

## Assessment of Nutritional Status in Dairy Cows Using Biological Indicators

V.Meenalochani\*, C.Chandrasekaran, M.Murugan, M.Purusothaman,  
P.Vasanthakumar

**Abstract:** *The present investigation was conducted to assess biological indicators reliable for assessing the nutritional status of dairy cows under field conditions. The serum biological indicators for assessment of energy includes glucose, cholesterol and NEFA in Jx in this study varied from 42.73 to 46.78 mg/dl, 142 to 177 mg/dl and 0.56 to 0.61 mmol/l respectively at different physiological stages. In HFx serum biological indicators for assessment of energy includes glucose, cholesterol and NEFA varied from 37.76 to 52.17 mg/dl, 96 to 109 mg/dl and 0.41 to 0.72, mmol/l respectively at different physiological stages and were within the reference range value. As the deficit percent of TDN in both breeds varied from 6.8 to 14.6 per cent, it was not well expressed in serum NEFA level but in HFx during late lactation it was more than 0.7 mmol/l. indicating as critical alarm for the occurrence of metabolic diseases at later stages. The serum biological indicators for assessment of protein status includes total protein, albumin, globulin, BUN and MUN in Jx varied from 8.80 to 9.61 g/dl and 3.15 to 3.48 g/dl and 5.64 to 6.13 g/dl and 15.47 to 16.37 mg/dl and 8.9 to 8.81 mg/dl, respectively and was comparable during different physiological stages. In HFx total protein, albumin, globulin, BUN and MUN varied from 7.88 to 8.89 g/dl and 3.04 to 3.73 g/dl and 4.84 to 5.31 g/dl and 15.26 to 16.66 mg/dl and 8.51 to 9.58, mg/dl respectively at different physiological stages. As the deficit percent of intake CP in both breeds varied from 19 to 34.7 it was well indicated by the concentration of milk urea nitrogen ( 8.51 to 9.58 mg/dl ) as the MUN value below 11 per cent was indicative of protein deficiency. The biological indicator for assessment of mineral status by calcium in Jx varied from 7.26 to 7.56 mg/dl and in HFx 8.48 to 9.44 mg/dl at different physiological stages. Though the deficiency of calcium intake varied from 8.2 to 28.8 percent in Jx and 8.2 to 23.4 in HFx at different physiological stages it was not identified in the serum due to the existence of homeostasis mechanism. The excess phosphorus intake was not identified by serum level though it varied from 4.25 to 5.71 mg/dl and varied from 5.7 to 6.54 mg/dl in HFx at various physiological stages as the excess intake being excreted through faeces and urine. Hence mineral deficit cannot be identified by serum biological indicators.*

**Keywords:** Dairy cows - Nutritional status – biological indicators

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### I. Introduction

Majority of dairy cows are reared under grazing system with limited supplementation of green fodder, dry fodder and concentrate feed. But optimum productive and reproductive efficiency of livestock could be achieved only if the animals fed with the required quantity of feedstuffs and all nutrients in proper proportion(NRC, 2001).The actual intake of the animal for its production capability cannot be ascertained and hence the energy and protein status of the animals under field conditions are very difficult to assess.

In the dairy Industry, the use of metabolic profiles for assess in the nutritional and health status of cows is wide spread (Grunwaldt et al.,2000). The blood metabolites give an immediate indication of an animal's nutritional status at the point of time ( Pambu- Gollah et al.,2000) is becoming popular. (Ndlovu *et al.*, 2007). Serum concentrations of metabolites such as glucose, cholesterol, non esterified fatty acids(NEFA), Total protein, albumin, globulin, blood urea nitrogen (BUN), milk urea nitrogen(MUN), and minerals are commonly used to assess the nutritional status of dairy cows. Thus the present investigation was done to assess the nutritional status of Jersey cross & Holstein Friesian cross dairy cows using blood metabolites under field conditions of Dharmapuri district.

### II. Materials And Methods

The study was conducted in Dharmapuri district with 101 Jx and 112 HFx cows .The data regarding nutritional status of animals were collected from the selected farmers through face to face interview with the help of well structured questionnaire. It includes recording of body measurements, feeding pattern, quantity of feed and fodder offered during 24 hours, milk yield, Besides, this the amount nutrients intake from fodders were calculated by considering average nutritive values of the respective fodders.(Ranjhan 1998). The mean nutrient deficit in terms

of Dry matter(DM), total digestible nutrient(TDN),crude protein(CP) calcium (Ca)and phosphorous (P) intake at various physiological stages for both the breeds was calculated as per NRC (2001) feeding standard were and were furnished in table 1 and 2 .

A total 213 lactating dairy cows of Jx and HFx were used for the analysis of blood metabolites.Ten ml of blood was collected from the jugular vein of each animal and serum was separated for analysis of glucose, cholesterol, and non esterified fatty acids, to assess the energy status and total protein, albumin, globulin, blood urea nitrogen were estimated for assessing the protein status, calcium and phosphorous were measured for assessment of mineral status with commercial kits and as per manufacturers protocol. The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994) and the data was subjected to analysis of variance (ANOVA) and t-test. Further, means were compared using Duncan's multiple range test by using the soft ware package SPSS version 12 (SPSS,1996).

### **III. Results**

The per cent dry matter deficit in Jx was 13.8, 4.7, 16.9 in early, mid, late lactation respectively and significantly higher (+1.6) during late gestation. In HF x dry matter deficit per cent was 10.9, 2.5, 11.0 and 10.3 during early, mid and late lactation and late gestation , respectively. The deficit per cent of TDN in Jx was 14.6,7.6 10.6 and 13.4 in early, mid and late lactation and late gestation, respectively. In HFx the deficit per cent of TDN during early, mid and late lactation and late gestation was 12.3, 6.8, 9.8 and 7.7 in HFx and was significantly higher in early lactation in both breeds. (table 1and 2)

Hence,the study revealed that the percent of deficit was noticed in DMI, TDN, CP and calcium intake and excess was found in phosphorus intake. Among the nutrients intake, deficit of crude protein was significantly high and deficit per cent was felt during early lactation on both breeds comparing with the different physiological stages.

The serum biological indicators for assessment of energy status includes Glucose, Cholesterol,and Non esterified fatty acid. These values for the dairy cows in early, mid and late lactation, late gestation were furnished in table 3 .The mean glucose value for early, mid and late lactation, late gestation (table -3) were within the reference range values. (table-4). But the glucose concentration in Jx was significantly less and was comparable in other physiological stages. But,in HFx it was significantly less during early lactation and significantly high during late gestation, even though the TDN deficit was noticed in all physiological stages.

There was no significant difference in serum cholesterol level between physiological stages, even though the per cent of TDN deficit was noticed in both breeds. But the cholesterol concentration in Jx was less in late lactation and highest in early lactation, whereas in HFx, the concentration was less in late gestation and highest in mid lactation. The serum NEFA concentration was comparable in Jx during various physiological stages and agreed with the reference value in Table 4. In HFx it was significantly higher during late lactation suggesting, restricted feeding practice was adopted by the farmers as the milk yield decreased during late lactation. Further, Ospina *et al.* (2010) stated that NEFA levels more than 0.7 mmol/l was defined as critical alarm as it leads to occurrence of various metabolic diseases in later stages.

Further, the per cent deficit of CP was 34.7, 24.4, 31.2 and 30.6 in Jx and 30.0, 19, 31.4 and 22 in HFx during early, mid and late lactation and late gestation, respectively. The deficit per cent was significantly less during mid lactation in both breeds. The serum total protein value for late gestation, early, mid, and late lactation, were 9.61, 8.80, 9.31 and 8.94 g/dl in Jx and 8.89, 8.31, 8.50 and 7.88 g/dl, respectively and is given in Table 1.The serum total protein, albumin, globulin, blood urea nitrogen, milk urea nitrogen value for late gestation, early, mid, and late lactation, were furnished given in table 3. Theserum taoatl protein values were slightly higher than the reference range values of table 4 ,though the animals consumed diet deficient in protein. In HFx cows, the concentration was significantly less in late lactation and significantly higher in late gestation. The concentration was comparable in Jx and significantly lower in late gestation in HFx.

The serum albumin values in Jx and HFx during late gestation, early, mid and late lactation, were furnished in table 3 and was within the reference range values (1.6-5.5 g/dl) in table 4. Further, it was comparable in the physiological stages in Jx, whereas in HFx significantly it higher in late gestation and was comparable at various lactation stages.The serum globulin values in various physiological stages were furnished in table 1 and agreed with the reference range value. table 4.

The serum BUN value were in table 3 and was within the reference value given (2-34 g/dl) and the variation was comparable in Jx and in HFx there was significant difference between various lactation stages.The MUN values in milk for Jx and HFx, are presented in table 3 and were within the reference value (7.0- 17.6 mg/ dl) as per the Table 4. The serum calcium (6.5 to 12.4 mg/dl) and phosphorus values (2.6 to 8.6 mg/dl). were within the

reference values and presented in the table 3. Further, the deficit per cent of calcium was significantly higher in early lactation in both breeds.

#### **IV. Discussion**

The serum glucose concentration in both breeds agreed with the reports of Herdt (2000) stated blood glucose was under hormonal control, hence the level of glucose was insensitive index of energy intake. The result was in contrary with the findings of Farver (1997) as he stated there was no significant difference in glucose values as noticed between stages of lactation and Sivaraman (1998), stated that glucose level was highest during late lactation and less during mid lactation.

But the cholesterol concentration in this report was in contrary with the values of Rowlands *et al.* (1980) who reported that there exist significant difference in cholesterol concentration between physiological stages. Ospina *et al.* (2010) stated that NEFA levels more than 0.7 mmol/l was defined as critical alarm as it leads to occurrence of various metabolic diseases in later stages. Hence, NEFA acted as a good biological indicator for energy deficit diet in field conditions and corroborated with the report of Radostitis *et al.* (1994) that in marginal energy imbalance, blood glucose and cholesterol were unreliable indices of adequacy of energy, due to its excessive variability, but non esterified fatty acids was a sensitive indicator for assessment of energy status in dairy cows.

The serum total protein was comparable in Jx between physiological stages, and was similar with the report of, Filipejova and Kovacic. (2009) and Mohebbi Fani *et al.* (2005) who observed there was no significant difference between lactation and gestation. Though the CP deficit per cent ranged between 24.4 and 34.7 in the study area it did not influence the serum total protein value. The globulin value varied among themselves it was comparable in both breeds. The results agreed with the findings of Sivaraman. (1998) and Hayashi *et al.* (2005), who stated in globulin level there existed no significant difference in various physiological stages. The BUN value in both breeds was less during late lactation showed the reduction in milk yield during late lactation forced the farmers to adopt restricted feeding. This study was similar with the findings of Sivaraman. (1998) as he stated there existed no significant difference on BUN concentration at various physiological stages.

The MUN value in Jx was comparable between physiological stages, and in HFx it was significantly higher in mid lactation. This result agreed with the findings of Hoff *et al.* (1997) and Hwang *et al.* (2000), reported that MUN value less than 11 mg/dl was suggestive of protein deficiency, as the per cent of protein deficiency was more in the study area, the result obtained is justified. Further, the research report agreed with Wambugu *et al.* (1996) as MUN value of 8.7 mg/dl was noticed in protein deficit diet fed animals and 17.6 mg/dl in the adequately protein fed animals when compared to control group. As the MUN value ranged between 8.51 and 9.58, as agreed with protein deficiency in the study area (19 to 34.7 per cent) and it was concluded that MUN acts as a good biological indicator of protein deficiency.

In Jx, the serum calcium level was comparable at various physiological stages and in HFx it was significantly higher in mid lactation. The reason may be due to the increased milk yield in mid lactation in HFx when compared to other lactation stages prompting increased osteoclastic activity. This report was in agreement with that of Sivaraman. (1998) as he stated that though the concentration of calcium varied among themselves, it was not significant. Further, calcium level was very low in early lactation for both the breeds as most of the farmers were unaware of feeding needed calcium to the cows during lactation. The concentration of phosphorus was significantly high in late gestation and it was comparable between various stages of lactation in Jx. In HFx though the values varied among themselves during various physiological stages, they were non significant.

#### **V. Conclusion**

The biological indicators were not significantly reliable for assessing the nutritional status of dairy cows in field conditions except for NEFA for assessment of energy status and MUN for assessment of protein status. Hence the report agrees with the findings of Maurya and Singh (2015) and Madziga *et al.*, (2013) stating that physiological status and breed have no significant impact on biological indicators and hence the biochemical profiles must be combined with traditional methods for assessment of nutritional status

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**Table -1:** Percentage deficit /excess of nutrient intake of Jx cows at different physiological stages in relation to NRC (2001) feeding standards.

Parameters	Early lactation (n=30)	Mid lactation (n=30)	Late Lactation (n=23)	Late gestation (n=18)
Body weight ( kg)	333 ± 4 <sup>b</sup>	347 ± 11 <sup>b</sup>	337 ± 4 <sup>b</sup>	377 ± 12 <sup>a</sup>
Dry matter (DM)				
Requirement ( kg/day )	9.23 ± 0.06	11.17± 0.27	9.56± 0.10	8.26± 0.28
Intake (kg/day)	7.95 ± 0.11	10.69± 0.34	7.93± 0.17	8.38± 0.31
Shortage( kg/day)	1.28 ± 0.11	0.48± 0.14	1.63± 0.17	0.12± 0.14
Shortage (%)	13.8 ± 1.22 <sup>a</sup>	4.7 ± 1.46 <sup>b</sup>	16.9 ± 1.84 <sup>a</sup>	+1.6 ± 2.81 <sup>c</sup>
Total digestible nutrient (TDN)				
Recommended (g/day )	6127.74± 34.73	6613.35± 184.45	5125.07± 68.97	5068.12± 97.76
Intake (g/day)	5229.45±63.65	6113.35± 202.67	4580.49± 53.88	4387.52±158.43
Shortage (g/day)	898.29 ± 59.51	475± 0.60.72	554.58± 64.84	675.60± 126.21
Shortage (%)	14.6 ± 0.97 <sup>a</sup>	7.6 ± 1.11 <sup>b</sup>	10.6 ± 1.16 <sup>ab</sup>	13.4 ± 2.59 <sup>a</sup>
Crude protein (CP)				
Recommended (g/day)	1210.05± 4.72	1304.06± 41.74	935.99± 15.76	909.49± 13.06
Intake (g/day)	792.72± 11.07	992.36± 54.22	641.99± 10.77	632.39± 29.18
Shortage (g/day)	417.33± 9.7	311.70± 41.08	293.99± 13.54	277.10± 25.70
Shortage (%)	34.7 ± 0.87 <sup>a</sup>	24.4 ± 3.20 <sup>b</sup>	31.2 ± 1.11 <sup>ab</sup>	30.6 ± 2.94 <sup>ab</sup>
Calcium (Ca)				
Recommended ( g/day)	48.75± 0.27	52.82± 1.54	38.6± 0.58	38.76± 0.86
Intake ( g/day)	34.71± 0.75	46.92± 1.86	35.43± 0.70	29.39± 0.55
Shortage ( g/day)	14.03± 0.73	5.9± 1.68	3.17± 0.87	9.37± 0.87
Shortage(%)	28.8 ± 1.50 <sup>a</sup>	11.2 ± 2.86 <sup>b</sup>	8.2 ± 2.24 <sup>b</sup>	24.2 ± 2.35 <sup>a</sup>
Phosphorous (P)				
Recommended (g/day)	21.02± 0.07	24.72± 0.73	17.68± 0.28	15.02± 0.25
Intake (g/day)	27.96± 0.59	32.77± 2.88	21.65± 0.47	19.91± 0.55
Excess	6.91± 0.55	8.05± 2.51	3.97± 0.54	4.89± 0.52
Excess (%)	32.7 ± 2.64 <sup>ab</sup>	32.6 ± 8.41 <sup>b</sup>	22.5 ± 3.26 <sup>a</sup>	32.8 ± 3.67 <sup>a</sup>
Average milk yield (liters)	8.5± 0.03	9.6± 0.4	5.1± 0.4	--

<sup>ab</sup>Means in a row with different literal differ significantly (P≤ 0.05)

**Table-2:** Percentage deficit /excess of nutrient intake of Hfx cows at different physiological stages in relation to NRC (2001) feeding standards

Parameters	Early lactation (n=30)	Mid lactation (n=30)	Late Lactation (n=23)	Late gestation (n=18)
Body weight ( kg)	408 ± 4 <sup>b</sup>	437 ± 12 <sup>b</sup>	406 ± 8 <sup>b</sup>	465 ± 7 <sup>a</sup>
Dry matter (DM)				
Requirement ( kg/day )	10.42± 0.07	13.19± 0.28	11.16± 0.25	9.69± 0.10
Intake (kg/day)	9.28± 0.15	12.85± 0.31	9.92± 0.26	8.69± 0.19
Shortage ( kg/day)	1.14± 0.14	0.34± 0.21	1.24± 0.16	1.00± 0.20
Shortage (%)	10.9 ± 1.42 <sup>a</sup>	2.5 ± 1.27 <sup>b</sup>	11.0 ± 1.44 <sup>a</sup>	10.3 ± 2.09 <sup>a</sup>
Total digestible nutrient (TDN)				
Recommended (g/day )	6872.85± 47.90	8114.78 ± 168.07	6080.95± 180.35	5738.26± 47.90
Intake (g/day)	6027.35± 89.89	7580.37± 188.04	5490.41± 192.12	5294.53± 112.32
Shortage (g/day)	845.50± 84.02	534.41± 64.44	590.53± 59.31	443.73± 108.28
Shortage (%)	12.3 ± 1.20 <sup>a</sup>	6.8 ± 0.87 <sup>b</sup>	9.8 ± 1.06 <sup>ab</sup>	7.7 ± 1.91 <sup>b</sup>
Crude protein (CP)				
Recommended (g/day)	1277.06± 11.05	1621.97± 34.66	1123.18± 40.01	997.34± 6.32
Intake (g/day)	893.43± 16.80	1302.26± 50.13	772.31± 36.57	779.42± 15.74
Shortage (g/day)	383.63± 16.03	319.71± 33.96	350.88± 15.95	217.92± 16.08
Shortage (%)	30.0 ± 1.18 <sup>a</sup>	19 ± 2.20 <sup>b</sup>	31.4 ± 1.53 <sup>a</sup>	22.0 ± 1.60 <sup>b</sup>

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Calcium (Ca)				
Recommended ( g/day)	58.95± 0.77	68.90± 1.5	48.32± 1.42	44.72± 0.44
Intake ( g/day)	45.12± 1.52	53.27± 2.5	43.34± 3.35	41.01± 0.67
Shortage ( g/day)	13.83± 1.54	15.63± 3.02	4.98± 0.31	3.71± 0.34
Shortage (%)	<sup>a</sup> 23.4 ± 2.60	<sup>ab</sup> 21.1 ± 4.31	<sup>bc</sup> 10.2 ± 8.28	<sup>c</sup> 8.2 ± 1.56
Phosphorous (P)				
Recommended (g/day)	24.49± 0.19	31.13± 0.66	21.74± 0.68	16.80± 0.12
Intake (g/day)	38.34± 1.19	32.62± 2.17	28.57± 2.85	26.84± 1.55
Excess	13.85± 1.14	1.49± 2.03	6.83± 2.29	10.04± 0.67
Excess (%)	<sup>a</sup> 56.8 ± 4.56	<sup>c</sup> 4.7 ± 7.74	<sup>b</sup> 31.3 ± 10.28	<sup>a</sup> 59.8 ± 5.32
Average milk yield (liters)	10.12± 0.12	12.11± 0.4	6.9± 0.4	--

<sup>ab</sup>Means in a row with different literal differ significantly (P≤ 0.05)

**Table-3:** Blood biochemical parameter of dairy cows at various physiological status .

Parameters	Physiological stages			
	Early Lactation	Mid Lactation	Late lactation	Late gestation
Jersey crossbred				
Glucose (mg/dl)	46.75 <sup>a</sup> ± 1.16	46.78 <sup>a</sup> ± 1.05	42.73 <sup>b</sup> ± 1.36	46.68 <sup>a</sup> ± 0.86
Cholesterol (mg/dl)	189.00 ± 15.00	175.00 ± 13.00	142.00 ± 13.00	177.00 ± 21.00
NEFA (mmol/lit)	0.61 ± 0.05	0.56 ± 0.05	0.57 ± 0.06	0.57 ± 0.08
Total protein (g/dl)	8.80 ± 0.19	9.31 ± 0.45	8.94 ± 0.19	9.61 ± 0.18
Albumin (g/dl)	3.15 ± 0.09	3.23 ± 0.13	3.26 ± 0.12	3.48 ± 0.09
Globulin (g/dl)	5.64 ± 0.22	6.40 ± 0.52	5.77 ± 0.22	6.13 ± 0.23
BUN (mg/dl)	15.56 ± 0.46	15.53 ± 0.55	15.47 ± 0.49	16.37 ± 1.07
MUN (mg/dl)	8.81 ± 0.07	8.69 ± 0.06	8.75 ± 0.04	--
Calcium (mg/dl)	7.29 ± 0.30	7.35 ± 0.24	7.26 ± 0.20	7.56 ± 0.25
Phosphorus (mg/dl)	4.61 <sup>b</sup> ± 0.15	4.73 <sup>b</sup> ± 0.18	4.25 <sup>b</sup> ± 0.20	5.71 <sup>a</sup> ± 0.26
Holstein Friesian crossbred				
Glucose (mg/dl)	37.76 <sup>d</sup> ± 0.22	47.76 <sup>c</sup> ± 0.34	50.06 <sup>b</sup> ± 1.41	52.17 <sup>a</sup> ± 0.19
Cholesterol (mg/dl)	108.00 ± 6.42	109.00 ± 7.11	104.00 ± 7.00	96.00 ± 4.69
NEFA (mmol/lit)	0.60 <sup>a</sup> ± 0.04	0.41 <sup>b</sup> ± 0.02	0.72 <sup>a</sup> ± 0.11	0.61 <sup>a</sup> ± 0.06
Total protein (g/dl)	8.31 <sup>bc</sup> ± 0.10	8.50 <sup>ab</sup> ± 0.12	7.88 <sup>c</sup> ± 0.19	8.89 <sup>a</sup> ± 0.17
Albumin (g/dl)	3.17 <sup>b</sup> ± 0.07	3.20 <sup>b</sup> ± 0.08	3.04 <sup>b</sup> ± 0.07	3.73 <sup>a</sup> ± 0.18
Globulin (g/dl)	5.14 ± 0.11	5.31 ± 0.14	4.84 ± 0.20	5.15 ± 0.25
BUN (mg/dl)	16.66 <sup>b</sup> ± 0.19	18.13 <sup>a</sup> ± 0.28	15.26 <sup>c</sup> ± 0.68	15.38 <sup>c</sup> ± 0.40
MUN (mg/dl)	8.92 <sup>bc</sup> ± 0.17	9.58 <sup>a</sup> ± 0.10	8.51 <sup>c</sup> ± 0.10	--
Calcium (mg/dl)	8.48 <sup>b</sup> ± 0.07	9.44 <sup>a</sup> ± 0.06	8.71 <sup>b</sup> ± 0.20	8.49 <sup>b</sup> ± 0.18
Phosphorus (mg/dl)	6.54 ± 0.27	6.06 ± 0.28	5.74 ± 0.28	6.09 ± 0.24

**Table-4:** Reference value of biological indicators for energy/protein status

S.no	Glucose (mg/dl)	Cholesterol (mg/dl)	NEFA (mmol/l)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	BUN (mg/dl)	MUN (mg/dl)	Ca (mg/dl)	P (mg/dl)	Reference
<b>Text books</b>											
1	35-55	39-177	--	5.7-8.1	2.1-3.6	3.6-4.5	6-27	--	9.7-12.4	5.6-6.5	Radostitis <i>et al.</i> (1994)
2	45-75	55-88	0.2	6.74-7.46	3.03-3.55	7.5-8.8	20-30	--	--	--	Jerry Kaneko <i>et al.</i> (1997)
3	40-80	75-150	--	--	--	--	8.0-20	--	8.0-10.5	4.0-7.0	William O.Reece (2005)
4	42-75	62-193	--	5.5-7.5	2.6-4.0	2.1-3.7	8.8-26	--	8.7-11.8	2.9-6.2	Merck Veterinary Manual (2011)
<b>Research publications</b>											
5	40-95	53-417	0.17-0.69	--	1.6-5.5	--	2.0-30.7	--	6.5-12.3	2.6-8.6	Kida (2002)
6	55-57	263-271	0.20-0.32	6.8-7	3.3-3.4	3.5-4.2	4.9-7.1	--	--	--	Hayashi <i>et al.</i> (2005)
7	51-77	43-331	0.04-0.34	--	3.2-4.1	--	8-27	--	8.2-10	5.2-7.9	Zimpro performance panel (2011)
8	--	--	--	--	--	--	15.1-15.6	--	--	--	Baker <i>et al.</i> (1995)
9	--	--	--	--	--	--	15-16	--	--	--	Ferguson and Chalupa (1989)
10	--	--	--	--	--	--	11-17	--	--	--	Hwang <i>et al.</i> (2000)
11	--	--	--	--	--	--	8.7-17.6	--	--	--	Wambugu <i>et al.</i> (1996)
12	--	--	--	--	--	--	--	--	6.86-7.91	3.42-4.43	Alacam <i>et al.</i> (2008)
13	--	--	--	--	--	--	--	--	8.7-11	4.5-8.0	Anderson and Rings (2009)
<b>Indian work</b>											
14	55-30	160-20	--	7.05	3.03	4.04	11.85	--	9.01	4.34	Arunaman (2002)
15	49.5-59.0	97.7-126.4	--	6.22-6.98	3.14-3.89	3.31-3.52	--	--	9.11-10.27	4.22-5.02	Ramakrishna (2003)
16	--	--	--	--	--	22-34	--	--	--	--	Lazarus and Thiagarajan (1980)
17	--	--	--	--	--	--	7-14	--	--	--	Somer (1991)
18	--	--	--	--	--	--	11-16	--	--	--	Wadhaawa <i>et al.</i> (2005)
19	--	--	--	--	--	--	4.67-9.45	--	--	--	Bakshi and Wadhwa (2011)