

SPSS® 20.0 for Windows was used for statistical analysis of data. Pearson's correlation coefficient was used to obtain correlation of heart rate and body weight with various electrocardiographic and echocardiographic parameters and regression equation derived.

RESULTS

Correlation of Heart Rate with Electrocardiographic Parameters

Negative correlation was observed between heart rate and heart weight in case of Rajapalayam dogs ($P<0.01$) and Chippiparai dogs ($P<0.05$). All the other parameters showed no correlation with regard to heart rate (Table 1).

Correlation of 2D Echocardiographic parameters with Body weight

In the present study, the above 2D Echocardiographic parameters were correlated with body weight of Rajapalayam and Chippiparai dogs. Linear regression analysis was done and regression equation was obtained for these parameters with body weight.

In Rajapalayam dogs, Left ventricular diameter at diastole and systole (LVDd and LVDs) showed high positive correlation with body weight. Left atrial posterior wall thickness in systole (LAPWs) and Right ventricular diameter (RVD) values showed positive correlation with body weight, whereas, Heart rate (HR) and Aortic root diameter (AO) were found to be having high negative correlation in relation with body weight. Statistical analysis revealed no significant correlation between Left ventricular posterior wall thickness at diastole and systole (LVPWd, LVPWs), Interventricualr septal thickness at diastole and systole (IVSd, IVSs), Aortic area (AOA), Left atrial diameter in diastole and systole (LADd, LADs), Left atrial posterior wall thickness in diastole (LAPWd) and Right ventricular posterior wall thickness (RVPW) with body weight (Table 2).

In Chippiparai dogs, HR and IVSs values showed positive correlation with body weight, whereas, RVDd was found to be having high negative correlation in relation with body weight. Statistical analysis revealed no significant correlation between LVDd, LVDs, LVPWd, LVPWs, IVSd, AO, AOA, LADd, LADs, LAPWd, LAPWs and RVPW with body weight (Table 3).

DISCUSSION

Correlation of Heart Rate with Electrocardiographic Parameters

Though, the values of heart rate were within the normal range (upto 220 bpm for puppies and 70-160 bpm for adult dogs) given by Tilley (1992), there was high significant difference observed between young and adult age groups which might be due to increase in body weight.

The difference in body weight could also affect the heart rate (Vailati *et al.,* 2009) as there is large basal metabolic rate in animals of higher surface area (small body weight compared to the animals with small surface area (large body weight). Regarding body weight and heart rate similar findings were reported by Ferasin *et al.* (2010) and Gugjoo *et al.* (2014).

Another possible reason for difference in heart rate could be the effect of adrenergic system on heart rate (Bavegems *et al.* 2009).

Negative correlation was observed between heart rate and heart weight in case of Rajapalayam dogs ($P<0.01$) and Chippiparai dogs ($P<0.05$). All the other parameters (P wave amplitude, P wave duration, R wave amplitude, QRS interval, and PR interval) showed no correlation with regard to heart rate. This is in contrast to the findings of Hanton and Rabemampianina (2006) where positive correlation observed in P wave amplitude and negative correlation in PR interval.

Correlation of 2D Echocardiographic parameters with Body weight

LVDs is determined by the degree of myofibre shortening which, inturn is affected by the ionotropic state of the myocardium and by afterload. LVDd and LVDs were found to be positively correlated with body weight in Rajapalayam dogs as reported in previous studies of O'Leary *et al.* (2003) and Muzzi *et al.* (2006). This might be due to increase in cardiac size in relation

with increased body weight of animals. As cardiac size increases, the left ventricular internal diameter may also increase.

In the present study, no significant correlation was observed between body weight and IVS in both Rajapalayam and Chippiparai dogs, whereas, positive correlation was reported by Gooding *et al.* (1986), Lombard (1984), Sisson and Schaeffer (1991), Bayon *et al.* (1994) and Kayar *et al.* (2006). Page *et al.* (1993) found a weak relationship between body weight and IVS.

Larger AO diameter in younger animals might be due to increased physical activity as reported by Snoeckx *et al.* (1982) and Vanoverschelde *et al.* (1993). Pearson's correlation revealed high negative relation with body weight in case of Rajapalayam dogs and no correlation in Chippiparai dogs. This is in contrast to the findings of Boon *et al.* (1983), Kayar and Uysal (2004) and Kayar *et al.* (2006) who had reported a positive correlation between aortic diameter and body weight.

Correlation of LAD/AO ratio with body weight revealed there was significant relation in Rajapalayam breed. A similar finding was recorded by Vollmar (1999) who reported that a significant increase in Left atrial dimension resulted in increased LAD/AO ratio with advance in age due to reduction in Aortic root dimension. However, the present finding was in contrast with the findings of Kayar *et al.* (2006) and Saxena (2008).

High negative correlation was observed for RVD in relation with body weight in Chippiparai dogs whereas positive correlation reported n Rajapalayam breed.

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Table 1 Correlation of heart rate with various electrocardiographic parameters in rajapalayam and chippiparai

Parameters	Rajapalayam (n=12)	Chippiparai (n=12)
P amplitude (mV)	-0.33 NS	0.25 NS
R amplitude (mV)	0.46 NS	-0.52 NS
P duration (sec)	-0.17 NS	0.16 NS
PR interval (sec)	0.21 NS	0.06 NS
QRS interval	-0.20 NS	0.21 NS
Heart Weight (g)	-0.75**	-0.63*

NS - No significant correlation between HR and ECG parameters (P>0.05)

* - Significant correlation between HR and ECG parameters (P<0.05)

** - Significant correlation between HR and ECG parameters (P<0.01)

Table 2 Correlation of body weight with various 2D echocardiographic parameters in rajapalayam dogs

Parameters	Rajapalayam (n=12)	Regression Equation	R^2
LVDd (cm)	0.94**	37.09 (x) - 83.50	0.89
LVDs (cm)	0.88**	81.91 (x) -180.82	0.80
LVPWd (mm)	0.31 NS	1.82 (x) +11.49	0.09
LVPWs(mm)	0.14 NS	0.87 (x) + 19.86	0.01
IVSd (mm)	0.54 NS	4.63 (x) - 9.14	0.29

IVSs (mm)	0.19 NS	2.64 (x) + 8.20	0.05
LADd (cm)	-0.29 NS	-8.52 (x) +47.17	0.06
LADs(cm)	-0.14 NS	-4.02 (x) + 34.86	0.01
AO (cm)	-0.73**	-27.24 (x) + 89.51	0.51
AOA (cm^2)	-0.36 NS	-5.42 (x) + 47.71	0.12
LAPWd (mm)	0.45 NS	9.28 (x) -16.06	0.19
LAPWs (mm)	0.65*	12.66 (x) -24.37	0.41
RVD (cm)	0.69*	19.47 (x) - 17.48	0.48
RVPW (mm)	0.31 NS	2.58 (x) + 9.33	0.09

NS - No significant correlation between body weight and parameters (P>0.05)

* - Significant correlation between body weight and parameters (P<0.05)

** - Significant correlation between body weight and parameters (P<0.01)

Table 3 Correlation of body weight with various 2D echocardio graphic parameters in chippiparai dogs

Parameters	Chippiparai (n=12)	Regression Equation	R^2
LVDd (cm)	0.03 NS	0.28 (x) + 13.97	0.41
LVDs (cm)	0.02 NS	0.16 (x) + 14.23	0.01
LVPWd (mm)	-0.26 NS	-2.25 (x) +30.99	0.07
LVPWs (mm)	-0.31 NS	-2.95 (x) +35.52	0.10
IVSd (mm)	0.00 NS	-0.12 (x) + 15.34	0.00
IVSs (mm)	0.69*	2.90 (x) - 2.39	0.48
LADd (cm)	0.39 NS	2.94 (x) + 9.71	0.15
LADs (cm)	0.51 NS	4.72 (x) + 8.76	0.27
AO (cm)	0.53 NS	7.68 (x) + 1.21	0.28
AOA (cm^2)	0.51 NS	2.60 (x) +8.12	0.26
LAPWd (mm)	-0.37 NS	-10.23 (x) + 29.23	0.14
LAPWs (mm)	-0.40 NS	-9.76 (x) + 25.46	0.16
RVD (cm)	-0.86**	-7.47 (x) + 29.23	0.73
RVPW (mm)	0.34 NS	2.12 (x) + 3.15	0.11

NS - No significant correlation between body weight and parameters (P>0.05)

* - Significant correlation between body weight and parameters (P<0.05)

** - Significant correlation between body weight and parameters (P<0.01)

Evaluation of stability of reconstituted live attenuated Paste-Des-Petits Ruminants, Sheep Pox and Goat Pox vaccines

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ABSTRACT

In the present study, the thermostability of live attenuated peste-des-petits ruminants (PPR), sheep pox and goat pox vaccines were assessed for their stability at 5±3°C, 25±1°C and 36±1°C in reconstituted form. All the vaccine batches maintained the minimum infectious titre in their reconstituted form (2.5 $\log_{10}$ $CCID_{50}$/dose for PPR and 3 $\log_{10}$ $CCID_{50}$/dose for sheep/goat pox) when stored at 36±1°C for at least 20 hrs. These live attenuated vaccines can be used within 8 hrs of reconstitution for immunization of animals in tropical field situations or during cold chain failures by delivering the required quantity of vaccine dose as they are found to be stable in their reconstituted form at ambient as well as at higher temperatures.

Key words: Peste-des-petits ruminants virus, Sheep pox virus, Goat pox virus, Vaccines, Stabilizers, Reconstitution, Thermostability

The diseases caused by Peste-des-petits ruminants virus (PPRV), Sheep pox virus (SPV) and Goat pox virus (GPV) cause substantial loss to farming community throught the world. Very effective vaccines are available against PPR and Capripox to provide strong and long lasting immunity. It is necessary that each animal vaccinated against PPR should receive a minimum recommended dose of $10^{2.5}$ cell culture infective dose 50 ($CCID_{50}$) whereas a minimum infectious dose of 10^3 $CCID_{50}$ is recommended for Sheep pox virus (SPV) and Goat pox virus (GPV) (Indian Pharmacopoeia 2018). However, one of the key issues in effective implementation of the existing live PPR vaccine is limited thermotolerance that requires the maintainance of a continuous cold chain (Baron *et al*. 2017). In contrast, Capripox viruses are generally considered to be thermoresistant and have differing sensitivity to heat between isolates (Rao and Bandyopadhyay 2000).

In India, with respect to PPR and Capripox viral diseases, the control strategy is mostly aimed at vaccination using the conventional live attenuated vaccines. Most of these live attenuated vaccines lose their potency if not stored under a controlled cold chain which remains a major hurdle

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in mass immunization programs (Kumru *et al.* 2014).Under such situations, studies on thermal stability of PPR and Capripox virus vaccines is important when these vaccines are to be used in tropical climatic conditions and in areas lacking cold chain infrastructure. Therefore, the present study focuses to evaluate the thermostability of PPR, sheep pox and goat pox vaccine viruses in reconstituted form at various temperatures which are usually encountered during storage and transport under field settings.

The vaccine batches of PPRV, SPV and GPV were prepared by propagating the viruses on Vero cell line. The virus was harvested at appropriate time and mixed with the stabilizers. The composition of PPR and SPV vaccine formulation consisted of 5.1 % lactalbumin hydrolysate (LAH, BD), 5.05 % sucrose (Himedia) and 0.63% gelatin hydrolysate (Himedia), whereas, the formulation for GPV vaccine consisted of 5% LAH,10% Sucrose, 1% sodium glutamate and 0.63% gelatin hydrolysate. One millilitre of virus-stabilizer mixture was dispensed in sterile 2 ml capacity glass vials and lyophilized using automated bench top freeze-dryer (Labocon, LFD-BT-102). The vaccine vials were tested for vacuum by spark test and moisture by Karl Fischer volumetric titration. All the vaccine vials used in the study showed to contain the vacuum as tested by vacuum spark test. The residual moisture (RM) levels observed in the vaccine vials ranged from 1.8-2.2%, which was within the acceptable limit of 3%.

For stability studies of reconstituted PPRV, SPV and GPV vaccine, the freeze dried vaccine vials was reconstituted in 100 ml of diluent representing 100 doses of vaccine stored at respective stability temperatures of 5 ± 3°C, 25±1°C and 36±1°C. The sample from each vial was titrated on 0, 4, 8, 12, 16, 20 and 24 hours interval and infectivity titres were calculated using Spearman-Karber method.

The reconstituted PPR vaccine when stored at 5±3°C retained titre of 2.75 $\log_{10}$ $CCID_{50}$/dose at the end of the study period of 24 hrs, whereas, reconstituted SPV and GPV vaccines retained an infectivity titre of 3.5 $\log_{10}$ $CCID_{50}$/dose at the end of 24 hours of storage at 5 ± 3°C (Table). The PPR vaccine lost 0.75 $\log_{10}$ $CCID_{50}$ virus titre at the end of 24 hrs of exposure at 25±1°C, whereas, reconstituted SPV and GPV vaccine maintained infectivity titre of 3.25 $\log_{10}$ $CCID_{50}$ /dose till 24 hrs of incubation at 25°C. Similar results were observed with reconstituted thermo-adapted PPR vaccine virus (Ta PPRV Jhansi/2003) where it maintained the required titre for 48 hrs at 25±1°C (Riyesh *et al*. 2011).

Reconstituted PPR vaccine virus titres rapidly dropped during storage at 36 ±1°C from an initial titre of 3.5 $\log_{10}$ $CCID_{50}$/dose to 2 $\log_{10}$ $CCID_{50}$/dose within the span of 24 hours. However, the SPV and GPV vaccine viruses could able to retain an infectivity titre of 3 $\log_{10}$ $CCID_{50}$/dose at 36±1°C till 24 hrs of observation period (Table). Both SPV and GPV vaccine viruses were found to be marginally superior to PPRV in terms of their thermostable nature.

Storage of reconstituted live attenuated vaccines beyond 6 hours at ambient temperature or higher is usually

not recommended since reconstitution affects both the safety and effectiveness of vaccines (WHO 2000). In the present study, all the reconstituted vaccine batches showed stability beyond 16 hrs at 36 ±1°C. However, we would recommend to store the reconstituted live attenuated PPR, SPV and GPV vaccines upto the point of use at 5±3°C and to vaccinate animals within 8 hours of reconstitution and the left over vaccine should be discarded at the end of each immunization session. Even if the reconstituted vaccines are exposed to higher temperatures for a brief period, the vaccines tend to be effective.

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Table. Stability of reconstituted PPR, sheep pox and goat pox vaccines at various temperatures

Vaccine	Temp (°C)	Virus titre (log_{10} $CCID_{50}$/dose)						
		Time (hrs) 0	4	8	12	16	20	24
PPRV	5 ± 3	3.5	3.25	3.5	3.25	3.5	3.5	2.75
	25 ±1	3.5	3.5	3.5	3.5	3.5	3.25	2.75
	36 ±1	3.5	3.5	3.25	2.75	2.75	3	2
SPV	5 ± 3	3.75	3.75	3.75	3.75	3.75	3.5	3.5
	25 ±1	3.75	3.75	3.5	3.5	3.5	3.25	3.25
	36 ±1	3.75	3.5	3.5	3.25	3.25	3.25	3
GPV	5 ± 3	4	3.75	3.75	3.75	3.75	3.5	3.5
	25 ±1	4	3.5	3.5	3.5	3.5	3.5	3.25
	36 ±1	4	3.5	3.5	3.5	3.5	3.25	3

Short Communication

Incidence of corneal pathologies in dogs - A retrospective prevalence study

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ABSTRACT

The incidence of corneal pathologies was compiled for a period of two years from October 2015 to October 2017 by scrutinizing the hospital record of Ophthalmology unit, Department of Veterinary Surgery and Radiology, Madras Veterinary College Teaching Hospital. The rate of incidence of pathologies of the cornea was documented in terms of age, gender and breed of dogs. Incidences of corneal ulcers,descemetocele, staphyloma, non-inflammatory keratopathies,corneal dystrophies and degenerations, pigmentary keratitis and chronic superficial keratitis were documented. Analysis of prevalence of corneal ulcers in dogs with two previous time frames from the same region were also elicited.

Keywords: Corneal ulcer, dogs, non -inflammatory keratopathy, pigmentary keratitis, corneal pathologies

INTRODUCTION

Corneal affections in dogs are common in clinical practice of canine patients. The range of corneal affections in dogs was documented including the various corneal pathologies and those with anterior corneal afflictions i.e. only including the epithelium and stromal layer. Corneal ulceration, or ulcerative keratitis, is one of the most common extra- ocular disease identified in dogs. A corneal ulcer when present results in a break in the corneal epithelium that exposed the underlying corneal stroma (Slatter and Hakason 1993). Clinical signs are varying degrees of lacrimation, blepharospasm, photophobia, conjunctival hyperemia, corneal oedema, and possibly miosis and aqueous flare and determined by the retention of topically applied fluorescein dye by the corneal stroma(Gelatt,2000). A detailed ophthalmic examination with indirect ophthalmoscope revealed involvement of stroma which was confirmed with the help of fluorescein dye. (Miller, 2001). Glove and Constatinescue (1997) reported that the cornea along with the lens focused light on to the retina to produce a combined refractive capacity of approximately 60 Diopter, out of which cornea accounts for 40 Diopter due to its curvature and high refractive index. Hence from the refraction point of view, corneal pathologies are to be treated with prime

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concern for restoring vision, preserving tectonicity and transparency of the cornea, controlling infection and in some cases, to salvage the globe. This document presents a retrospective study on the incidence of corneal pathologies in dogs at Madras Veterinary College Teaching Hospital, Chennai.

MATERIALS AND METHODS

Medical records and diagnostic ophthalmoscopic tests performed in dogs presented to the small animal ophthalmology unit of Madras Veterinary College, Chennai over a period of 24 months (Oct 2015-Oct 2017) for ocular affections were scrutinised. Incidence of various primary corneal pathologies in dogs with respect to age, breed and gender were documented. Analysis of prevalence of corneal ulcers in dogs with two different time frames from the same region was also done.

RESULTS AND DISCUSSION

A total of 3027(n) dogs were screened in this study. Among them, 1075 dogs (35.51%) suffered from various corneal pathologies. The incidence of various corneal pathologies causing corneal affliction with reference to age, breed, gender, and involvement of one or both the eyes were analysed and presented. A total of 3027 dogs were presented to small animal Ophthalmology Unit with ophthalmological complaints during the aforesaid period out of which 1075 dogs (35.51%) dogs were related to corneal pathologies based upon routine ophthalmic diagnostic methods and the remaining 2852(64.49%) were presented for other ocular pathologies at Madras Veterinary College Hospital. The percentage of dogs with bilateral corneal affections of non-inflammatory keratopathy was 13.34% and so mostly the affections were unilateral.Slit lamp examination with slit beam and indirect ophthalmoscope revealed the depth of the pathology involved in the corneal layers. The number of cases with an intact endothelium and Descemet membrane or pathology extending until epithelium and/or stroma were 1039 i.e. 96.65%. They all had an intact anterior chamber with normal endothelium and Descemet membrane. Thus, most dogs had affections of anterior cornea or afflictions extending till the deep anterior lamellae alone. This suggests that there are adequate appropriate chances of improvement in vision, corneal transparency and tectonicity by anterior lamellar surgeries.

The commonest corneal pathologies documented were corneal ulcers (52.9%), pigmentary keratitis syndrome (18.79%), non-inflammatory keratopathy (17.95%), chronic superficial keratitis (6.33%), corneal dystrophies (1.12%), corneal degenerations (0.28%), keratomalacia (0.28), corneal dermoid (0.09%), corneal cyst (0.28%), corneal perforation(0.74%), staphyloma (1.49%) and descemetocele (1.12%).

The frequently affected breeds of dogs were found to be Pugs (46.14) mostly, followed by Labrador retrievers (12.37%), Non-descript / native dogs (15.07%), Spitz (10.6%) and German Shepherd (3.44%). This higher incidence could be due to brachycephaly with shallow orbit housing a protruded eye ball. In addition, the hairs projecting from the facial fold, medial lower palpebralentropion and relatively

low corneal sensitivity were also other attributing reasons. The increased incidence in pug breed in the present study when compared to a study done during 2002 and 2012 in the same unit could be due to increased popularity or prevalence of the breed. Mostly, young dogs of until 3 years of age (61.11%) were affected with corneal pathologies. This could be due to increased activity and playfulness along with reduced acquired or learned reflexes. Thus, the present study highlights the need for periodical ophthalmic evaluation in this breed especially within three years age group.

Ramani *et al*., (2012) reported the presence of corneal ulcers in 526 dogs (14.4 %). This differed from the findings of Ancheril (2004) who reported must the year wise incidence of corneal ulcer was an average of 8.04%.Wilkie and Whittaker (1997) reported that older dogs appeared predisposed and yet dogs of any age could be affected. Moore (2003), had reported the corneal ulcer incidence was high in middle aged dogs with a mean age of 8.2 year. Ramani et al. (2012) found that in the age wise distribution, the dogs in the age group of three month to three year had the highest incidence of corneal ulcers (63.35%), 3-7 year age group had 21.7%, 7-10 year age group had 5.15% and 10-15 year age group had 9.3 % corneal ulcers. Ramani et al. (2012) documented the breed wise distribution of corneal ulcer highest in dogs and ---- in Pugs 37.26%, followed by Spitz 26.7%, Non-Descript 16.7% Boxer and Labrador both had the incidence rate of 4.94%, Terrier, 1.86% Rottweiler, Great Dane & Pekingese had the incidence rate of 1.24% Dachshund, Bulldog, Beagle and Cocker Spaniel had 0.62% of the incidence rate. Ancheril, (2004) found that incidence of corneal ulcer in spitz 51.85%, Non-Descript 22.22%, Lhasaapso 7.41%, Great Dane, German shepherd, Pug, Bull terrier & terrier as 3.7%. Moore (2003) reported that corneal ulceration was observed in over 45 different breeds of dogs with Boxer being the most common breed with 24.56% incidence, followed by mixed breed 11.03%, but a high number of cases occurred in Poodles, Golden retrievers, Corgie, Labradors, Springer spaniel and German Shepherds.

Wilkie and Whittaker (1997) reported that dogs of any sex could be affected by corneal ulcer. Ramani et al.,(2012) reported the incidence rate to be 60.2% in males and 39.8 % in females during the period 2009-2011, whereas, Moore (2003) had reported that the per cent ratio of corneal ulcers as 54.67% in male dogs and 45.34% in female dogs. Ramani et al. (2012) reported that out of 526 dogs related to corneal pathology due to corneal ulcer 283 (53.8%) were bilateral and the remaining 243 (46.2%) were unilateral.

CONCLUSION

The reports from this study are significant in comparison with those of Ramani et al. (2012) and Ancheril (2004) as all were done from the same place of study, the Ophthalmology unit attached to the Teaching Hospital Complex of Madras Veterinary College in various time periods. Hence, changes in incidence of corneal pathologies, corneal ulcers in dogs and also in relation to various other affections can be compared to note the incidence

with time from the same region. Thus, this is an appropriate prevalence study on the incidence of corneal affections in dogs.

Affections of corneal pathologies were found mostly to be confined to the epithelium and/or stroma (96.65%). Considering the importance of power of cornea in relation to visual acuity, anterior lamellar surgeries present a more pivotal role in improving the transparency of the cornea. Hence, an effective and timely diagnosis and treatment for abating such affections is the need of the hour in canine patients.

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Table1: Incidence of common primary corneal pathological conditions in dogs

S.No	CORNEAL PATHOLOGY/ DIAGNOSIS	NUMBER	PERCENTAGE
1	Ulcerative keratitis or Corneal Ulcers	569	52.90
2	Pigmentary keratitis syndrome	202	18.79
3	Non-Inflammatory Keratopathy	193	17.95
4	Chronic superficial keratitis	68	6.33
5	Corneal dystrophies	12	1.12
6	Corneal lipid degenerations	3	0.28
7	Keratomalacia	3	0.28
8	Corneal Dermoid	1	0.09

9	Corneal Cyst	3	0.28
10	Corneal perforation	8	0.74
11	Staphyloma	16	1.49
12	Descemetocele	12	1.12

Table 2: Breed wise prevalence of corneal pathologies/affections

S.No.	BREEDS AFFECTED COMMMONLY	NUMBER	PERCENTAGE(%)
1	GERMAN SHEPHERD	37	3.44
2	LABRADOR RETRIEVER	133	12.37
3	PUG	496	46.14
4	SPITZ	114	10.60
5	ND	162	15.07
6	OTHERS	133	12.37

Table 3: Age wise prevalence of ocular affections

S.No.	AGE GROUPS AFFECTED	NUMBER	PERCENTAGE(%)
1	0-3years	657	61.11
2	3-8 years	306	28.47
3	>8 years	112	10.42

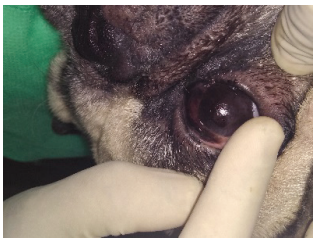
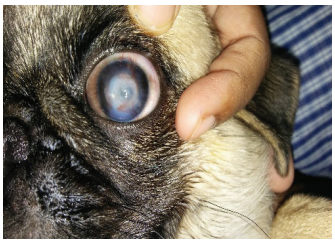
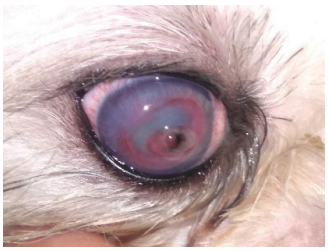
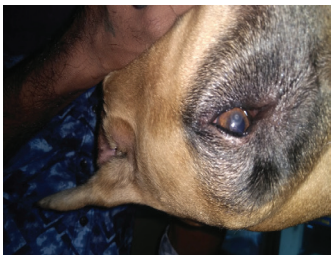
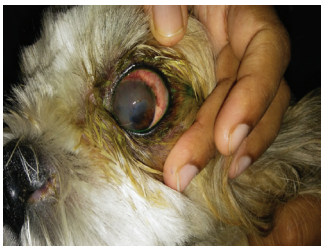
Table 4: Gender wise distribution of ocular affections

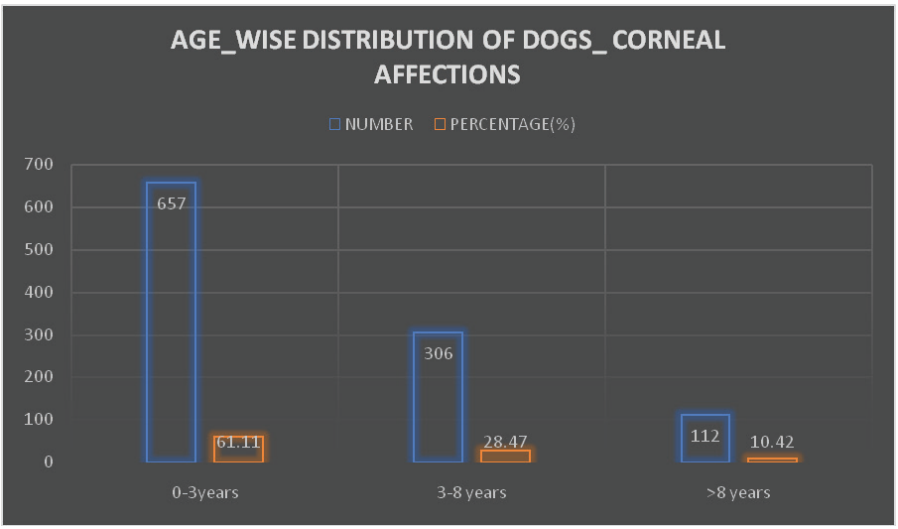
S.No.	GENDER	NUMBER	PERCENTAGE(%)
1	FEMALE	466	43.34
2	MALE	609	56.65

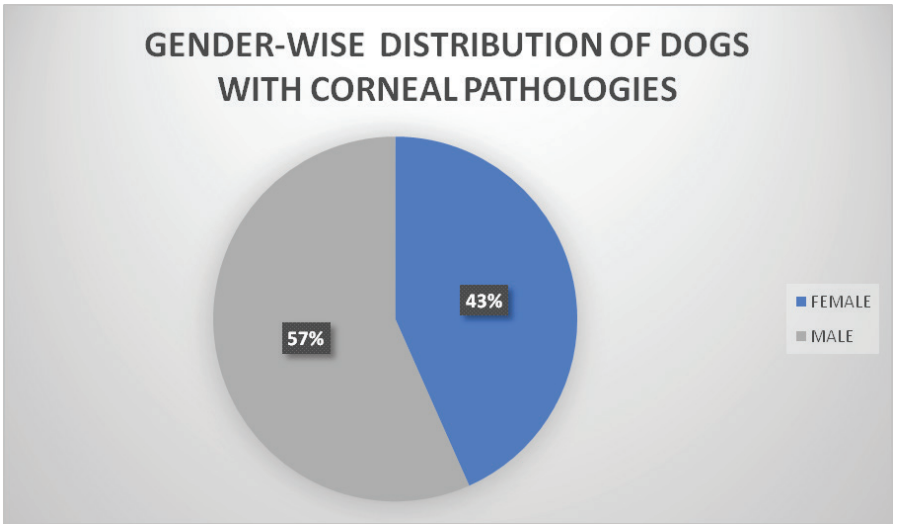
Table 5: Common corneal affections- distribution by number

Common Corneal Affections	BREED						GENDER		AGE (years)		
	GSD	Lab	Pug	Spitz	ND	Others	Female	Male	0-3	3-8	>8
Corneal Ulcer	7	31	266	35	56.	49	247	197	327	89	28
Pigmentary keratitis	1	2	129	6	6	7	90	61	69	69	13
Non-inflammatory keratopathy	17	82	48	55	62	56	182	138	180	97	43

IMAGES

		
OS-Pigmentary Keratitis 5y, male, Pug	OS -Descemetocele 11months, male, Pug	OD-Deep ulcerative keratitis- 3yr, female, Shih Tzu
		
OS-Non inflammatory keratopathy- 2y, female, Great Dane	OD- Corneal degeneration 10yr, female, Golden Retriever	OS-Chronic superficial keratitis- 2y, male, Shih Tzu




GENDER-WISE DISTRIBUTION OF DOGS WITH CORNEAL PATHOLOGIES
43%
57%
FEMALE
MALE

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