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Nutrient Digestibility and Growth Performance of Mehsana Buffalo Calves Fed Probiotics

Devchand A. Sadrasaniya, A.P. Raval, Ashok P. Patel, N. Emmanuel, Sanjay Joshi, Bharat B. Rajgor, Vijay Chaudhary, Suresh Patel, Ingle Pandurang, S.R.Bhagwat

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(Received on 14.04.2013; Accepted on 25.04.2013)

Abstract

Fourteen female Mehsana buffalo calves were divided into two groups of seven animals in each, viz., T_1 group fed basal diet and T_2 group fed basal diet supplemented with probiotics (containing Saccharomyces cerevisiae; 1.5 x 10^8 cfu/g and bacteria, Lactobacillus sporogens; 5 x 10^7 cfu/g) at the level of 5 g/h/d. The DM intake (kg/d) was significantly higher in probiotics supplemented group. The digestibility of DM, crude protein and NFE were significantly higher in T_1 group compared to T_2 group. The average body weight gain was significantly higher in T_1 group than T_2 group and feed conversion efficiency was lower in T_1 group than T_2 group. The cost of feeding per kg body weight gain was lower in probiotics supplemented group than the control group. Results revealed that supplementation of probiotics significantly influenced digestibility of DM, crude protein and NFE and growth performance of Mehsana buffalo calves.

Keywords: Probiotics; Growth performance; Digestibility; Buffalo calves.

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Introduction

The main objectives of application of probiotics in the rearing of young animals are improved survival, inhibition of diarrhoea, superior growth and better feed conversion efficiency [1]. Dietary use of probiotics is thus preferred to that of antibiotics to enhance nutrient utilization, improve feed efficiency and maintain health status because of their non-harmful effect on consumers [2]. The term "Probiotic" has been defined as a "live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance[3]. The present study, therefore, was undertaken to assess the effects of probiotics supplementation on nutrient digestibility and growth performance of Mehsana buffalo calves.

Materials and Methods

Fourteen growing female Mehsana buffalo calves of 8 to 10 month of age were selected for the experiment to observe the effects of probiotics supplementation. They were divided into two equal groups of seven animals in each group, viz., T_1 group fed basal diet (consisting of concentrate mixture and green fodder at 50: 50 ratio) to meet the nutrient requirement as per Kearl's feeding standard (1982) and T_2 group fed basal diet supplemented with probiotics (containing *Saccharomyces cerevisiae*; 1.5 x 10^8 cfu/g and bacteria, *Lactobacillus sporogens*; 5 x 10^7 cfu/g) at the level of 5 g/h/d for period of 90 days. Feed & dry matter intake recorded weekly and body weight changes measured fortnightly. At the end of experimental feeding a digestion trial of seven days duration was conducted to assess nutrient utilization.

Results and Discussion

The average DM intake (kg/d) was 3.28±0.03 and 3.56±0.13 in T_1 and T_2 groups, respectively, revealing significantly ($P < 0.05$) higher DM intake in probiotics supplementation group than the control group. However, DM intake (kg/100 kg BW) was without any significant variation between the groups (2.26±0.10 vs. 2.47±0.10). The digestibility of DM (67.72±1.82 vs. 62.25±0.65 %), crude protein (68.23±1.35 vs. 63.23±1.26 %) and NFE (75.90±1.58 vs.

Table 1: Effect of supplementation of probiotics on apparent digestibility of nutrients and growth performance of growing buffalo calves

Parameters	Dietary groups		P value
	T_1	T_2	
Digestibility (%)			
Dry matter	62.25^a±0.65	67.72^b±1.18	*
Organic matter	66.50±0.82	68.33±1.15	NS
Crude protein	63.23^a±1.26	68.23^b±1.35	*
Ether extract	66.01±1.62	69.19±2.45	NS
Crude fibre	55.45±3.79	57.69±5.77	NS
Nitrogen free extract	70.36^a±0.78	75.90^b±1.58	*
Growth performance (90 d)			
Intial BW (kg)	146.4±5.63	145.7±5.79	NS
Final BW (kg)	189.9±5.83	194.8±6.16	NS
Net BW gain (kg)	43.42^a±0.68	49.1^b±0.44	*
ADG (g/d)	482.4^a±4.17	545.1^b±4.93	*
Total DMI (kg)	294.9^a±2.49	320.4^b±2.39	*
FCR	6.80^b±0.08	6.53^a±0.07	*

^{ab} Means with different superscript in a row differ significantly (*$P < 0.05$; NS = non-significant)
T_1 : Control group T_2 : Supplemented probiotics @ 5 g/h/d
BW: body weight; ADG: average daily gain; DMI: dry matter intake; FCR: feed conversion ratio

70.36±0.78 %) were significantly ($P < 0.05$) higher in probiotics supplemented group as compared to that of control group (Table 1). The digestibilities of other nutrients were not affected by supplementation of probiotics. Findings of present study corroborate with authors.[4]. The initial and final body weights were 146.4 and 189.8 kg in T_1 and 145.7 and 194.8 kg in T_2 groups, respectively. The average daily weight gain was significantly ($P < 0.05$) higher in T_1 group (545.1 g/d) compared to that of T_2 group (482.4 g/d). The feed conversion efficiency was better in T_2 group (6.53) as compared to T_1 group (6.80). The cost of feeding per kg body weight gain was lower in probiotics supplemented group (Rs. 43.9 vs. 48.1) than the control group.

Conclusion

It was concluded that supplementation of probiotics (containing *Saccharomyces cerevisiae*; 1.5×10^8 cfu/g and bacteria, *Lactobacillus sporogens*; 5×10^7 cfu/g) at the level of 5 g/h/d in growing female Mehsana buffalo calves improved intake of DM, digestibility of DM, crude protein and NFE, growth performance and feed conversion efficiency.

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Milkability of Lactating Kankrej Cows in Different Months

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Abstract

An experiment was conducted on 20 lactating Kankrej cows divided in four groups according to lactation number one to four and initial stage of lactation with almost same production. Highly significant difference was observed for let down time, milking time, and milk yield and milk flow rate. Interaction of months with number of lactations was also found significantly different in all the traits except milk yield and milk flow rate.

Keywords: Milkability; Lactation; Cattle.

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Introduction

Milk plays a major role in economic significance in cattle and buffaloes. India has emerged as leading milk producing country in the world[1]. Milk harvesting is an art and science as well as it is the most important aspects on a dairy farm management [2]. Full co-operation of the milch animal is required for harvesting clean and maximum milk.

Materials and Methods

The experiment was done on twenty lactating Kankrej cows. Animals were divided into four groups according to number of lacatation one to four (L_1 to L_4). The research works was carried out at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, and Sardarkrushinagar. All animals reared under semi-loose housing system and two times (Morning & Evening) milking was done with full hand milking method in RCC milking parlour. All the animals allotted routine feeding and management practices followed at Livestock Research Station. The experiment was conducted for six months (August-2003 to January-2004).

Let down time and milking time were recorded with use of stop watch in seconds while, milk yield was recorded by electronic weighing balance in Kilogram. Milk flow rate (Kg/minute) was calculated by dividing total milk yield by total milking time per cow at each milking. The data so obtained were analysed using standard statistical methods[3]

Results and Discussion

Let down time

Month-wise let down time are presented in table: 1. The average let down time was observed 64.83 ± 3.4 seconds with a range from 51.75 to 86.30 seconds. It was lower than previously reported (73.19 Sec.) in same breed[4]. The difference due to months was highly significant. The let down time showed linear decreasing tendency as the parity advances. This is due to more acquaintance of cows with milking barn routine as the parity advances.

Milking time

Month-wise milking time is presented in table: 1. The average milking time was observed 252.87 ± 17.81 seconds with a range from 222.74 to 268.87 seconds. The difference due to months was highly significant. The maximum time was recorded in the month of December, while minimum time was recorded in August. The difference due to months was highly significant. The milking time recorded was lower than Gir (390 Sec.), Red Sindhi (390 Sec.) and Crossbred cows (270 Sec.).[5]. Normally milking time is proportional to milk yield. The Kankrej is a dual purpose breed; it produces less milk than other milch breeds (Gir and Red Sindhi etc.)

Table 1: Milking attributes recorded during different months in Kankrej cows

Month	Milking attributes			
	Let down time (Seconds)	Milking time (Seconds)	Milk yield /milking (Kilogram)	Milk flow rate (Kg/Milking)
August	62.82	222.74	3.580	0.970
September	71.72	260.44	3.870	0.860
October	69.85	253.99	4.130	1.000
November	63.31	259.51	4.390	1.040
December	54.51	268.87	4.370	0.980
January	66.78	251.67	4.150	0.980
Average	64.83 ± 3.4	252.87 ± 17.81	4.080 ± 0.47	0.970 ± 0.07
SEM	1.124	6.427	0.168	0.027
C.D.	3.393 **	17.815 **	0.465 **	0.075 **

** P < 0.01

Milk yield per milking

The overall average milk yield per time was recorded 4.080 ± 0.470 Kg (Table:1). The difference due to months was highly significant. It was lower than Sahiwal (7.2 Kg), Holstein Friesian (7.5 Kg) and Jersey (6.0 Kg)[6,7,8]. This might be due to the lower yield in Kankrej as dual purpose breed, while earlier observations were taken for milch breeds.

Milk flow rate

The overall average milk flow was recorded 0.97 ± 0.07 Kg/minute (Table: 1). The difference due to months was significant. It was higher than reported earlier (0.89 Kg/Min.) in same breed.[4] While, it was less than Tharparkar (1.6 Kg/Min.) and Sahiwal (1.6 Kg/min.) .[9] Milk flow rate was obviously less due to more milk yield in aforesaid breed than Kankrej cows.

Conclusion

Milking attributes of lactating Kankrej cows were recorded during different months. The difference due to months in all parameters were found highly significant.

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Influence of Supplementation of Bypass Fat on Nutrients Intake and Milk Yield and its Composition in Crossbred Lactating Cows

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Abstract

An on-farm trial of 7 days preliminary feeding and 90 days experimental period was conducted in village Mumanvas, Vadgam Taluka of Banaskantha district of Gujarat during September to November 2012. Twelve lactating crossbred cows of uniform body weight, milk yield and with 2nd and/or 3rd lactation number in initial stage of lactation were selected for experiment to study the effect of bypass fat supplementation. Six healthy animals in each group allotted to two dietary treatments. Completely randomized design was followed for making treatment groups i.e. T_1: control (concentrate mixture + green fodder + dry fodder) and T_2: treatment (T_1+bypass fat@10 g/kg milk yield). The nutrient requirement of animals meets out as per the ICAR (1998) guideline. Milk yield of morning and evening was recorded daily and sampled every fort night interval. At the end of experiment, digestion trial of 7 days was under taken. The average daily DMI (kg/h), CPI (g/h), DCP intake (g/h) and TDNI (kg/h) in T_1 and T_2 groups were 13.80±0.20 and 14.07±0.43; 1786.70±32.48 and 1871.35±76.35; 1222.32±48.95 and 1329.80±77.60; 8.39±0.15 and 9.33±0.32, respectively. The DMI, CPI and DCPI were found statistically non significant between treatment groups. While as, TDNI was found to be significantly ($P<0.05$) higher in bypass fat group. The cumulative DMI, CPI, DCPI and TDNI (kg/90d) in T_1 and T_2 were 1242.32 ±18.28 and 1265.94±39.06; 160.80±2.92 and 168.42±6.87; 110.01±4.40 and 119.68±6.98; 755.38±13.33 and 839.78±28.67, respectively. The TDNI was differ statistically ($P<0.05$) between treatment groups while statistically non significant difference between treatment groups in terms of cumulative DMI, CPI, DCPI.

The average daily production of whole milk, 4% FCM, fat and SNF in experimental crossbred cows in groups T_1 and T_2 were, 15.67±0.43 and 17.78±0.81, 15.30±0.74 and 18.55±0.90; 0.60±0.04 and 0.77±0.04 and 1.31±0.03 and 1.53±0.06 kg/h/d, respectively and all values were statistically ($P<0.05$) significant and higher in bypass fat fed group than control group. The cumulative yield (kg/h/90d) for whole milk, 4% FCM, fat and SNF were, 1410.11±38.96 and 1600.01±72.88; 1376.85±66.31 and 1669.32±80.60; 53.57±3.81 and 69.05±3.66 and 118.32±2.56 and 138.02±5.72 in T_1 and T_2 groups, respectively. The cumulative yield was significantly ($P<0.005$) higher in bypass fat fed group as compared to control.The percent of milk fat, total solid and SNF of experimental cows in groups T_1 and T_2 were, 3.82±0.11 and 4.32±0.12; 12.24±0.22 and 12.94±0.13 and 8.40±0.06 and 8.64±0.09, respectively. The fat percentage, total solid percentage and SNF percentage were statistically ($P<0.05$) significant and higher in the group which was fed bypass fat supplementation.

Keywords: Bypass fat; SNF; DCP; TDN; Nutrients; Crop residues; Palability; Digestibility

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Introduction

Livestock are an integral part of agriculture in India and likely to be the instrument of the economic growth and development of the country. The problem is not only about the feed availability, but also about feed quality and feed cost. The major feed sources available in India are crop residues including straws and stovers which are very inferior quality in terms of palatability, digestibility and supply of nutrients. To overcome the problem of feed shortage and to optimize essential nutrients supply to ruminants, we have to evolve newer feeds and have to device alternative technologies to increase nutrient supply through better utilization of feed resources. This can be achieved by modifying the feeds and feeding systems and by manipulating the digestive tract, especially rumen through active as well as passive manipulation. Supplementation of fat in the ration can increase its energy density. The levels of unprotected fat cannot be incorporated in the ration beyond 4%. High levels of unsaturated fat in diet are harmful to rumen microbes and causes inhibition of rumen fermentation, alter crude fibre digestibility, microbial protein synthesis and volatile fatty acid production. This can be successfully overcome by feeding protected fats to ruminants. Bypass fat technology involves feed management through passive rumen manipulation. Feeding of protected or inert fat or bypass fat is a means of rendering fats insoluble in the rumen from ruminal hydrolysis and bio-hydrogenation and make available in small GI tract for absorption resulting reduce negative energy balance and beneficial for various body function like production and reproduction. Bypass fat remains inert in the rumen and a good source of fatty acids for meeting energy needs of animals and fatty acids requirement for milk synthesis.

Diet	Group	Group	No
Home-made concentrate mixture + green fodder + dry fodder	Control	T_1	6
Home-made concentrate mixture + green fodder + dry fodder +Bypass fat @10g/kg of milk yield.	Bypass fat	T_2	6

In the light of above facts, the present experiment was conducted to study the effect of supplementation of bypass fat in the ration of lactating crossbred cows to study the influences of supplementing bypass fat during early lactation on nutrients intake and on milk yield and milk composition.

Materials and Methods

The present experiment was conducted on lactating crossbred cows at Mumanvas village of Vadgam Taluka of Banaskantha district, Gujarat between 16th September and 24thDecember 2012 for 90 days to know the effect of feeding commercial bypass fat (*Megalac*) to crossbred cows during early lactation. Twelve crossbred cows in early lactation (15-30d post-partum) were selected on basis of their 12-13 kg daily milk yield of last lactation. Initially both the groups of animals should had more or less similar average body weight, average milk yield and average fat yield to avoid biasness in experiment. The experimental animals were randomly allotted to two dietary treatments i.e. T_1: (control) and T_2: (treatment). Six animals in each follow

Table 1: Average DM intake of experimental cows during digestion trial

Animal No.↓	kg/d		kg/100 kg BW		kg/kg $W^{0.75}$	
Treatment→	T_1	T_2	T_1	T_2	T_1	T_2
1	13.89	13.98	3.66	3.68	3.17	3.15
2	12.86	12.98	3.06	3.25	2.90	2.84
3	14.20	15.27	3.16	3.40	3.32	3.08
4	13.92	14.04	3.76	3.70	3.18	3.17
5	14.22	15.29	3.00	4.02	3.46	3.05
6	13.73	12.84	3.62	3.21	2.87	3.11
Average	13.80±0.20	14.07±0.43	3.37±0.14	3.54±0.13	3.15±0.09	3.07±0.05

Table 2: Average daily crude protein intake of experimental cows during digestion trial

Animal. No.	g/day		g/100kg B.wt		g/kg $W^{0.75}$	
Treatment	T_1	T_2	T_1	T_2	T_1	T_2
1	1803.80	1868.60	474.68	491.74	408.56	423.24
2	1629.20	1668.90	407.30	397.36	359.90	373.17
3	1842.90	2083.40	409.53	462.98	400.11	452.33
4	1807.40	1864.60	475.63	503.95	412.09	422.33
5	1840.10	2082.10	484.24	438.34	394.17	471.60
6	1796.80	1660.50	449.20	436.97	406.98	371.29
Average	1786.70±32.48	1871.35±76.35	450.10±14.02	455.22±16.06	396.97±7.86	418.99±16.62

Completely Randomized Design.

The home-made concentrate mixture prepared daily by the farmer using cotton seed cake, compound concentrate mixture and maize bhardo for thirty lactating crossbred cows and equally distributed to experimental animals irrespective of milk yield. The ingredient composition of home-made concentrate mixture had been observed throughout the experiment. The wheat straw was fed *ad libitum.* The compound concentrate mixture was procured from Banaskantha District Co-operative Milk Producer's Union Limited and commercial bypass fat supplement (*Megalac*) from Optimaxnutricare limited, Rajkot.

All the experimental animals were individually offered a basal diet of green maize and wheat straw along with required quantity of concentrate mixture to reach out their protein and energy needs for maintenance and for milk production.[1] The bypass fat supplement *Megalac* was provided to cows of T_2 group @ 10gm/kg milk yield/animal as per recommendation of manufacturer.

The crossbred females of both groups were individually offered the daily allowance of home-made mixture at irrespective of their milk production during time of milking (both morning and evening) up to entire experimental period of 90 days. The green fodder was offered at 8.00 a.m. and 5.00 p.m; while the dry forage was offered two times a day after feeding green fodder. The leftover from all cows were weighed daily. The samples of concentrate, wheat straw and green were collected fortnightly for subsequent analysis of DM and DMI during experimental period. The feeding schedule was adjusted every fortnight according the milk yield of that animal for compensating nutrient requirement for maintenance and milk production. The clean, fresh and wholesome drinking water was made available to all the experimental animals *ad libitum* in water troughs fitted in front of them.

All the animals were housed in well ventilated *pukka shed.* The roof and floor was of cement concrete. All the animals had sufficient space for easy movement and were reared under stanchion system. All the experimental crossbred cows were hand milked twice daily (5.00 a.m. and 16.00 p.m.) and yields were recorded. The milk samples were drawn at fortnightly intervals from individual animals during both times of milking. After thorough mixing, a sample of 100-150 ml was taken for further analysis of fat, total solid and SNF content. The samples were stored at 4^0C with precaution of bottle, closely tight with rubber stopper. The fat content of milk samples was

Table 3: Average daily digestible crude protein intake of experimental cows

Animal. No. ↓	g/day		g/100kg B.wt		g/kg $W^{0.75}$	
Treatment →	T_1	T_2	T_1	T_2	T_1	T_2
1	1225.60	1343.00	322.53	353.42	277.60	304.19
2	987.90	1127.10	235.21	281.78	218.23	252.02
3	1328.80	1503.80	295.29	334.18	288.50	326.49
4	1274.00	1385.60	344.32	364.63	290.47	313.84
5	1247.90	1539.00	262.72	405.00	267.31	348.58
6	1269.70	1080.30	334.13	270.08	287.59	241.56
Average	1222.32±48.95	1329.80±77.60	299.03±17.55	334.85±20.95	271.62±11.25	297.78±17.28

Table 4: Average daily total digestible nutrient intake of experimental cows

Animal No. ↓	kg/day		kg/100kg BW		kg/kg $W^{0.75}$	
Treatment →	T_1	T_2	T_1	T_2	T_1	T_2
1	8.75	9.28	2.30	2.44	1.98	2.10
2	7.84	8.08	1.87	2.02	1.73	1.81
3	8.68	9.13	1.93	2.03	1.88	1.98
4	8.62	9.53	2.33	2.51	1.97	2.16
5	8.39	9.45	1.77	2.49	1.80	2.14
6	8.09	10.51	2.13	2.63	1.83	2.35
Average	8.39^{a}±0.15	9.33^{b}±0.32	2.05±0.10	2.35±0.11	1.87^{a}±0.04	2.09^{b}±0.07

a ,b means with different super scripts in rows differ statistically

estimated by digital electronic milk testing machine "Milkotester" (REMI made). Total solids i.e. dry matter content of milk sample was estimated by gravimetric or evaporation method. A known quantity of milk was driven off its water and the residue which was left out was expressed as percent of total solids. About 5 g of milk sample was transferred to a pre weighed clean, dry empty silica dish and the weight of dish with sample was taken. The dish was placed in a boiling water bath for 30 minutes. The dish was removed; bottom was wiped and kept in hot air oven at 98-100°C for about 3 hrs. Then the dish was transferred to a desiccator to cool for about 30 minutes and the weight was taken accurately. The difference in the weight between the residue plus dish and empty dish was expressed as per cent of total solids.

Solid- Not- Fat (SNF)

% SNF= total solids-percent fat content

Fat-corrected milk yield

For the conversion of whole milk into 4% FCM, the equation derived by Gaines and Davidson (1923) as given below was used:

4% FCM (kg) = (0.4× M +15× F)

Where, M=milk yield in kg,F= weight of fat contained in it.

The digestion trial of 7-days collection period was conducted at the end of the feeding trial during which quantity of feed offered, leftover of the ration and total faeces voided by the animals were recorded on 24 hrs basis (Plate 3.9). The samples of the feeds and faeces were collected and preserved for proximate analysis. Based on data of feed ingested and their nutritive value; the intake of DCP and TDN by individual animal were worked out. DCP of a feed stuff express the amount of crude protein which gets digested after ingestion of 100 gms of that specific feed stuff.

DCP = (CP% of the feed × digestibility of CP)/100

Likewise DCF, DNFE, DEE were calculated and total digestibility of nutrient was calculated as:

TDN% = % DCP +%DCF +%DNFE +(%EE× 2.25)

Daily representative samples of concentrate mixture, green fodder (maize) fed to the animals and faeces were collected during digestibility trial and the pooled samples were analyzed for proximate principles. [2]

Table 5: Cumulative intakes of DM, CP, DCP and TDN (kg/h) of experimental cows

Ani. No. ↓	DM		CP		DCP		TDN	
Treat. →	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	1250.05	1257.92	162.34	168.17	110.30	120.87	787.20	835.37
2	1157.74	1168.10	146.63	150.20	88.91	101.44	705.28	727.23
3	1277.65	1374.46	165.86	187.51	119.59	135.34	780.88	821.87
4	1252.49	1263.80	162.66	167.82	114.66	124.71	775.67	857.84
5	1280.03	1376.01	165.61	187.39	112.31	138.51	755.67	850.78
6	1235.97	1155.35	161.71	149.45	114.27	97.22	728.04	945.59
Average	1242.32± 18.28	1265.94± 39.06	160.80± 2.92	168.42± 6.87	110.01± 4.40	119.68± 6.98	755.38^{a}± 13.33	839.78^{b}± 28.67

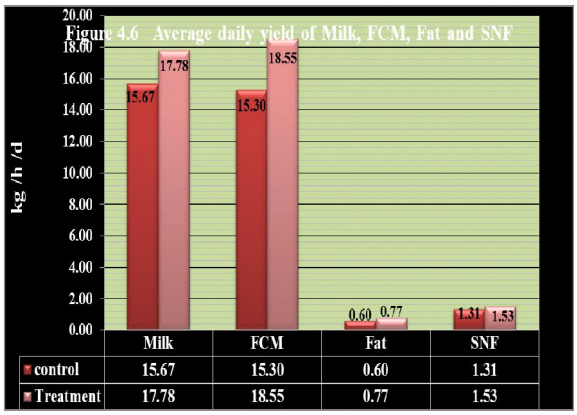
a, b means with different super scripts in rows differ statistically

Table 6: Average daily whole milk, 4% FCM, fat and SNF (kg/h/d) yield of experimental cows

Animal No.↓	Milk production		FCM		Fat		SNF	
Treat.→	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	16.25	20.48	18.26	21.56	0.79	1.35	1.35	1.76
2	15.03	15.80	15.26	17.54	0.58	1.28	1.28	1.40
3	14.98	19.43	14.33	20.59	0.56	1.28	1.28	1.70
4	15.73	16.10	14.52	15.78	0.55	1.30	1.30	1.43
5	14.56	16.57	13.09	17.10	0.48	1.24	1.24	1.42
6	17.45	17.90	16.33	18.71	0.62	1.44	1.44	1.49
Average	15.67^a ±0.43	17.78^b± 0.81	15.30^a ±0.74^a	18.55^b ±0.90	0.60^a ±0.04	1.31^b± 0.03	1.31^a± 0.03	1.53^b ±0.06

a, b means with different superscripts in rows differ statistically

Figure Average Daily Yield of Milk, FCM, Fat and SNF



The data generated during the experiment was analyzed, using methods given in Snedecor and Cochran (1994).[3]

Results and Discussion

The average daily DMI in terms of kg/h/d, kg/100kg BW and kg/$W^{0.75}$ were 13.80±0.20 and 14.07±0.43;3.38±0.14 and 3.54±0.1 and 3.15±0.09 and 3.07±0.05 in T1 and T_2 groups, respectively and the treatment differences were statistically (P<0.05) non significant. Similar results were also reported by Schneider *et al* (1988), Garg and Mehta (1998), Tyagi and Thakur (2007), Shankhpal *et al* (2009a), Shelke and Thakur (2011), Mudgal *et al* (2012) and Ranjan *et al* (2012).[4-10] However, Moallen *et al* (2007)[11] observed increase in DMI due to supplementation of bypass fat. The average daily CPI in terms of g/h/d; g/100kg BW and g/$W^{0.75}$ were 1786.70±32.48 and 1871.35±76.35; 450.10±14.02 and 455.22±16.06 and 91.46±2.92 and 89.13±1.84 in T_1 and T_2 groups, respectively. Thus, supplementation of bypass fat in ration of experimental cows indicating non significant difference between the groups in terms of crude protein intake.

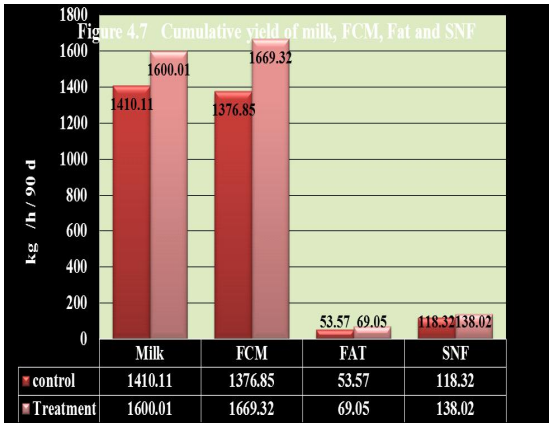
The average DCP intake in terms of g/h/d; g/100 kg BW and g/$W^{0.75}$ of experimental cows in T_1 and T_2 groups during digestion trial were 1222.32±48.95 and 1329.80±77.60; 299.03±17.35 and 334.85±20.95 and 271.62±11.25 and 297.78±17.28, respectively; which was statistically non significant between the treatment groups. Thus, supplementation of

Table 7: Cumulative yield of whole milk, 4% FCM, fat and SNF (kg/h) of experimental cows

Animal No.↓	Milk production		FCM		Fat		SNF	
Treatment	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	1462.50	1842.75	1643.79	1940.72	70.74	80.28	121.39	158.03
2	1352.97	1422.00	1373.63	1578.42	51.84	67.32	115.10	126.17
3	1348.47	1784.25	1289.79	1853.41	49.95	75.96	115.29	153.36
4	1415.97	1449.00	1306.72	1420.44	49.32	55.71	117.20	128.71
5	1310.22	1491.03	1177.83	1539.17	43.56	62.91	111.63	127.96
6	1570.50	1611.00	1469.33	1683.75	55.98	72.09	129.33	133.81
Average	1410.11 ^{a}± 38.96	1600.01^b ±72.88	1376.85^a± 66.31	1669.32^b ±80.60	53.57^a ±3.81	69.05^b ±3.66	118.32^a± 2.56	138.01^b ±5.72

a, b means with different super scripts in rows differ statistically

Figure Cumulative yield of whole milk, 4% FCM, fat and SNF (kg/h) of experimental cows



bypass fat in ration of experimental animals did not affect digestible crude protein intake. The average daily TDNI in terms of kg/h/d; kg/100 kg BW and kg/$W^{0.75}$ were 8.39±0.15 and 9.33±0.32;2.05±0.10 and 2.35±0.32 and 1.87±0.04 and 2.09±0.07 in T_1 and T_2 groups, respectively. The TDNI in terms of kg/h/d and kg/$w^{0.75}$ were statistically (P<0.05) differed. Whereas, in terms of kg/100kg BW the treatment different statistically (P>0.05) similar. Shelke and Thakur (2011) found significant effect on TDN intake with group fed rumen protected fat at 2.5% of DMI (2.03 kg/d) than in control group (1.75 kg/d). However, Shankhpal *et al* (2009a) reported non-significant difference for average TDNI (7.91, 8.56 and 9.11 kg/h).[8] Thus, supplementation of bypass fat in ration of experimental cows significantly increased TDNI.

The cumulative intake of DM, CP, DCP and TDN were (1242.32±18.28 and 1265.94±39.06; 160.80±2.92 and 168.42±6.87; 110.01±4.40 and 119.68±6.98; 755.38±13.33 and 839.78±28.67 kg/head/90d) in T_1 and T_2 groups, respectively; suggest differ statistically (P<0.05) in terms of cumulative TDNI while as statistically non significant difference between the treatment groups for cumulative DMI, CPI and DCPI. Thus, supplementation of bypass fat in ration of experimental cows increased the cumulative TDN intake while has no adverse effect on cumulative DMI, CPI and DCPI.

DMI percent of requirement for T_1 and T_2 was 112.49±4.61 and 118.06±4.28, respectively. The experimental crossbred cows received 12.49 and 18.06 percent more DM than requirement.

The DCP percent of requirement in T_1 and T_2 groups was 129.42±5.62 and 119.75±7.72, respectively. The experimental crossbred cows received 29.42 and 19.75 percent more DCP than requirement. The TDN intake percent of requirement in T_1 and T_2 was 105.76±2.72 and 105.37±5.35, respectively which was 5.76 and 5.37 percent more than the requirement.

The average daily production of whole milk, 4% FCM, fat and SNF in experimental crossbred cows in groups T_1 and T_2 were,15.67±0.43 and 17.78±0.81, 15.30±0.74 and 18.55±0.90; 0.60±0.04 and 0.77±0.04 and 1.31±0.03 and 1.53±0.06 kg/h/d, respectively and all values were statistically (P<0.05) significant between the treatment groups. Thus, the values of whole milk production, 4% FCM, fat and SNF yield were higher in bypass fat fed group than control group.

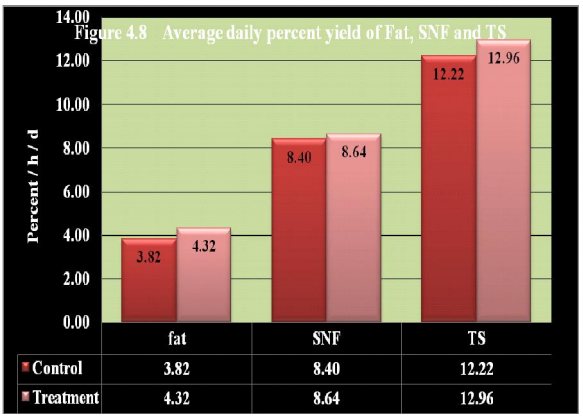
Andrew *et al* (1991), Garg and Mehta (1998), Garg *et al* (2002), Sahu *et al* (2010), Bhanderi *et al* (2011) and Shelke *et al* (2012) also observed increase in whole milk yield in dairy animals fed bypass fat.[12,13,4,14-16] Similarly, Schneider *et al* (1988), Sklan *et al* (1992), Wu *et*

Table 8: Average percent of milk fat, SNF and Total solid of experimental cows

Animal No.↓	Fat %		SNF%		TS%	
Treat. →	T_1	T_2	T_1	T_2	T_1	T_2
1	4.28	4.35	8.30	8.58	12.58	12.93
2	3.82	4.73	8.51	8.87	12.33	13.60
3	3.63	4.27	8.55	8.60	12.18	12.87
4	3.55	3.87	8.28	8.88	11.83	12.75
5	3.69	4.22	8.52	8.58	12.21	12.80
6	3.93	4.48	8.24	8.31	12.17	12.79
Average	3.82^a±0.11	4.32^b±0.12	8.40^a±0.06	8.64^b±0.09	12.22^a±0.10	12.96^b±0.13

a, b means with different superscripts in rows differ significantly

Figure Average percent of milk fat, SNF and Total solid of experimental cows



al (1994), Mishra *et al* (2004), Tyagi and Thakur (2007), Shelke and Thakur (2011), Parnerkar *et al* (2011) and Desai (2012) reported a significant increase in whole milk and FCM yield in dairy animals fed bypass fat which is in agreement of present findings.[17-19,7,9,20,10,21] Parnerkar *et al* (2010), Sahoo *et al* (2010) and Ranjan *et al* (2012) found a significant increase in FCM yield in dairy animals fed bypass fat.[22,6,15] On contrary, Saxena *et al* (2009), Ranjan *et al* (2012) and Wadhwa *et al* (2012) reported no difference in milk yield when buffaloes were fed bypass fat.[23,6,24]

The cumulative yield (kg/h/90d) for whole milk, 4% FCM, fat and SNF were, 1410.11±38.96 and 1600.01±72.88; 1376.85±66.31 and 1669.32±80.60; 53.57±3.81 and 69.05±3.66 and118.32±2.56 and 138.02±5.72in T_1 and T_2 groups, respectively. The cumulative yield was significantly (P<0.005) higher in bypass fat fed group as compared to control.

The average percent of milk fat, Total solid and SNF of experimental cows in groups T_1 and T_2 were, 3.82±0.11 and 4.32±0.12;12.24±0.22 and 12.94±0.13 and 8.40±0.06 and 8.64±0.09, respectively. The milk fat percent, Total solid percent and SNF percent were found to be statistically (P<0.05) significant between the treatment groups and was higher in T_2thanT_1 group. Thus, supplementation of bypass fat in ration of experimental animal increased fat, Total solid and SNF percent of milk.

Similar results were also reported by Schneider *et al* (1988), Sklan *et al* (1992), Mishra *et al* (2004), Garg *et al* (2008), Shankhpal *et al* (2009a), Shelke and Thakur (2010), Parnerkar *et al* (2010), Bhanderi *et al* (2011) and Shelke *et al* (2012).[13,25,18,22,7-9,16] However, Kent and Arambel (1988), Andrew *et al* (1991), Wu *et al* (1994), Harrison *et al* (1995), Moallem *et al* (2007), Sirohi *et al* (2007), Tyagi and Thakur (2007), Lounglawan *et al* (2008), Purushothaman *et al* (2008) and Wadhwa *et al* (2012) observed similar milk fat percent in bypass fat and control group.[12,26-28,11,29,30,10,24,21]

Conclusion

The average daily DMI of crossbred cows did not differ in control and treatment groups. The CPI and DCPI were found to be similar in both treatment groups. The TDNI was statistically (P<0.05) significant and found higher in bypass fat fed group. Supplementation of bypass fat increased the digestibility coefficient of Ether extract, but digestibility coefficient of DM, OM, CP, CF and NFE were statistically (P<0.05) similar. The feed conversion efficiency in terms of DM, CP,DCP and TDN to whole milk and 4% FCM was statistically (P<0.05) significant and higher in bypass fat supplemented group as compare to control group. Provision of bypass fat to cows resulted in statistically (P<0.05) significant and higher in daily yield of whole milk, fat, SNF and 4% FCM as compare to control group. The daily percentage of milk fat, total solid and SNF were statistically (P<0.05) significant and higher in bypass fat fed group than control group.

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Poultry Welfare Issues: An Overview

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Abstract

Keeping animals well-nourished and free from discomfort, pain and stress are essential to sustain a good welfare state. Though it is must to increase production for fulfillment of our need but it's our prime duty to avoid suffering of animals as part of humanity. Poultry welfare improves health and maximizing efficiency which resulted in meat and poultry products that are affordable for all sector of society so poultry welfare issues be included in future international trade talks. Managemental strategies based on good current scientific information and proactive attitude are the key to success.

Keywords: Discomfort; Stress; Suffering; Humanity; Welfare.

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Introduction

Now day's human population is increasing like anything irrespective of natural resources such as land and water. Human population keeps on growing but land and water remains constant which results into scarcity of food. To meet the need of hunger human increased food of animal origin by manifolds. Poultry meat is the most preferred and cheapest source of protein. The world's poultry population is 648.83 billion and India ranks third in egg production and fifth in poultry meat production. Likewise in livestock sector also we are successful in increasing animal production for our selfishness. But, if we put humans above animals and treat animals badly then that is wrong. People who disregard animals and abuse them are generally capable of abusing humans too, but our father of Nation Mahatma Gandhi said that, "The greatness of a nation and it's moral progress can be judged by the way it's animals are treated."

Animal welfare is a controversial topic in modern animal agriculture , due to discrepancy of opinions regarding how animals should be maintained and treated. So animal welfare is important as every living thing on this earth is of equal value.

History and Background

As per the Indian tradition and culture, animals always had a respect and a special place in society. Each Hindu God or Goddess is seen with an animal, so they were seen as religious symbols of something great and treated with compassion as part of god's creation. The doctrine of Karma, which is important to Jains, Buddhists and Hindus, teaches that any improper behaviour will have to be paid for in future life ,so cruel acts to animals should be avoided.

- King Ashoka promoted the kindness among all living life on this planet during 274-232 BC.
- Martin was founder of first animal welfare organization "Society for the Prevention of Cruelty to Animals" in 1824.
- United Kingdom- first country, to implement law to protect animals (Cruelty to Animals Act, 1876)
- The issue of farm animal welfare under modern production methods was first brought to the attention of the general public with the publication of a book by Ruth Harrison in 1964 called "Animal Machines."[1]
- An improved understanding of motivation, cognition and the complexity of social behaviour in animals has led in the last 30 years to the rapid development of animal welfare science.

Meaning of welfare

The welfare of any animal, including human beings, is understood to be a combination of multiple dimensions including physical and mental health, experienced or perceived well-being, and the ability to satisfy drives or needs. [2]

According to oxford English dictionary welfare means 'well being; happiness & thriving or successful progress in life'

FREEDOM	INFLUCING FACTORS
1. Freedom from hunger and thirst	By ready access to fresh water and a diet to maintain full health and vigour
2. Freedom from discomfort	By providing an appropriate environment including shelter and a comfortable resting area
3. Freedom from pain, injury or disease	By prevention or rapid diagnosis and treatment
4. Freedom to express normal behaviour	By providing sufficient space, proper facilities and company of the animals own kind
5. Freedom from fear and distress	By ensuring conditions and treatment that avoid mental suffering

In 2008 the OIE adopted a definition on animal welfare: "Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/ killing.

According to Saunders comprehensive dictionary animal welfare means-The avoidance of abuse & exploitation of animals by humans by maintaining appropriate standards of accommodation, feeding & general care, the unnecessary discomfort & pain (Saunders comprehensive vet. Dictionary, 2007).

These definitions clearly show that an animal can experience both good and poor welfare and that there are important factors that influence its welfare. These factors are often summarized by Farm Animal Welfare Council as the Five Freedoms, (1993) which are given below together with the factors that influence the animal.

Need Of welfare

Animal welfare plays a vital role in the economic status of a country. For many centuries, western culture largely excluded animals from the realm of morality. Physical, physiological, and psychological similarities between humans and many nonhuman animals make it logical to conclude that these nonhumans can experience pain, suffering, and enjoyment[3,4]. Indian government though given general guidelines of prevention of cruelty of animals, specific guidelines for welfare measures is not available for the poultry industry. Globalization, international trade, and dramatic increases in the demand for animal protein for decades to come may magnify these concerns from both practical and ethical perspectives and thus create a need for objective debates [5]. Many developing countries rely on animals for their livelihoods so improving their welfare is crucial. It is an integral part of an ideal sustainable system cause an efficient production system can not be based on keeping unhealthy, underfed animals.

Natural behaviour of chickens

i. Wing flapping:
ii. Dust bathing: Keeps chickens feathers and skin in healthy condition.
iii. Exploring: By exploration birds acquire information about the surrounding environment.
iv. Exercising: Normal movements causes stresses and strains to bone and muscle that keep the skeletal system healthy.
v. Scratching and foraging: They have natural urge to foraging and scratching.
vi. Nesting: They have strong maternal instinct so highly motivated to gain access to a nest site when they are about to lay an egg.
vii. Roosting and perching: Important to avoid injury from more aggressive flock mates.

Welfare issues of layers

The commercial laying hen produces more than 300 or more eggs a year; in contrast, their ancestral parent species, the jungle fowl, lays 4 to 6 eggs per year (Natural Encounters Inc., 2006). Unnaturally high level of productivity is metabolically taxing, often causing hens to suffer from "production diseases," including osteoporosis and accompanying bone fractures, and can lead to reproductive disorders. Research suggests that the problem of osteoporosis may be worsening, possibly due to industry's continuous push toward maximizing productivity.

The welfare issues of layers are:

1) Behavioral restriction
2) Housing
3) Mutilations

4) Forced molting
5) Disposal of spent laying hens

Behavioural restriction

In cages hens are so intensively confined that they have no opportunity to exercise so, lack of exercise in cages leads to bone fragility and impaired bone strength and also detrimental to the psychological well being. Dust bathing deprivation leads to stress because it is pleasurable activity of birds. Caged hens prior to oviposition are restless, show stereotypic spacing and escape behaviour. The lack of appropriate foraging substrate may lead to redirected pecking and to the development of abnormal feather pecking.

Housing

Types of housing:

- Free range
- Semi-intensive
- Intensive
  - i) Deep litter
  - ii) Cage system
    - a. Reverse cages
    - b. Battery cages
    - c. Flat deck cages
    - d. Furnished cages

Free-range birds with shelters are able to express additional behaviors such as freedom of movement, running, flying, and the scratching of soil, and have the opportunity to be exposed to a wide variety of environmental stimuli. [6]

The traditional cage is the most common housing system because of the advantages of a more disease-free bird (e.g., prevention of coccidiosis;[6,7] , less bird aggression and cannibalism and stress with smaller group sizes of 6 hens or less, less ammonia and dust providing an improved environment for birds and workers [8,9], , ease of bird inspection[10] , cleaner eggs and economics [11] compared with alternative housing systems such as deep littered housing.

The major welfare shortfalls of traditional cages is that hens are not able to express normal behaviors such as nesting, dust bathing, foraging, perching, scratching needed to prevent broken claws[12] , wing flapping, stretching, body shaking, walking, running, and freedom of movement and escape leading to inappropriate behaviors such as repetitive stereotypic (e.g., pacing before oviposition) and vacuum activities (e.g., hens going through the motions of dust bathing.[13]

Managemental practices to improve welfare of layers in housing

Opportunities to improve welfare include the elimination of practices such as backfilling cages with different aged birds to maintain a full house. Bird welfare is compromised when backfilling is done every month to replace mortality. Research has shown that mixing birds of different ages or from other flocks increases susceptibility to disease, it should be avoided. Selection for less cannibalism[13] and feather pecking osteoporosis.[14] Benefits of environmental enrichment include reduced aggressive behavior and improved livability, feather condition, and egg production.[15] Fearfulness is reduced by use of objects such as rattles, balls, colorful plastic bottles, strings or drawings on the wall .[16] Installation of a solid side partition consisting of sheet metal or plastic rather than a wire partition reduces feather damage due to wear and pecking between cages by 15 to 20%.[17]

Cages modified with enrichments of a perch, nest, claw abrasives, and dust bath offer opportunity for expression of bird behavior, hens show improved bone mineral density. [18] Redesign of furnished cages with egg collection closer to the darkest area of the nest box allows eggs to roll shorter distances thus reducing the incidence of broken shells. Behavioral studies indicate that 25 ppm of ammonia is aversive to laying hens. [19] To improve bird welfare and avoid keratoconjunctivitis and respiratory disease;[20], atmospheric ammonia should be kept below 20 ppm in poultry houses .[21] Use of genetic marker technologies should advance

the selection of birds with stronger skeletons and lesser cannibalistic and aggressive tendencies. [22]

Mutilations

The Animal Welfare Act 2006 defines a mutilation as "a procedure which involves interference with the sensitive tissues or bone structure of the animal, otherwise than for the purposes of medical treatment."

The practice of beak trimming in the poultry industry occurs for over 60 yr. to prevent excessive body pecking, cannibalism, and to avoid feed wastage;[23] & this routine industry practice is usually undertaken at 1 d of age. It typically involves the removal of the upper, lower or both, mandible. After beak trimming, several anatomical, physiological, and biochemical changes occur in cut peripheral nerves and damaged tissues .[23,24]. The effect of beak trimming on bird well-being depends on multiple factors, including the amount of beak that is trimmed and the quality of the procedure.[25]. There are welfare concerns regarding beak trimming, and some countries including Norway, Sweden, Finland, and Iceland have banned its use. The commonly used methods for beak trimming are hot blade & infrared energy treatment and third i.e. Laser technique .

Welfare concerns regarding beak trimming

Loss of normal function (feeding, behavior, water intake & preening) due to reduced ability to sense materials with beak.

Significant decreases in activities such as feeding, drinking, environmental pecking, preening and head shaking.[26]. When beak trimming is performed at an early age, food intake and BW are significantly reduced during the first several weeks after treatment.*[27]* If the pin of the nipple drinker requires some force to displace it for getting a few drops of water, chick with sensitized beaks may avoid the nipple drinkers.

Short –term pain & debilitation

An injury discharge has been recorded from the intra-mandible nerve but the discharge is short lasting.[28] Another indicator of the short term stress was a significant increase in heart rate of debeaked hens compared with controls. [29]

Neuromas & scar tissue

A neuroma is a proliferative mass of Schwann cells and neurites (nerve processes) that may develop at the proximal end of a severe nerve. Scar tissue was also identified adjacent to the mass of regenerating nerve fibers.[30]

Long term pain

The presence of neuromas and spontaneous discharges near the tip of trimmed beaks were presented as evidence of long-term pain.

Management to improve welfare during beak trimming

In response to welfare concerns, several management techniques, such as reducing light intensity or modifying housing environments, have been used to prevent feather pecking and aggression. However, these methods have limited success and provide no guarantee of controlling feather pecking. [31] It has been known for some time that beak trimming at a young age (i.e., d 1) is preferable because it is less stressful, has better production outcomes, and results in the formation of fewer neuromas in the beak.[23]

One substitute is an infrared beak treatment (IR-BT), which is purported to have a less negative effect on well-being than HB-BT in broiler breeder chicks.[32]

One of the perceived advantages of this method of trimming relative to HB-BT is the elimination of open wounds and potential bleeding sites that may lead to inflammation,

infection, and pain. No occurrence of open wounds was recorded for birds in the IR-BT treatment.[31]

Forced molting

Definition: Molting is a natural biological process of all birds to renew their feathers, but molt induction is a contentious practice because of the methodology used. Normally wild chickens molt once a year and it requires about 4 months for a hen to drop her feathers & grow a new set. The artificial program takes 6-8 wks. Methods to induce forced molting.

1) Water withdrawal
2) Feed withdrawal
3) Light reduction

Feed deprivation is most common method used in India & other countries except European Union which has already banned this practice Molting by feed withdrawal has received a great criticism from animal welfare activists.

Welfare implications of forced molting

Hunger is an extremely powerful motivation & chickens have evolved to forage & consume food throughout the day. Food deprivation results in a classical physiological stress response. Frustration of feeding leads to signs of extreme distress such as increased aggression formation of stereotyped pacing & stereotypic pecking. Forced molting is stressful leading to increased mortality during the first 2 wk of molt. [10] **D**ecreased skeletal integrity. Increase in salmonella organisms in the environment of molted compared with non molted flocks.

Managemental practices to improve welfare

Development of effective alternative methods of molting that never demand starvation is essential to replace the existing method. Central Avian Research Institute in collaboration with department of biotechnology has been researching on this issue.

Non feed withdrawal molting regimens

In a field trial with Ad.lib feeding of diet, high in corn gluten wheat middlings, corn or combination of 71% wheat middlings & 23% corn, showed 40% lower mortality when molting induced by wheat middlings alone. Diet with increased zinc acts on the appetite centers located in the brain which causes the birds to reduce their feed intake which in turn precipitates the molt. 25 kg Zinc oxide containing 73% zinc to a ton (2000 lb) of feed that contains 3.5% Ca and drugs viz. Methalibure, Enheptin, Progesterone, Chlormodione, and Iodine resulted in increased resistance to salmonella. livability & improved skeletal integrity. This suggests that non feed withdrawal methods of molting may be more welfare friendly than the more conventional feed withdrawal molting regimens.

Disposal of spent laying hens

The 74 wks old hen either sent for slaughter as spent laying hens or force molted and kept for a second laying year.

Welfare concerns

Fragile skeleton: 20% of hens from battery cages have freshly broken bones just before stunning. [33]

Poorly designed cages: Small doors of cages results in hens getting limbs caught as they are being removed. 25% caged hens suffered broken bones during removal from cages. [34]

Low value: Only a few processing plants are prepared to accept spent hens, so long journeys require to reach processing plants.

Management to improve welfare

Cages should be designed so that the whole front opens up, lead to lower risk of damage. [35] Use portable CO_2 gas stunning and killing cabinet .[36] Development of light body weight hybrid strains.

Welfare issues of broiler

1) Behavioral restriction
2) Feed restriction in broiler
3) Stocking density and group size
4) Catching ,transport & slaughter

1) Behavioural restriction

Feed restriction in broiler

To prevent health and fertility problems associated with excessive weight gain, broiler breeders are severely feed restricted during rearing, which may affect welfare**.** Under commercial conditions broiler breeders are feed restricted during rearing to limit growth rate, and they may receive one small meal a day or be fed once every 2 days .[37,38]

Welfare implications of feed restriction

Frustration and stress Ascites: It is the condition in which rapidly growing broiler chickens do not have the heart and lung capacity needed to distribute oxygen throughout the body .[39] Symptoms include accumulation of fluid in the abdominal cavity, an enlarged flaccid heart, shrunken liver, lameness, heart failure and mortality

Stocking density and group size

Deterioration of footpad and hock condition and increased stress are important welfare concerns in high-density broiler production. Their tibias were also longer and less symmetric in length.[40]

Lameness is considered to be one of the more serious welfare problems facing the broiler industry. It is known to be related to a range of issues including tibial dyschondroplasia [41], general leg weakness[42], and severe footpad dermatitis. It has also been connected to an overall lack of activity.[43]Aside from the welfare implications, footpad dermatitis can also have adverse effects on production because damaged feet cannot be sold and affected broilers may take longer to gain weight. The detrimental effect of high density was especially pronounced at 18 birds / m2 .[40] Crowding cause poor walking ability . [44]thigh sores and scabs, and scratches on the back from birds walking over one another .[45] hock and foot-pad dermatitis, lesions on the back of the legs and feet, respectively, which may be superficial or progress into deep ulcers may also develop indirectly by deteriorating litter quality. Air quality continues to deteriorate at even higher stocking densities, and, when overcrowded, broiler chickens may experience more bruising and heightened fearfulness, Rest is important for young, growing animals, and crowding also increases the frequency with which birds disturb and walk over each other, interrupting resting patterns.

Catching, transport & slaughter

There are severe welfare problems associated with pre-handling, handling and transport of broiler chickens as assessed by many research workers. Transit birds are exposed to a wide variety of potential stressors such as the withdrawal of food and water, fractures, bruises, pain, stocking density, social disruption, transport micro-environment, motion, acceleration, vibration, noise and restriction of behavior. When birds raised for meat reach market weight, they must be caught and crated for transport from production facilities to slaughter plants. Chickens are typically carried inverted by a single leg, three or four birds per hand before they are put into transport crates. Fasting before and during transportation is a major stressor. Manual catching, as well as handling and loading for transportation, have been identified by researchers as "major sources of stress and trauma to the birds.". [46]

Issues related to welfare during slaughter

- *Uncrating:* Moreover, hanging upside-down is a physiologically abnormal posture for chickens. Handling, inversion, and shackling are traumatic and stressful, as reported in multiple studies that measured physiological indicators of stress. Because of this, approximately 90%

of birds flap their wings vigorously which may lead to dislocated joints and broken bones.

- *Pre-stun shocks:* Some birds inadvertently experience painful electric shocks prior to being stunned in the electrified water bath. This can happen when a bird's leading wing makes contact with the water before the head or if wing-flapping occurs at the entrance to the stunner.
- ***Ineffective stunning***

Management to improve welfare

Automated catching machines -14% decrease in bruising among birds caught by machine significantly lower incidences of leg and wing fractures and dislocations: Leg, wing, and rump injuries were 50%, 22%, and 27% lower, respectively, and the percentage of birds with one or more injuries was 30% lower than those caught manually. [47]

Newer systems, including Controlled Atmosphere Stunning (CAS) and Controlled Atmosphere Killing (CAK) methods employing naturally occurring gases, are increasingly seen as better alternatives for improved animal welfare, worker conditions, and carcass quality.

Animal welfare organizations & acts

During the nineteenth century animal welfare organizations came into being. The first such organization in the world, the Society for the Prevention of Cruelty to Animals (SPCA) was formed in 1824 by Arthur Broome in England and became the Royal SPCA (RSPCA) in 1840 as a result of the patronage of Queen Victoria.

The organizations working for animal welfare are:

- People For Animals (PFA) – India's largest organization
- International Fund For Animal Welfare (IFAW) One of largest animal welfare & conservation charities in the world.
- World Society For The Protection Of Animals (WSPA)
- People for the Ethical Treatment of Animals (PETA)
- Farm Animal Reform Movement (FARM)
- In Defense of Animals (IDA)- 1996
- National Institute of Animal Welfare (NIAW) in Ballabhgarh, Haryana
- Pet Animal Welfare Society (PAWS)-1998
- Society for animal protective legislation (SAPL)
- World Organization For Animal Health (OIE): In 2004, the OIE integrated animal welfare as part of its Terrestrial Animal Health Code and has published the OIE Guiding Principles on Animal Welfare (OIE, 2004).
- Animal Welfare Society: The animal welfare society was conceptualized in 2004. It is recognized by the Animal Welfare Board of India.
- Animal Welfare Association: The animal welfare association is an organization, which is a home to most of the orphaned animals. Every year, almost 9,500 animals receive care from animal welfare association.
- Animal Welfare Organizations: It is spread all across India, and they are doing a commendable job as far as animal welfare is concerned.
- Animal Welfare Board of India: It was established by Central Government. The Animal Welfare Board of India has over 2500 organizations registered with it which are involved in the field of animal welfare."
- There is one bird welfare organization "ASHA FOUNDATION" at Ahmadabad.

There are also various welfare laws and acts passed, across the world to protect animals against ill treatment .The Prevention of Cruelty to Animals (PCA) Act 1960 (59 of 1960) was enacted in December 1960 with the object of preventing infliction of unnecessary pain an suffering to animals. The Animal Welfare Board of India (AWBI) had formulated a draft Animal Welfare Act, 2011, and submitted it to the

Ministry of Environment and Forests to replace the outdated Prevention of Cruelty to Animals Act, 1960. Apart from this, there are various NGO's working for animal's rights, animal's protection and also their well being.

Impact on food availability & safety

Within this global scenario, a major challenge for all parties ought to be implementing a "clean, green, and ethical" animal agriculture, while guaranteeing that food is produced under high animal welfare standards. The industry and producers in the developed countries have followed the animal welfare "rules"; in some instances, this is due to legislation and penalties they can be subjected to, while in other cases, there is a genuine concern about animal welfare and potential implications on productivity .[5]

The fast-food retailers realized very quickly that they were in a public relations battle for the hearts and minds of consumers, one that involves convincing people of the moral acceptability of their products. In 2000, McDonald's Corporation announced that it had established an animal welfare program with specific requirements that it intended its suppliers to meet. Shortly thereafter, Burger King, Wendy's International, and Kentucky Fried Chicken introduced similar animal welfare programs.[48] That improvements in farm animal welfare can often improve productivity and food safety, and hence lead to economic benefits.

Conclusion

Keeping animals well nourished, free from discomfort, pain and stress are essential to sustain a good welfare state. Though it is must to increase production for fulfillment of our need but it's our prime duty to avoid suffering of animals as part of humanity. Poultry welfare improves health and maximizing efficiency which resulted in meat and poultry products that are affordable for all sector of society so poultry welfare issues be included in future international trade talks. Managemental strategies based on good current scientific information and proactive attitude are the key to success.

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Effect of Dietary fat on Reproduction in Cattle

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Abstract

Feeding supplemental fat to dairy cows no longer has energy density as a primary consideration in the ration. Essential fatty acids, notably linoleic (omega-6) and linolenic (omega-3) acids have direct effects on physiological processes such as cellular membrane integrity, hormonal pathways, and immune function. Clearly, they are important to uterine health, and that is accompanied by earlier ovulations PP and more cycles during the voluntary waiting period. decline in fertility and there is increasing evidence to assume that reduced oocyte and embryo quality are two major players in this "disappointing fertility syndrome" nutrition has the potency to alter the micro-environment of the oocyte and the embryo, making it more hostile to optimal fertilization and pre implantation embryonic growth .Data reviewed shows that supplementation with different sources of lipids and fatty acids improve reproductive performance of the female ruminant. However, it is important to consider that the optimum response will be achieved when under nutrition status of the female is not extremely severed. A nutrient balance (protein: energy) in the ration consumed by the animal is fundamental to obtain maximum benefit from supplementation with fat, The feeding of additional energy in the form of fat reduces the cow's negative energy status so that she returns to oestrus earlier after calving and therefore conceives sooner.

Cows fed fat produce or secrete more progesterone, a hormone necessary for the implantation and nutrition of the newly formed embryo. Specific individual long chain fatty acids found in some fats inhibit the production or release of prostaglandin by the uterus. This prevents the regression of the corpus luteum (CL) on the ovary so that the newly formed embryo survives. Based on the experiments done at this time, it appears that dietary fats may increase the size and the life span of the CL. The larger size of the dominant follicle in fat-supplemented cows may result in a larger CL. More CL cells produce more progesterone. Greater progesterone should improve implantation and nutrition of the embryo. In addition, certain fatty acids such as linoleic acid and those found in fish may partially suppress secretion of Prostaglandin by the uterus at the time of conception so that the CL is not regressed and embryo survival is potentially enhanced.

Keywords: Essential fatty acid; Fertility; Nutrient-balance; Embryonic growth; Oestrous; Micro-environment.

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Introduction

Nutrition has an important impact on the reproductive performance of cattle. Energy is the major nutrient required by adult cattle and inadequate energy intake has a detrimental impact on reproductive activity of the female bovine. Cows under negative energy balance have extended periods of an ovulation. Postpartum anoestrus, as well as infertility, is magnified by losses of body condition during the early postpartum period. Resumption of ovulatory cycles is associated with energy balance, and the underlying mechanisms seem to be associated with metabolic signals and regulatory hormones primarily insulin and insulin-like growth factor-1(IGF-1), which link nutritional status with gonadotropin secretion, re-coupling of the growth hormone-IGF system, and follicle maturation and ovulation.

Feeding diets that promote increases in plasma glucose and insulin may improve the metabolic and endocrine status of cows in early lactation. Nevertheless, feeding excess of starch to promote increase in insulin and glucose might suppress intake of early lactating dairy cows, thereby pre including benefits to cyclicity. Feeding behaviour of dairy cows during the transition period, particularly a decline in feed intake before calving is associated with risk of postpartum uterine disease. Because metritis and more chronic forms of uterine diseases have profound negative effects on pregnancy in dairy cows, providing adequate bulk space and an environment to maximize feed intake might potentially improve fertility of dairy cows. Specific nutrients and dietary ingredients have been implicated on reproduction in cattle.

Addition of moderate amounts of supplemental fat to the diet improves energy intake, modulates PGF2α secretion by the uterus, affects ovarian dynamics, enhances luteal function and embryo quality, and has moderate positive effects on fertility. More specifically, some fatty acids (FA) might impact fertilization rate and embryo quality in dairy cows. Early research confirmed that nutrition played an important role in reproduction. This fact sheet presents some of the available research information relating nutrition to reproduction in dairy cows and it provides a basis from which to evaluate potential contribution of nutritional factors to impaired reproductive performance in field situation. However, two facts about the relationship between nutrition and reproduction must be kept in mind Nutrition is only one possible cause of reproductive problems. Other possibilities should not be neglected. Some, like poor oestrous detection and poor sanitation and hygiene at calving, should be ruled out before looking for a nutritional cause for breeding problems in herds where obvious nutritional problems are not apparent.

Relatively little information is known regarding to the complex interaction between nutrition and reproduction. Often the best recommendation that can be made is to feed a ration that is balanced for energy estimate of feed intakes are required. Metabolic hormone secretion documented effects of supplemental lipids on metabolic hormones have been mixed. The consumption of polyunsaturated plant oils has been shown to increase serum insulin and GH concentrations in both dairy and beef cows However, it is reported [1]that no differences in serum concentrations of glucose, NEFA, GH, IGF-I, insulin, or IGF-I binding proteins in primiparous beef heifers supplemented with high linoleic or high oleic safflower seeds. Bellows *et al* (2001) also found no differences in concentrations of IGF-I, glucose, or NEFA after feeding primiparous beef heifers sunflower seeds for 68 day before calving compared to a control diet without added fat.[1] It was also reported that plasma NEFA is nearly always elevated in cows fed supplemental fat and blood glucose concentrations generally are not changed under conditions of supplemental fat.

What are dietary fat

Fats consist of a wide group of compounds that are generally soluble in organic solvents and generally insoluble in water. Fats are triglycerides, triesters of glycerol and any of several fatty acids. Fats may be

either solid or liquid at room temperature, depending on their structure and composition. Although the words "oils", "fats", and "lipids" are all used to refer to fats."Lipids" is used to refer to both liquid and solid fats, along with other related substances,

Types of fat

Saturated and unsaturated are the types of fat. Unsaturated fats are sub-classified into the monounsaturated and polyunsaturated fat, polyunsaturated fat mostly found in nuts, seeds, fish, algae, leafy greens, Whole food sources are always best, as processing and heating may damage polyunsaturated fat.

Reproductive problems associated with dietary fat

Under nutrition inhibits oestrous behaviour by reducing responsiveness of the central nervous system to estradiol by reducing the estrogens receptorα content in brain (Hileman *et al*, 1999). Cows with low BCS at 65 d postpartum are more likely to be anovular. (Santos *et al*, 2008) Prolonged postpartum anovulation or anoestrus extend the period from calving to first AI and reduces fertility during the first postpartum service.

Nutrition in fertility and Postpartum uterine health

Nutritional factor

Over conditioning at the time of calving has been related to a higher incidence of infections in some studies, since over conditioned cows may exhibit poor uterine muscle tone, fatigue earlier during the calving process, and experience a higher incidence of difficult births. On the other hand, severely under conditioned cows appear to be more susceptible to infection than cows in proper condition. Monitor body condition in late lactation so that cattle calve with body condition scores between 3+ to 4- on a scale of 1 to 5. Calcium is important for proper uterine smooth muscle contraction. Low levels of blood calcium may contribute to retained placenta resulting in uterine infection. Low calcium may also delay uterine involution maintenance of healthy uterine tissue. Other nutritional factors may be indirectly related to maintaining uterine health, so feeding a balanced dry cow ration is critical. Adequate tissue levels of proper vitamins and minerals must be present prior to calving and throughout the postpartum period.

Postpartum uterine health

Before parturition the uterine lumen is sterile and if bacterial invasion occurs, there is usually resorption of the foetus or abortion.During parturition, the physical barriers of the cervix, vagina and vulva are compromised providing the sum for bacteria to ascend the genital tract from the environment as well as the animal's skin and faeces. Indeed, bacterial contamination of the uterine lumen is almost ubiquitous in cattle and notably greater than in other mammals including ruminants such as sheep. The reasons for the species differences are not clear, as sheep and cattle inhabit similar environments and the progress of uterine involution is similar . Surprisingly, the level of hygiene of the environment during and immediately after parturition appears to have little effect on the qualitative or quantitative uterine bacterial flora.[2]Percent animals with uterine bacteria Day postpartum.Proportion of uteri contaminated with bacteria during the first 60 days postpartum drawn from the data.[2] However, the functional capacity of neutrophils is reduced after parturition in many cattle and this may predispose to the establishment of uterine disease. Later, macrophages are likely to be important in the uterine immune response. In addition, an early ovulation and formation of a corpus luteum after calving (<19 days versus >3days) increased the risk for prolonged luteal cycles before service in dairy cattle.Metritis is a severe inflammatory condition to postpartum period.

Effect of uterine health on fertility

Infection causes damage to the uterine tissues and features of endometrial damage such as

increased inflammation in the stratum compactum are associated with poor reproductive performance.[1] Endometritis causes infertility at the time the uterine infection is present and sub fertility even after successful resolution of the disease. In typical studies the conception rate is about 20% lower for cows with endometritis, the median calving to conception interval 30 days longer and there are 3% more animals culled for failure to conceive (Borsberry and Dobson, 1989; LeBlanc *et al*, 2002). Furthermore, cows with a purulent cervical discharge have lower submission rates (McDougall, 2001). As well as the effects on fertility, uterine infection is associated with lower milk yields particularly if associated with retained placenta.

Nutritional manipulation to increase energy intake strategy to increase energy intake

The extent and duration of postpartum negative energy balance is influenced by genetic potentiality for milk production, dietary energy density and dry matter intake. Nutritional management strategies can be employed to minimize the extent and duration of negative energy balance. In view of the fact that dry matter intake during the early lactation period goes down, increasing energy density of the ration is the only available option to improve energy intake, which can be achieved through supplementation of grains or fat. Diets containing high levels of grain may cause metabolic disturbances, such as rumen acidosis, and may ultimately result in low milk and milk fat production. To avoid these problems, fat can be added to increase the energy density of the diet. Fat supplementation also has other potential benefits, such as increased absorption of fat-soluble nutrients and reduced dustiness of feed. In addition, feeding fat to dairy cows generally improves fertility.

Dietary supplementation of fat

Vegetable oils as such are not recommended for ruminants because the unsaturated fatty acids are toxic to rumen bacteria, especially to fibre degrading bacteria. Unsaturated fat supplementation reduces fibre digestion, thereby defeating the major objective of increasing the availability of energy. Therefore, the supplementation of fat for dairy cows is achieved by means of bypass fats, which pass the rumen without any degradation. Rumen bypass fats can be either rumen-protected or rumen-stable fats. These are inert in the rumen and are digested in the lower GI tract; hence they are not harmful to rumen bacteria.

Rumen-stable and rumen-protected fats

The protected fats are mostly either calcium salts of long-chain fatty acids or saturated fats. Protection does not mean stability; usually protection depends on the conditions of the rumen and its p^H. Rumen-protected calcium-soap or calcium salts of long-chain fatty acids were developed to improve milk production. Being a chemical reaction product, they have many disadvantages. Because of the pungent soap taste, there is usually poor acceptance of the feed. A further disadvantage is that larger amounts of feed concentrate, low p^H values in feed and in the rumen, impair the stability of calcium soaps resulting in the release of the unsaturated fatty acids. These unsaturated fatty acids may negatively influence milk fat formation and may also disturb ruminal digestion, as described earlier. Recent development in fat supplementation for dairy cows is rumen-stable fats, which are fractionated triglycerides, rich in saturated fatty acids, mainly palmitic acid. Rumen-stable fats are stable at various p^H conditions. Their fatty acids are largely saturated so that they pass through the rumen almost unchanged. As a result, the fats reach the small intestine where they are broken down by enzymes and, subsequently, utilised by the body as an efficient source of energy. Animals, during the 2 week period before calving for cows that went on to develop puerperal metritis, and Quimby *et al* (2001), with feedlot steers, indicated that reduced feeding behavior can be used to detect animal morbidity approximately 4.1 days earlier than identification by pen riders. This work provides clear evidence that reduced feeding time and DMI during the period before

calving increases the risk of cows being diagnosed with metritis after calving. However, whether a reduction in intake and feeding time before calving is a cause of metritis, or an effect of something else going on during the prepartum period, is not known. Cows that developed postpartum metritis also engaged in fewer aggressive interactions at the feed bunk during the week prior to calving and avoided the feed bunk during period when competition for feed was highest. Nutritional efforts to minimize the extent and duration of NEB may improve reproductive performance. The first and most important factor that affects energy intake in dairy cows is feed availability.[3] Therefore, dairy cows should have continual access to a high quality, palatable diet to assure maximum DMI. However, DMI is limited during late gestation and early lactation, which can compromise total energy intake and reproductive performance. Several nutritional management strategies have been proposed to increase energy intake during early lactation. Feeding high quality forages, increasing the concentrate: forage ratio, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows.

A number of studies have demonstrated the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on reproductive parameters in dairy cattle. In early postpartum dairy cattle under NEB, reduced expression of hepatic growth hormone receptor 1A (GHR-1A) is thought to be responsible for the lower concentrations of IGF-I in plasma of cows . Because IGF-I is an important hormonal signal that influences reproductive events such as stimulation of cell mitogenesis, hormonal production, and embryo development, among other functions; increasing concentrations of IGF-I early postpartum are important for early resumption of cyclicity and establishment of pregnancy. It is interesting to note that insulin mediates the expression of GHR-1A in dairy cows[4] which results in increased concentrations of IGF-I in plasma. Because IGF-I and insulin are important for reproduction in cattle, feeding diets that promote greater insulin concentrations should benefit fertility.

Resumption of postpartum cyclicity

The onset of lactation creates an enormous drain of nutrients in high producing dairy cows; which, in many cases, antagonizes the resumption of ovulatory cycles. During early postpartum, reproduction is deferred in favour of individual survival. Therefore, in the case of the dairy cow, lactation becomes a priority to the detriment of reproductive functions. During periods of energy restriction, oxidizable fuels consumed in the diet are prioritized toward essential processes such as cell maintenance, circulation, and neural activity . Homeorhetic controls in early lactation assure that body tissue, primarily adipose stores, will be mobilized in support of milk production. Therefore, the early lactation dairy cow that is unable to consume enough energy-yielding nutrients to meet the needs of production and maintenance, will sustain high yields of milk and milk components at the expense of body tissues. This poses a problem to reproduction, as delayed ovulation has been linked repeatedly with energy status.[4] Energy deprivation reduces the frequency of pulses of luteinizing hormone (LH); thereby impairing follicle maturation and ovulation. Furthermore, under nutrition inhibits oestrous behaviour by reducing responsiveness of the central nervous system to estradiol by reducing the estrogens receptor α content in the brain. Generally, the first postpartum ovulation in dairy cattle occurs 10 to 14 d after the NEB.[4]

Severe weight and BCS losses caused by inadequate feeding or illnesses are associated with anovulation and anoestrus in dairy cattle. In fact, cows with low BCS at 65 d postpartum are more likely to be anovular which compromises reproductive performance at first postpartum insemination. Prolonged postpartum anovulation or anoestrus extends the period from calving to first AI and reduces fertility during the first postpartum service. In fact, anovular cows not only have reduced oestrous detection and conception rates, but also have compromised embryo survival . On the other hand, an early return to cyclicity is important in reared to early conception. The timing of the first postpartum ovulation

determines and limits the number of oestrous cycles occurring prior to the beginning of the insemination period. Typically, in most dairy herds, fewer than 20 % of cows should be anovulatory by 60 d postpartum. Oestrous expression, conception rate, and embryo survival improved when cows were cycling prior to an oestrous synchronization program for first postpartum insemination. Resumption of ovarian activity in high producing dairy cows is determined by energy status of the animal. Therefore, feeding management that minimizes loss of body condition during the early postpartum period and incidence of metabolic disorders during early lactation should increase the number of cows experiencing a first ovulation during the first 4 to 6 wk postpartum.

Fat and its importance in reproduction

Fat to dairy cattle usually improved the risk for pregnancy, although responses have not been consistent . When fat feeding improved production and increased body weight loss, primiparous cows experienced reduced pregnancy risk at first AI; although pregnancy to AI was extremely high in the unsuplemented cows. However, Ferguson *et al* (1990) observed a 2.2 fold increased risk of pregnancy at first AI and all AI in lactating cows fed 0.5 kg/d of fat, which tended (P = 0.08) to enhance the proportion of pregnant cows at the end of the study (93 vs. 86.2 %).[5] In grazing cows, supplementation with 0.35 kg of FA improved the risk of pregnancy after the first postpartum AI; although a similar proportion of cows were pregnant at the end of the study.[6] Feeding calcium salts of long chain fatty acids (Ca-LCFA) of palm oil improved pregnancy of dairy cows , although the authors did not report statistical significance. On the other hand, others did not observe improvements in fertility of dairy cows supplemented with Ca-LCFA or oilseeds ; which might be attributed to increased milk yield and body weight losses. Because the benefits of feeding fat may originate specific FA , others have evaluated whether feeding FA differing in the degree of saturation might influence fertility of cows. The essential FA of the n-6 and n-3 families are available in much smaller supply to ruminants than non ruminants because of microbial bio-hydrogenation of FA in the rumen [7]suggesting that their supplementation may benefit reproduction.

Three recent studies explored the role of n-6 and n-3 FA supplementation to lactating dairy cows on risk of pregnancy after the first postpartum AI; When cows were fed 0.75 kg of fat from flaxseed, a source rich in C18:3 n-3, or sunflower seed, a source rich in C18:2 n-6; pregnancy tended (P = 0.07) to be greater for cows fed n-3 FA. However, a similar response was not observed by others when cows were fed flaxseed as the source of n-3 FA. Similarly, feeding n-3 FA from fish oil as Ca-LCFA did not improve risk of pregnancy in high producing, lactating dairy cows when compared with a source rich in saturated FA [7] or with Ca-LCFA of palm oil evaluated the effect of feeding cows pre- and postpartum Ca-LCFA of either mostly saturated and monounsaturated FA or a blend of C18:2 n-6 and trans-octadecenoic FA. He observed that cows fed unsaturated FA had 1.5 times greater risk of pregnancy either at 27 or 41 d after AI compared with cows fed mostly saturated FA.

Improvements in pregnancy risk when cows were fed C18:2 n-6 and trans-octadecenoic FA were supported by improved fertilization and embryo quality in non-superovulated lactating dairy cows.[8]Because n-3 FA can suppress uterine secretion of PGF2α ,[9,10] it is thought that they have the potential to improve embryonic survival in cattle.[9] In 3 of 5 experiments, feeding n-3 FA either as flaxseed rich in C18:3 n-3 or fish oil rich in eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) reduced pregnancy losses in lactating dairy cows after the first postpartum AI. On the other hand, when n-6 FA were fed as Ca-LCFA, pregnancy losses were similar to those observed for cows fed Ca-LCFA of palm oil . Collectively, these data suggest that feeding fat to dairy cows generally improves fertility and responses are observed when the energy density of the ration increased with fat feeding. Also, these data suggest that fertility responses to fat feeding are altered according to the type

of FA supplemented in the diet. Feeding n-3 FA from oilseeds has improved pregnancy risk in some, but not all studies; however feeding n-3 FA as Ca-LCFA containing fish oils does not seem to influence risk of pregnancy. On the other hand, feeding Ca-LCFA rich in n-6 and transoctadecenoic FA improved pregnancy in lactating dairy cows. Although feeding n-3 FA has not consistently improved pregnancy risk, it has reduced pregnancy losses in dairy cows.

Action of EFA

Polyunsaturated fatty acids (PUFA) are precursors to cholesterol, which is an antecedent to steroid hormones. The corpus luteum (CL) uses cholesterol to make progesterone (P4), and P4 is itself a precursor to estradiol (E), but it is also an inhibitor of Estrogens secretion. Linoleic acid (along with its elongated product, eicosapentanoic acid) is a proven inhibitor of cyclooxygenase in endometrial tissues (Haag, 2001). Consequently, endometrial secretion of the prostaglandin (PG) PGF2α from the uterus can be suppressed. This action might also be aided by the effect fat has in repressing E17β secretion ; thereby reducing PGF2α secretion and decreasing the sensitivity of the CL to PGF2α. This helps prolong the life and functionality of the CL and allows it to increase P4 concentrations. Higher pre-breeding concentrations of P4 have a positive impact on conception rates, discussed subsequently

Cholesterol-progesterone concentrations

Dietary fat supplementation increases circulating concentrations of cholesterol and progesterone and the lifespan of induced corpora lutea (**CL**) in cattle. Cholesterol serves as a precursor for the synthesis of progesterone by ovarian luteal cells. Progesterone prepares the uterus for implantation of the embryo and also helps maintain pregnancy. Increased concentrations of plasma progesterone have been associated with improved conception rates of lactating ruminants.Increased concentrations of cholesterol from fat supplementation may lead to an increase in progesterone synthesis or reduced rate of clearance from the blood [11], primary follicles exposed to adverse conditions associated with the metabolically challenging period of NEB early postpartum may be less capable of producing adequate amounts of estrogens and progesterone (after ovulation). Moreover, such follicles would be doomed to contain an inferior oocyte, which will then ovulate approximately 60-80 days postpartum. Early embryonic death is a major cause of reproductive failure in dairy cows. There are four major factors impinging on embryo quality in the specific case of high producing dairy cows: gamete quality, corpus luteum quality combined with the circulating progesterone concentration, uterine involution, and nutrition. However, only those that are related to NEB

LH secretion and Follicular development

Secretion of LH from the pituitary and follicular growth in cattle are regulated partially by the energy status of the animal. Energy provided by fat supplementation increases LH secretion in animals deficient in energy. A mechanism independent from energy by which dietary fatty acids affect LH secretion has not been established.[9] In some studies, LH dynamics were stimulated by fat supplementation but were unchanged or decreased in others . The mechanism by which supplemental fat would stimulate LH release is not known unless a glucose-sparing effect occurs at the mammary gland, providing greater glucose to signal the hypothalamic-pituitary control system to secrete more LH. Similarly, fat supplementation may increase glucose production through increased propionate production. This increase in glucose may have a positive effect on LH release.

Supplemental fat stimulated programmed growth of a preovulatory follicle, increased total number of follicles, and increased the size of preovulatory follicles.[9] Increased size of preovulatory follicles may be due in part to increased concentrations of plasma LH, which stimulates the latter stage of follicular growth. The ovulation of larger follicles may result in the formation of larger corpora lutea with increased steroidogenic capacity and result in

greater progesterone production, which has been associated with higher conception rates.

Prostaglandin synthesis

Prostaglandins play an important role in restablishing oestrous cycles both immediately after parturition and thereafter, until conception occurs. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is responsible for uterine involution after parturition. Greater postpartum $PGF_{2\alpha}$ concentration is associated with faster uterine involution. The uterus releases $PGF_{2\alpha}$ during each oestrous cycle to regress each new CL if the cow is not pregnant and initiate a new oestrous cycle. During the period of CL regression, concentrations of $PGF_{2\alpha}$ and progesterone are inversely related. If the cow conceives, release of $PGF_{2\alpha}$ from the uterus is prevented in order to preserve the CL and maintain pregnancy. Prostaglandin is important for uterine involution after parturition, but increased production and release after conception may lead to luteolysis and increased embryonic mortality.

Linoleic acid is substrate for the syntheses of $PGF_{2\alpha}$. Linoleic acid can be desaturated and elongated to form arachidonic acid, which is a precursor for $PGF_{2\alpha}$. Regulatory enzymes for this conversion, include δ_6-desaturase and cyclooxygenase. Linoleic acid can inhibit $PGF_{2\alpha}$ synthesis by competitive inhibition with these key enzymes.[3] In contrast, Grant *et al* (2003) found that supplementing beef cows postpartum with high-linoleate safflower seed increased PGF-metabolite from 25 to 80 d postpartum and tended to decrease first-service conception rates. Filley *et al* (2000) also demonstrated that feeding calcium salts of palm oil increased plasma linoleic acid and PGF-metabolite in beef heifers. Arachidonic, and two fatty acids found in fishmeal, eicosapentaenoic (EPA) and docosahexanoic (DHA), have been shown to inhibit cyclooxygenase activity as well .[9] Heifers with low-luteal phase progesterone supplemented with fishmeal had lower PGF-metabolite concentrations after an oxytocin challenge. However, fishmeal had no effect in heifers with high-luteal phase progesterone. Linolenic acid was also present in the endometrial $PGF_{2\alpha}$ synthesis inhibitor isolated. [12]Linolenic acid has also been shown to be a strong inhibitor of $PGF_{2\alpha}$ synthesis. The amount and probably type of particular fatty acids reaching the target tissues likely influence whether $PGF_{2\alpha}$ synthesis is stimulated or inhibited. It has also been suggested that reductions in intrafollicularand serum[13] concentrations of estradiol associated with fat supplementation may play a role in modulating luteal responsiveness to prostaglandin.

Dietary PUFA and Oocyte maturation

Reproductive Effects of Dietary Fat Ovarian Follicle Development A variety of fat sources have influenced the size and number of ovarian follicles. Normally follicle development progresses through stages of recruitment, selection, and dominance during each oestrous cycle .[12] In the initial days of the oestrous cycle, a group of follicles grow up from which a single follicle (called the dominant follicle) continues to grow while the others undergo atresia. At approximately day 10 to 11 of the cycle, this dominant follicle regresses, and this process of recruitment and selection reoccurs. A second dominant follicle arises and ovulates. This is the normal 216 [12] sequence for cows having a two follicular wave oestrous cycle. Three consecutive dominant follicles would arise if cows experienced a three wave oestrous cycle. As follicles are recruited and grow in diameter, they increase from a detectable size of 3 mm up to about 15 to 18 mm before regressing or ovulating. Several studies involving either dairy or beef cows have reported that fat supplementation increased the number of follicles of different class sizes. An increase in the number of smaller follicles may reflect a greater pool of follicles available for subsequent development. A greater number of larger follicles may indicate an altered selection process. In addition to the increased numbers of follicles due to fat supplementation, the size of the dominant follicle has commonly been increased. Larger follicles usually occur under conditions of low concentrations of progesterone and high estradiol 17-β.

As will be discussed later, this hormonal profile is just the opposite of what is seen typically when fat is supplemented. The impact of larger ovarian follicles due to the feeding of supplemental fat on conception rate has not been defined. If a follicle becomes too large (> 25 mm), it can become cystic and fail to ovulate.

Only one study reported greater occurrence of cystic follicles when fat was fed (Salfer *et al*, 1995). The size of a healthy follicle may have no relationship to the amount of estradiol it secretes or to the secretion of progesterone by the subsequent CL formed. The mechanism by which dietary fats stimulate ovarian activity has yet to be determined. Mechanisms by Which Fats May Improve Fertility Several hypotheses have been proposed regarding the mechanism(s) by which fat supplementation improves reproductive performance. These include 1) an amelioration of a negative ES thus leading to an earlier return to oestrus postpartum and therefore improved fertility, 2) an increase in progesterone production/ secretion favourable to improved fertility, and 3) a stimulation or inhibition of PGF2α production/release which influences the persistence of the CL.

Early postpartum study

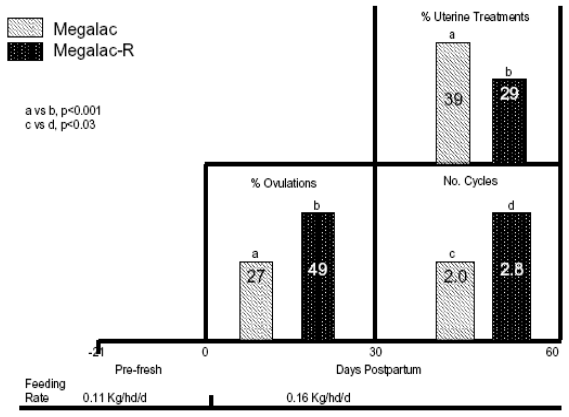
Importance of EFA early postpartum

Research at The University of Arizona has centered on evaluating the role of EFA in the early PP interval. Since EFA are precursors for steroids and PG, they should be related to re-initiation of cyclicity PP. Data in Figure 4 confirm that EFA directly affects postpartum reproductive events. Holstein cows were fed 0.11 kg/hd/d of Megalac® (Arm & Hammer Animal Nutrition, Princeton, NJ) 21 d prepartum, then received 0.16 kg/hd/d of Megalac® or Megalac®-R through the voluntary waiting period. Megalac®-R contains 4.5 fold the amount of linoleic acid and 23.5 fold the amount of linolenic acid compared to Megalac®. Those extra fats in Megalac®-R doubled the incidence of ovulations by 30 DIM, contributed to almost 1 additional cycle by 60 DIM, and markedly improved uterine health by 60 DIM compared to cows fed Megalac® (Figure 4). All of those physiological responses have been reported to reduce services per conception and days open in previous studies. A point to consider here: many people are extending voluntary waiting periods with timed A.I. programs. If uterine health and cyclicity are hastened by feeding EFA, then timed A.I. should occur earlier postpartum to capitalize upon the physiological advantage of cows being ready for breeding earlier

Figure 4. Reproductive events in dairy cows fed Megalac® or Megalac®-R from 3 wk prepartum through the voluntary waiting period. The percent of ovulations observed during the first 30 DIM; the number of estrual cycles during the first 60 DIM; and the percent uterine treatments prior to 60 DIM are portrayed Research at The University of Arizona has centered on evaluating the role of EFA in the early PP interval

Role of EFA in maternal recognition of pregnancy

Pregnancy recognition in the bovine occurs between d 15 and 18 post-oestrus with the conceptus maintaining the CL of pregnancy. Not unexpectedly, then, it is estimated that 40 % of embryonic death loss occurs during d 8-17 post-oestrus and could largely be a result of inability to maintain the CL. This is accomplished through the production of interferon-tau (INF) which inhibits the ability of the uterine endometrium to produce pulsatile PGF2α which is responsible for the degradation of luteal tissue.[12]There is evidence indicating that the length of the conceptus is directly related to the production capacity of INF.[14] Of equal importance is the observation that inclusion of n-3 fats in the diet via fish oil or Ca-LCFA increases the length of the conceptus, likely improving the ability to synthesize INF and enhancing postpartum and more cycles during the voluntary waiting period. These events lead to improved fertility earlier in the postpartum interval because they reduce services per conception and days open.



Effect of omega-3 fatty acid on male reproduction

Prostaglandins may also play an important role in male reproduction, as there are several effects of PG on sperm motility and quality.[15] Arachidonic acid and, subsequent production of PG and leukotrienes, may also be involved in mediating the stimulatory actions of luteinising hormone on testicular steroid synthesis.

No published studies have specifically examined the effects of n-3 supplementation on male fertility and semen quality in sheep and cattle. In non-ruminant studies, total sperm number.[16] and sperm motility was improved when boars were supplemented with fish oil. In addition, total sperm count and sperm motility were negatively related to serum concentrations of n-6 in a study in humans . Semen quality may be improved when the concentration of PUFA in sperm membranes is increased following supplementation with long-chain n-3.[15] However, PUFA are also associated with increased oxidative stress, which can reduce semen quality.[15] so several mechanisms need to be considered when examining the overall effects.

Optimum level of fat

As stated, the amount of supplemental fat needed to elicit a positive or, in some cases, a negative effect on reproductive function is largely unknown and titration studies are needed in all situations in which supplemental fat has been fed. Dose-response studies indicate that the amount of added plant oil necessary to maximize positive ovarian effects is not less than 4%, 3% added dietary fat (DM basis) has often positively influenced the reproductive status of the dairy cow. Lower levels of added dietary fat (2%) have also been shown to elicit a positive reproductive response.[17] and, in studies with fishmeal, less than 1% added fat produced a positive reproductive response[17] , indicating that both the amount and types of fatty acids are important. Feeding large quantities of fat (>5% of total DMI) has not been recommended due to potential negative effects on fiber digestibility and reduction in DMI (Coppock and Wilks, 1991).

Disorder fat cow syndrome

Excess energy (concentrates, corn silage, some hays) fed during the dry period may cause obese cows near calving time. These "too fat" cows are more susceptible to a number of other metabolic problems (milk fever, ketosis, displaced abomasums , retained placenta, metritis),and the chance of dying is more likely .It is not uncommon in some operations for overweight Holstein cows to weight 1,600 to 2,000 pounds, which frequently creates problems. Feeding strategy is recommended to restore lost body condition during late lactation. Not only will this practice help avoid severely overweight cows, but feed conversion into body tissue is more efficient during late lactation, compared to the dry period.

Fatty liver

Fatty liver syndrome is the accumulation of fat within the cow's liver. Fatty liver occurs as a result of the cow breaking down too much fat for the liver to process properly. Fat mobilisation occurs as a result of negative energy balance. The broken down far is then converted back to fat in the liver to prevent them becoming toxic. Thus the liver becomes fat when the cow is losing condition, the more loss in condition the more fat in the liver. Fatty liver can develop within 24 hours of an animal going off feed. This is typically around calving

time. Once it is deposited in the liver, the concentration of fat in the liver does not fall until the cow gets into positive energy balance, which can be over ten weeks after calving, particularly if the fatty liver is severe. Fat cows (Body Condition Score > 3.5)

Infertility

Caused by nutritional problems include of that may be too fat or too thin. Causes other than nutrition must be considered when obvious nutritional problems are lacking. Cow body condition evaluation is important because extremely thin or too-fat cow's reproductive efficiency is considerably reduced. The too-fat cows have more problems post-calving (retained placentas, metritis , cystic ovaries) while the too-thin cows usually have breeding problems due to prolonged time lapse before resuming normal heat cycles (30-40 days post-calving).Maintain and record body condition scores which rate 1 as too thin and 5 as too fat. Lactating cows, at peak production, should not drop below 2.5 and should be dried off at 3.5, and maintain this score throughout the dry period.

Conclusion

Feeding supplemental fat to dairy cows no longer has energy density as a primary consideration in the ration. Essential fatty acids, notably linoleic (omega-6) and linolenic (omega-3) acids have direct effects on physiological processes such as cellular membrane integrity, hormonal pathways, and immune function. Clearly, they are important to uterine health, and that is accompanied by earlier ovulations PP and more cycles during the voluntary waiting period. decline in fertility and there is increasing evidence to assume that reduced oocyte and embryo quality are two major players in this "disappointing fertility syndrome" nutrition has the potency to alter the micro-environment of the oocyte and the embryo, making it more hostile to optimal fertilization and pre implantation embryonic growth. Data reviewed shows that supplementation with different sources of lipids and fatty acids improve reproductive performance of the female ruminant. However, it is important to consider that the optimum response will be achieved when under nutrition status of the female is not extremely severed. A nutrient balance (protein: energy) in the ration consumed by the animal is fundamental to obtain maximum benefit from supplementation with fat, The feeding of additional energy in the form of fat reduces the cow's negative energy status so that she returns to oestrus earlier after calving and therefore conceives sooner.

Cows fed fat produce or secrete more progesterone, a hormone necessary for the implantation and nutrition of the newly formed embryo. Specific individual long chain fatty acids found in some fats inhibit the production or release of prostaglandin by the uterus. This prevents the regression of the corpus luteum (CL) on the ovary so that the newly formed embryo survives. Based on the experiments done at this time, it appears that dietary fats may increase the size and the life span of the CL. The larger size of the dominant follicle in fat-supplemented cows may result in a larger CL. More CL cells produce more progesterone. Greater progesterone should improve implantation and nutrition of the embryo. In addition, certain fatty acids such as linoleic acid and those found in fish may partially suppress secretion of Prostaglandin by the uterus at the time of conception so that the CL is not regressed and embryo survival is potentially enhanced.

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In Vitro Gas Production Technique for Evaluation of Feed Resources

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Abstract

The *in vitro* rumen fermentation method in which gas production and microbial mass production are concomitantly measured has several major advantages: i) it has the potential for screening a large number of feed resources, for example in breeding programmes for the development of varieties and cultivars of good nutritional value, ii) it could also be of great value in the development of supplementation strategies using locally available conventional and unconventional feed constituents to achieving maximum microbial efficiency in the rumen; iii) it has an important role to play in the study of rumen modulators for increasing efficiency of microbial protein synthesis and decreasing emission of methane, an environmental polluting gas, and iv) it provides a better insight into nutrient-antinutrient and antinutrient-antinutrient interactions, and into the roles of various nutrients (by changing the composition of the incubation medium) with respect to production of fermentative gases, SCFA and microbial mass. The method is also being used increasingly to screen plant-derived rumen modulators. These products have a lower toxicity to animals and humans, and are environment friendly. Consequently, they are becoming increasingly popular with consumers.

Further studies are required on: i) the development of simple approaches for identifying the incubation time in the *in vitro* gas system at which the PF (a measure of the proportion of fermented substrate which leads to microbial mass production) is maximum, ii) the effect of nitrogen in the incubation medium on the PF, and iii) the *in vivo* significance of the PF so obtained. The results of the limited experiments conducted so far have shown that simple models employing gas kinetic parameters and the PF are capable of predicting the dry matter intake of roughages and level of emission of methane by ruminants. Experiments also need to be done to test whether, for any given feed, the microbial protein synthesis as derived from digestion kinetic parameters (including PF) *in vitro* is sufficient to explain the observed microbial protein supply to the small intestine *in vivo*. At present, the simplest way of determining the latter parameter is to calculate it from the level of urinary purine derivatives. This validation excercise should be conducted for a wide range of feed constituents and diets which should enable the above mentioned simple technique of measuring gas and microbial mass to be a routine and powerful tool for feed evaluation thus avoiding the need for time-consuming, laborious and expensive feeding studies. Lately, much emphasis has been given to the development of statistical or mathematical models that best fit the gas production profiles and describe the gas evolution with high accuracy. Experiments must be designed to understand the biological significance of the various statistical and functional parameters being calculated using these models, and also to incorporate a measure of microbial mass into these mathematical descriptions.

Research and development efforts are required to establish a feed library for unconventional feedstuffs that includes information on nutritive values in addition to routine composition analysis. In the case of tannin-containing feedstuffs, there is a need to incorporate approach(s) measuring the biological activities of tannins as well as measuring tannin levels by chemical methods.

Keywords: Scarcity; Unconventional feed resources; Ligno-cellulosic stovers; UMMB; Soil erosion; Food security.

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Introduction

A major constraint to livestock production in developing countries is the scarcity and fluctuating quantity and quality of the year-round feed supply. Providing adequate good-quality feed to livestock to raise and maintain their productivity is and will be a major challenge to agricultural scientists and policy makers all over the world. Increase in population and rapid growth in world economies will lead to increase in demand for animal products; an increase of approximately 30 % in both meat and milk production is expected in the coming 20 years. At the same time, the demand for food crops will also increase. Future hopes of feeding the millions and safeguarding their food security will depend on the enhanced and efficient utilization of unconventional resources that can not be used as food for humans, as feed for livestock. In addition, a large area of land in the world is degraded, barren or marginal and the amount is increasing every year. This also calls not only for identification and introduction of new and lesser-known plants capable of growing in poor soils, which can play a vital role in the control of soil erosion in addition to providing food and feed. In developing countries, livestock are fed mainly on agro-industrial by-products containing a larger proportion of ligno-cellulosic feeds like cereal straws, stovers, sugarcane by-products and similar other feeds. These feeds are poor in protein, energy, minerals and vitamins. Addition of foliage from tree leaves or supplementation with seed meals or even urea can improve the utilization of low quality roughages mainly through the supply of nitrogen to rumen microbes. The use of simple but robust techniques for evaluation of the nutritional quality of these feed resources will contribute to their efficient utilization.

Both growth and milk yield of ruminants are largely limited by forage quality which is mainly reflected in low voluntary intake and digestibility. The importance of these parameters in animal nutrition has long been recognized. The determination of intake and digestibility of feed stuffs *in vivo* is time-consuming, laborious, and expensive, requires large quantities of feed and is unsuitable for large-scale feed evaluation. Therefore, many attempts have been made to predict intake and digestibility using laboratory techniques. Much effort has been directed towards the development of regression equations to predict digestibility from forage chemical composition, but a regression equation that satisfactorily predicts a wide range of forages has not yet been derived.

In this seminar we are going to highlight the potential of a novel approach using an *in vitro* rumen fermentation technique for evaluation of the nutritional quality of conventional and unconventional feed resources.

Methods for evaluation of feed resources

Overview

Recent advances in ration balancing include manipulation of feed to increase the quantity and quality of protein and energy delivered to the small intestine. Selection of fibrous feeds based on high efficiency of microbial protein synthesis in the rumen along with high dry matter digestibility, and development of feeding strategies based on high efficiency as well as high microbial protein synthesis in the rumen will lead to higher supply of protein post-rumen. This concept of feed evaluation has an extra element of the efficiency of microbial protein synthesis in addition to the more conventional one of the dry matter digestibility. The limited supply of protein post-rumen under most feeding systems in developing countries is an important limiting factor which prevents an increase in animal productivity.

There are a number of methods used to determine net microbial protein synthesis in the rumen based on the use of microbial markers. They require the use of post-rumen cannulated animals to determine flow of digesta. The cannulation approach is tedious and has several limitations[1]to its applicability under most research conditions in developing countries. A

simpler technique for determination of microbial protein supply to the intestine is based on the determination of total urinary purine derivatives.[2]This approach is being thoroughly investigated under a Joint FAO/ IAEA Coordinated Research Project.[3] Although the method is based on the collection of urine for determination of purine derivatives (allantoin and uric acid for cattle, and allantoin, uric acid, xanthine and hypoxanthine for sheep), the approach is being further simplified using spot urine samples. This technique does not require cannulated animals, but it involves feeding the diets under investigation to animals and therefore is not suitable for screening large numbers of samples or for developing feed supplementation strategies using various feed constituents.

A) In vivo methods

Total collection technique (direct method or conventional digestion trial)

The total collection (conventional digestion trial) is the most reliable method of measuring a feed's digestibility. Unfortunately, it is somewhat time consuming, tedious, and costly.

Usually, the animal is restrained in an individual cage so that a quantitative collection of feces can be made. Accurate records of feed intake, refusals and fecal output are kept, and a sub sample of each (usually 10% of daily output in the case of feces) is retained for analysis. When estimates of nitrogen balance are desired, urine output is also measured.

Three animals per feed are required as a minimum. The animals are usually allowed from 7 to 21 days (d) to adjust to the feed, followed by a collection. Samples can then be dried, ground, and analyzed for the nutrients of interest. Digestibility of any given nutrient can be calculated as follows:

Nutrient intake

$$\text{Nutrient digestibility (\%)} = \frac{\text{(Nutrient intake - Nutrient in feces)}}{\text{Nutrient intake}} \times 100$$

The most common arrangement for collecting the excreta of animals for digestibility experiments is through the use of metabolic crates. A metabolic crate is actually a stall or box large enough for the animal set on legs from 50 cm to 1 m high. It is so planned as to permit the quantitative collection of feces and urine. However, a common criticism of digestion estimation by total collection technique is that feed intake by animals is sometimes abnormally low and erratic. This lack of appetite is in many cases attributable to the fact that the animal may be too nervous or frightened to eat, resulting from the close confinement made necessary by the very nature of the equipment used. It is important that the experimental animals must be sufficiently comfortable during the adjustment period. The space allowed to the animal must be large enough to permit considerable freedom of movement. But conducting a digestion experiment may normally entail appreciable annoyance to the animal.

Some individual animals are temperamentally unsuited to be used in such experiments and are too nervous to be used in digestion trials. Mostly captive wild animals fall into this category. Even though conventional digestion trials are the standard with which all other measures of digestibility are compared, the values obtained still vary ± 1 to 4 % as a result of animal-to-animal variation, sampling procedures and analytical errors.

B) Indirect method

Apparent digestibility of a diet can be estimated using a natural constituent of the feed as an indicator. Acid insoluble ash (AIA) can be used in this way.The ratio between the concentration of AIA in the feed and the concentration of AIA in the faeces gives an estimate of digestibility.

For example, the digestibility of neutral

$$\text{Digestibility of DM} = 100 - \frac{[100\ (\text{AIA concentration in diet})}{(\text{AIA concentration in faeces})]}$$

detergent fibre (NDF) is calculated as:

$$\text{Digestibility of DM} = 100 - \left[\frac{100 \text{ (AIA concentration in diet X NDF concentration in faeces)}}{\text{(AIA concentration in faeces X NDF concentration in diet)}}\right]$$

In vitro methods

In vitro methods for laboratory estimations of degraded feeds are important for ruminant nutritionists. An efficient laboratory method should be reproducible and should correlate well with actually measured *in vivo* parameters. *In vitro* methods have the advantage not only of being less expensive and less time-consuming, but they allow to maintain experimental conditions more precisely than do *in vivo* trials. Three major biological digestion techniques are currently available to determine the nutritive value of ruminant feeds: 1) digestion with rumen microorganisms [4]or using a gas method[5] 2) *in situ* incubation of samples in nylon bags in the rumen [6], and 3) cell-free fungal cellulose[7] These biological methods are more meaningful since microorganisms and enzymes are more sensitive to factors influencing the rate and extent of digestion than are chemical methods. [8] The nylon bag technique has been used for many years to provide estimates of both the rate and extent of disappearance of feed constituents. This technique provides a useful means to estimate rates of disappearance and potential ruminal degradability of feed stuffs and feed constituents whilst incorporating effects of particulate passage rate from the rumen. The disadvantage of the method is that only a small number of forage samples can be assessed at any one time, and it also requires at least three fistulated animals to account for variations due to animal. It is therefore of limited value in laboratories undertaking routine screening of a large numbers of samples. It is also laborious, and requires large amounts of samples. Substantial error could result in values obtained at early stages of digestion due to a low weight loss; and for poor quality roughages, adherence of microbes at early stages can even lead to higher weights and thus distortion of results if kinetic modelling does not incorporate the lag-phase. [9,10]

The technique[4] is used widely because of its convenience, particularly when large-scale testing of feedstuffs is required. This method is employed in many forage evaluation laboratories and involves two stages in which forages are subjected to 48 h fermentation in a buffer solution containing rumen fluid, followed by 48 h of digestion with pepsin in an acid solution. The method was modified by Goering and Van Soest (1970)[11], in that the residue after 48 h incubation was treated with neutral detergent solution to estimate true dry matter digestibility. Although the method [4] has been extensively validated with *in vivo* values[8] , the method appears to have several disadvantages. The method is an end-point measurement (gives only one observation) thus, unless lengthy and labour-intensive time-course studies are made, the technique does not provide information on the kinetics of forage digestion; the residue determination destroys the sample and therefore a large number of replicates are needed. The method is therefore difficult to apply to materials such as tissue culture samples or cell-wall fractions.

Both the Tilley and Terry and nylon bag methods are based on residue determinations and may result in overestimation of dry matter digestibilities for tannin-rich feeds, since tannins are solubilised in both these systems but might be indigestible and do not contribute to nutrient supply to animals.[12]

In vitro gas production technique

The gas measuring technique has been widely used for evaluation of nutritive value of feeds. More recently, the increased interest in the efficient utilization of roughage diets has led to an increase in the use of this technique due to the advantage in studying fermentation kinetics. Gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs. Several gas measuring techniques and *in vitro* gas methods are in use by several groups. Advantages and disadvantages of these methods are discussed. [13]The *in vitro* gas method based on syringes [14,5]appears to be the most suitable for use in

developing countries. Other *in vitro* methods such as Tilley and Terry and nylon bag methods are based on gravimetric measurements which follow disappearance of the substrate (the components which may or may not necessarily contribute to fermentation), whereas gas measurement focuses on the appearances of fermentation products (soluble but not fermentable products do not contribute to gas production). In the gas method, kinetics of fermentation can be studied on a single sample and therefore a relatively small amount of sample is required or a larger number of samples can be evaluated at a time. The *in vitro* gas method is more efficient than the *in sacco* method in evaluating the effects of tannins or other anti-nutritive factors. In the *in sacco* method these factors are diluted in the rumen after getting released from the nylon bag and therefore do not affect rumen fermentation appreciably. In addition, the *in vitro* gas method can better monitor nutrient-antinutrient and antinutrient-antinutrient interactions.[15]

A simple *in vitro* approach is described below which is convenient and fast, and allows a large number of samples to be handled at a time. It is based on the quantification of substrate degraded or microbial protein produced using internal or external markers and of gas or short chain fatty acid (SCFA) production in an *in vitro* rumen fermentation system based on syringes. [5]This method does not require sophisticated equipment or the use of a large number of animals (but one or preferably two fistulated animals are required) and helps selection of feeds or feed constituents based not only on the dry matter digestibility but also on the efficiency of microbial protein synthesis.

In the method of Menke *et al* (1979)[5], fermentations are conducted in 100 ml capacity calibrated glass syringes containing feed stuff and a buffered rumen fluid. The gas produced on incubation of 200 mg feed dry matter after 24 h of incubation together with the levels of other chemical constituents are used to predict digestibility of organic matter determined *in vivo* and metabolizable energy.

For roughages, the relationships are:

ME (MJ / Kg DM) = 2.20 + 0.136 Gp + 0.057 CP, R^2= 0.94

OMD (%) = 14.88 + 0.889 Gp + 0.45 CP + 0.0651 XA, R^2=0.92

Where ME is the metabolisable energy; OMD organic matter digestibility; CP, crude protein in %; XA, ash in %; and Gp, the net gas production in ml from 200 mg dry sample after 24 h of incubation and after correction for the day-to-day variation in the activity of rumen liquor using the Hohenheim standard

Aiple et al (1996) compared three laboratory methods (enzymatic, crude nutrient and gas measuring technique) as predictors of net energy (as estimated by equations based on *in vivo* digestibility) content of feeds and found that for predicting net energy content of individual feeds, the gas method was superior to the other two methods.[16]

The method of Menke *et al* (1979)[5] was modified by Blümmel and Ørskov (1993) [17]in that feeds were incubated in a thermostatically controlled water bath instead of a rotor in an incubator. Makkar *et al* (1995b) and Blümmel *et al* (1997)[14,18] modified the method further by increasing the amount of sample from 200 to 500 mg and increasing the amount of buffer two-fold as a result the incubation volume increase from 30 ml in the method [5] to 40 ml in the modified method. In the 30 ml system, the linearity between the amount of substrate incubated and the amount of gas produced is lost when the gas volume exceeds 90 ml because of the exhaustion of buffer of the medium; and in 40 ml system, the linearity is lost when the gas volume exceeds 130 ml.[19] The exhaustion of the buffer decreases p^H of the incubation medium; consequently the fermentation is inhibited. If the amount of gas production exceeds 90 ml using the 30 ml system and 130 ml using the 40 ml system, the amount of feed being incubated should be reduced.

The main advantages of the modified method (the 40 ml system and incubation in a water bath) are: i) there is only a minimum drop in temperature of the medium during the period of recording gas readings on incubation of syringes in a water bath. This is useful for studying the kinetics of fermentation where gas

volume must be recorded at various time intervals, ii) because of large volume of water in the water bath and also its higher temperature holding capacity, drastic drop in the temperature of the incubation is prevented in case of power breakdown for a short duration, and iii) an increase in amount of sample from 200 to 500 mg reduces the inherent error associated with gravimetric determination needed to determine concomitant *in vitro* apparent and true digestibility.

When a feed stuff is incubated with buffered rumen fluid *in vitro*, the carbohydrates are fermented to produce short chain fatty acids (SCFA), gases and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate. Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation. The contribution of fat to gas production is negligible. When 200 mg of coconut oil, palm kernel oil and/or soybean oil were incubated, only 2.0 to 2.8 ml of gas were produced while a similar amount of casein and cellulose produced about 23.4 ml and 80 ml gas.

The gas produced in the gas technique is the direct gas produced as a result of fermentation and the indirect gas produced from the buffering of SCFA. For roughages, when bicarbonate buffer is used, about 50% of the total gas is generated from buffering of the SCFA and the rest is evolved directly from fermentation. At very high molar propionate the amount of CO_2 generated from buffering of SCFA is about 60% of total gas production. Each mmol of SCFA produced from fermentation releases 0.8-1.0 mmol of CO_2 from the buffered rumen fluid solution, depending on the amount of phosphate buffer present. Highly significant correlation has been observed between SCFA and gas production.

Gas is produced mainly when substrate is fermented to acetate and butyrate. Substrate fermentation to propionate yields gas only from buffering of the acid and, therefore, relatively lower gas production is associated with propionate production. The gas that is released with the generation of propionate is only the indirect gas produced from buffering. The molar proportion of different SCFA produced is dependent on the type of substrate. Therefore, the molar ratio of acetate of propionate has been used to evaluate substrate related differences. Rapidly fermentable carbohydrates yield relatively higher propionate as compared to acetate, and the reverse takes place when slowly fermentable carbohydrates are incubated. Many workers reported more propionate and thus lower acetate to propionate ratio in the ruminal fluid of cows fed a high grain diet. If fermentation of feeds leads to a higher proportion of acetate, there will be a concomitant increase in gas production compared with a feed with a higher proportion of propionate. In other words, a shift in the proportion of SCFA will be reflected by changes in gas production.

The gas produced on incubation of cereal straws[17] , a wide range of feeds including many dairy compound feeds and their individual feed components whose protein and fat contents vary greatly[20],and tannin containing browses[17]in absence or presence of polyethylene glycol (a tannin complexing agent) in the buffered rumen fluid was closely related to the production of short chain fatty acids (SCFA) as per Wolin (1960) stoichiometry[22], which is as follows:

Fermentative CO_2 = A/2+P/4+1.5B

where A, P and B are moles of acetate, propionate, and butyrate respectively.

Fermentative CH_4 = (A+2B)-CO_2

where A and B are moles of acetate and butyrate respectively and CO_2 is moles of CO_2 calculated from previous equation.

Assumption: one mole of SCFA releases one mole of CO_2 from bicarbonate-based buffer described as buffering CO_2 and therefore, mmol of buffering CO_2 is equal to mmol of total SCFA generated during incubation.

Gas volume = mmol of gas x gas constant (R) x T

Where

R = the ratio between molar volume of gas to

temperature (Kelvin zero; K) i.e. (22.411/273 = 0.082),

T = incubation temperature (Kelvin); 273 + 39° C = 312 K

Total volume of gas (ml) calculated from SCFA production = (BG + FG) x CF

BG = gas volume (ml) from buffering of SCFA,

FG = fermentative gas (ml) (CO_2 + CH_4),

CF = correction factor for altitude and pressure which is 0.953 for Hohenheim at altitude 400m above sea level[23]

(The volume of 1 mmol of gas at 39° C in Hohenheim would be;

1 x 0.082 x 312 x 0.953=24.4 ml).

The origin and stoichiometry of gas production have been described in details[13,14]

The *in vitro* gas production measured after 24 h incubation of tannin containing browses in the presence or absence of PEG was strongly correlated with gas volume stoichiometrically calculated from SCFA. The relationship between SCFA production (mmol) and gas volume (ml) after 24 h of incubation of browse species with a wide range of crude protein (5.4-27 %) and phenolic compounds (1.8-25.3 % and 0.2-21-4 % total phenols and total tannins as tannic acid equivalent respectively) was [21]:

In the absence of PEG

SCFA = 0.0239.Gas -0.0601; R^2 = 0.953; N = 39; P<0.001 (I)

In the presence of PEG

SCFA = 0.0207.Gas + 0.0207; R^2 = 0.925; N = 37; P<0.001 (II)

These relationships are similar to that obtained for wheat straw.[24]

The SCFA production could be predicted from gas values using the above relationship. The level of SCFA is an indicator of energy availability to the animal. Since SCFA measurement is important for relating feed composition to production parameters and to net energy values of diets, prediction of SCFA from *in vitro* gas measurement will be increasingly important in developing countries where laboratories are seldom equipped with modern equipments to measure SCFA.

The stoichiometric balance also allows calculation of the CH_4 and CO_2 expected from the rumen fermentation if the molar proportions and amount of SCFA are known.

Kinetics of fermentation feedstuffs can be determined from fermentative gas and the gas released from buffering of SCFA. Kinetics of gas production is dependent on the relative proportion of soluble, insoluble but degradable, and undegradable particles of the feed. Mathematical descriptions of gas production profiles allows analysis of data, evaluation of substrate- and media-related differences, and fermentability of soluble and slowly fermentable components of feeds. Various models have been used to describe gas production profiles. France *et al* (1993; 2000) [25,26]combine modelling of the gas profiles with estimates of substrate loss and ruminal rate of passage and derive estimates of ruminal extent of degradation thus linking gas production to events in the rumen proper.

Procedure

Preparation and weighing the feed sample

Before weighing, grind the dry material through a 1 mm screen. Avoid very fine grinding because of observed differences in digestibility (*in vivo*) and gas production (*in vitro*) between coarse and finely ground roughage.

For fresh samples, use a cutting mill, a slow rotating meat cutter or a pair of scissors to chop the roughage.

Weigh about 200 mg DM of the sample on a weighing boat. 2 Push the piston (greased with vaseline to ensure easy movement and precise fitting) down the cylinder. Close the silicon rubber tube attached to the capillary attachment (needle) of the syringe with a plastic clip. Fermentation is carried out in this glass syringe.

Rumen fluid

Not more than 15 minutes before the trial starts, collect rumen fluid (about 1 litre) in equal proportions from two rumen-fistulated donor cows/small ruminants under the same feeding regime (at Debre Zeit, grass hay given *ad libitum* and a total of 2.4 kg cotton seed cake given in two meals daily). Filter the sample through two layers of cheese cloth into a warm flask (kept in a bucket of water at 37–38°C) and flush with carbon dioxide (CO_2). Take the rumen fluid before the morning feed or before feeding the diet supplement.

Solutions

Prepare five different solutions as media and mix with rumen liquor.

The composition of the solutions are as follows:

Solution A (Micro mineral solution)

13.2 g calcium chloride ($CaCl_2.2H_2O$)

10.0 g manganese chloride ($MnCl_2.4H_2O$)

1.0 g cobalt chloride ($CoCl_2.6H_2O$)

8.0 g iron chloride ($FeCl_3.6H_2O$)

made up to 100 ml with distilled water.

Solution B (Buffer solution)

39.0 g sodium hydrogen carbonate ($NaHCO_3$)
or

35.0 g $NaHCO_3$ + 4.0 g ammonium hydrogen carbonate ($(NH_4)HCO_3$)

made up to 1 liter with distilled water.

Solution C (Macro mineral solution)

5.7 g disodium hydrogen phosphate (Na_2HPO_4)

6.2 g potassium dihydrogen phosphate (KH_2PO_4)

0.6 g magnesium sulphate ($Mg\ SO_4.7H_2O$)

made up to 1 litre with distilled water.

Resazurin solution

100 mg resazurin made up to 100 ml with distilled water.

Reducing solution

4 ml sodium hydroxide (1N NaOH)

625 mg sodium sulphide ($Na_2S.9H_2O$)

added to 95 ml distilled water.

The reducing solution must be freshly prepared each time shortly before the rumen fluid is taken from the animal. The other solutions can be made up and stored.

Preparation of media

Pour 400 ml distilled water, 0.1 ml solution A, 200 ml solution B, 200 ml solution C and 1 ml resazurin into a Buckner flask. You will observe a bluish colour. Add 40 ml reducing solution while mixing with a magnetic stirrer. Flush the mixture with CO_2 gas while the reducing solution is being added. The colour will change from bluish through a reddish colour (oxidised) to colourless (reduced).

Add the rumen fluid. The ratio of rumen fluid to buffer medium is 1:2 (v/v).

Preparing syringes for incubation

Place the glass syringes containing the substrates in a water bath at 38–39°C an hour before incubation starts.

During incubation, remove the glass syringe from the water bath and firmly fix the rubber tube on to the needle of the automatic syringe.

Pipette 30 ml of the rumen fluid/medium mixture with an automatic syringe into each of the pre-warmed glass syringes. Bring any air bubbles trapped in the syringe to the surface by gentle shaking and remove them through the capillary attachment by careful upward orientation and pushing the piston. Close the clip on the tube, read the initial volume and record it as V0. Place the syringe back in the

syringe rack for incubation in the water bath at 38–39°C.

Twenty-four hour incubation

Incubate the feeds in triplicate in at least two different sessions (with different rumen fluids), yielding six parallel measurements. Include four glass syringes containing rumen fluid/ media mixture without substrate (blank), three glass syringes containing Sululta hay (200 mg DM), i.e. the standard, and three syringes containing 140 mg DM Sululta hay and 60 mg starch in every set to control differences in composition and activity of the rumen fluid (control incubations). The readings from the blank, grass hay and grass hay + starch are GP0, GPH and GPHS, respectively. The exact reading where the end mark on the piston lies is regarded as the initial volume (V0).

Read the position of the piston 6 h after incubation begins and record it as intermediate volume (Vint.).

Move the piston gently beforehand to make sure that it is not sticking. If gas production exceeds 60 ml, open the clip and move the piston back to the 30 ml mark, while keeping it vertical, thus allowing most of the gas which has formed to escape. Record the exact reading before the piston is moved back to 30 ml as V1 for the next incubation hour. Continue the incubation and take the final reading after 24 hours (Vfinal).

Sequential incubation (3, 6, 12, 24, 48, 72, 96 and 120 h)

To determine the volume of gas produced at 3, 6, 12, 24, 48, 72, 96 and 120 hours, a slightly modified procedure is followed regarding the number of parallel measurements. Except for the blank which is incubated in triplicate, the substrates and both standards are all incubated in duplicate for every incubation period (time).

In the 3- and 6-hour incubations, the gas produced is not expected to exceed 60 ml and thus there is no V1. For the rest of the incubation periods conducted in series, i.e. 12, 24, 48, 72, 96 and 120 hours, consider the 12-hour reading as the first calibrated volume (1V1). During calibration reset the piston to the 30 ml position for all of the syringes except for the blanks. Since there is no calibration before the 12-hour reading, the net gas production at 3, 6 and 12 h incubation periods is simply the final reading of gas produced minus the sum of V0 and blanks at these hours.

For all other readings taken at and after 24 hours of incubation, calibrate only when the gas produced exceeds 60 ml. Release the gas produced and set the piston back to 30 ml (second calibration). The second calibrated volume (2V1) is the sum of the 1V1 and the most recent reading taken before the second calibration. Use the 2V1, like the 1V1, only for the calculation of the net gas production for the succeeding incubation hour. Likewise, if there is a need to release the gas for the third time, the 3V1 is the sum of the 2V1 and the most recent reading before the gas is released. The same method of calculation applies in this case.

Calculations

Use the volumes of gas recorded at different times to estimate the *in vitro* gas production during incubation of the feeds. Gas production (GP) is defined as the total increase in volume minus the blank (GP0). Subtract the mean blank value (GP0) from the recorded gas production of all samples and standards to give the net gas production. Relate the gas volume from which the blank value has been deducted to the weight of exactly 200 mg DM of the sample taken.

After collecting ample data for the standards, calculate the standard value for, in the case of Debre Zeit, the Sululta grass hay and the Sululta hay + starch. Estimate the mean gas production (in ml/200 mg DM) with each of the standard feeds at each incubation period and calculate the correction factor for the corresponding periods. Divide the standard value for the Sululta grass hay by the measured net value of the same standard hay for the particular incubation session to give the correction factor (FH). The correction factor for hay + starch standard (FHS) is derived similarly. Use the

mean of these two factors FH and FHS for correction of the sample measurements.

It is necessary to check from the standards included in every set how far the recorded values deviate from the standard values. The difference between FH and FHS is expected to be insignificant. The theoretically accepted values for FH and FHS lie between 0.9 and 1.1. If the factors do not fall within this range, the test must be repeated.

The general formula for calculating the corrected gas production is:

GP (ml/200 mg DM) = (XV1 - 30X + Vfinal - V0 - GP0) x 200 ((FH + FHS) 2)

weight in mg DM where:

X = the number of times that the gas is released from the syringe and the volume is set back to 30 ml

V0 = the initial volume of gas recorded before incubation starts

V1 = the volume of gas recorded before the gas is released from the syringe and the volume is set back to 30 ml

Vfinal = the final volume of gas recorded at the end of incubation time

GP0 = the mean blank value

FH = the correction factor for the standard grass hay

FHS = the correction factor for the grass hay/ starch standard

DM = dry matter.

Data from gas production may be processed like data obtained with the nylon bag technique. More often the following model is fitted to the data:

$Y = b (1–e–ct)$

where:

Y = the volume of gas produced with time (t)

c = the gas production rate

b = the potential extent of gas production.

The intercept is not included in the model with the understanding that no gas is produced from unfermented feed.

Applicability of the in vitro gas production technique

Determination of microbial mass

In vitro gas tests are attractive for ruminant nutritionists since it is very easy to measure the volume of gas production with time, but the measurement of gas only implies the measurement of nutritionally wasteful and environmentally hazardous products. In most studies the rate and extent of gas production has been wrongly considered to be equivalent to the rate and extent of substrate (feed) degradation. Current nutritional concepts aim at high microbial efficiency, which can not be achieved by measurement of gas only. *In vitro* gas measurements reflect only SCFA production. The relationship between SCFA and microbial mass production is not constant and the explanation for this resides in the variation of biomass production per unit ATP generated. This can impose an inverse relationship between gas volume (or SCFA production) and microbial mass production particularly when both are expressed per unit of substrate truly degraded. This implies that selecting roughages by measuring only gas using *in vitro* gas methods might result in a selection against the maximum microbial mass yield. Blümmel *et al* (1997) have demonstrated how a combination of *in vitro* gas production measurements with a concomitant quantification of the truly degraded substrate provides important information about partitioning of fermentation products, and the *in vitro* microbial mass production can be calculated as:

Microbial mass (mg) = mg substrate truly degraded - (ml gas volume x stoichiometrical factor)

For roughages, the stoichiometrical factor was 2.20. [14]

Partitioning factor

The parameters in the above equation also allow the calculation of a partitioning factor (PF). The PF is defined as the ratio of substrate truly degraded *in vitro* (mg) to the volume of

gas (ml) produced by it (equivalent to the reciprocal of parameter *Y*.[25] The above equation becomes

Microbial mass (units) = gas volume (PF - stoichiometrical factor)

A feed with higher PF means that proportionally more of the degraded matter is incorporated into microbial mass, i.e., the efficiency of microbial protein synthesis is higher. Roughages with higher PF have been shown to have higher dry matter intake. Different *in vitro* PF values are also reflected by *in vivo* microbial protein synthesis as estimated by purine derivatives (the higher the PF, the higher the excretion of urinary purine derivatives; and in methane production by ruminants (the higher the PF, the lower the methane output.[23] These results show that the PF calculated *in vitro* provides meaningful information for predicting the dry matter intake, the microbial mass production in the rumen, and the methane emission of the whole ruminant animal.

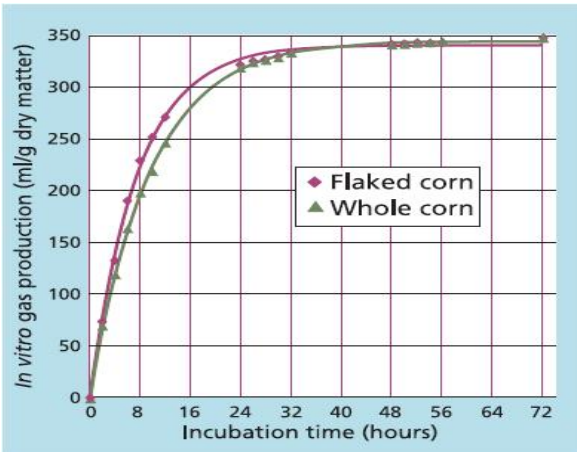
The procedures for the determination of truly degraded substrate and the calculation of the stoichiometrical factor; stoichiometrical relationship between SCFAs and gas volume; and relationship between SCFA production, ATP production and microbial mass yield can be obtained.[13,14] It may be noted that these procedures and relationships are valid for substrates consisting predominantly of structural carbohydrates, and the findings might not extend to substrates such as those high in soluble carbohydrate, protein or fat. Rymer and Givens (1999) have shown that, as observed by Blümmel *et al* (1997), good quality feeds (grass silage, wheat, maize, molasses sugarbeet feed and fishmeal) which produce large amounts of gas and SCFA yield small amounts of microbial mass per unit of feed truly degraded.[27,14]

It seems therefore justified to suggest that feeds or feed ingredients should be selected that have a high *in vitro* true degradability but low gas production per unit of truly degraded substrate. Dijkstra *et al* (2000) have described modelling of microbial protein synthesis *in vivo* from the *in vitro* gas parameters.[28]

Digestion kinetics of neutral detergent-soluble fraction

The gas measurement method has also been used to study digestion kinetics of the neutral detergent soluble fraction of forages, starch-rich feeds and other highly digestible carbohydrate components, which was obtained by subtracting the average gas production curve for the digestible neutral detergent fibre (NDF) from that of the unfractionated whole feed.[29]The subtraction procedure might give some useful information relevant to low-NDF fibre feeds, e.g., corn grain [29] but it is not suitable for forages rich in NDF.[30] Blümmel *et al* (1998b) examined the rate and extent of fermentation of whole roughage and extracted NDF, dry matter degradability of extracted NDF and the PF for whole roughage and the extracted NDF of 54 roughages.[30] The 24-h degradabilities of extracted NDF were higher than NDF degradabilities in whole roughages, and the PF values were lower for extracted NDF than for whole roughages (2.5 vs 3.1; i.e. the efficiency of microbial protein synthesis with extracted NDF was lower). Both the higher degradability and lower PF contributed to higher gas volumes obtained from extracted NDF compared with whole roughage. Supplementation of amino acids and sugars, which essentially constitute the solubles, may increase the efficiency of microbial synthesis from cell walls during fermentation (a situation similar to that in unfractionated forages) and

Fig 1: Kinetics of *in vitro* gas production during incubation of flaked and whole corn grain (Getachew et al. 2001)



the removal of solubles may result in lower microbial efficiencies. A considerable effect of cell solubles on partitioning of nutrients from the NDF raises doubts as to the significance *in vivo* of the kinetic parameters calculated using the subtraction procedure.[30]

Voluntary intake prediction

The main constraint to the utilisation of roughages by ruminants is voluntary feed intake so prediction of feed intake, particularly of fibrous roughage, is one of the important aspects of ruminant nutrition. *In vitro* gas production has been used to predict dry matter intake. Various workers have reported significant correlation between in vitro gas production and dry matter intake. Forage cell walls have considerable influence on voluntary feed intake through rumen fill mechanism.[8] Gas production from extracted neutral detergent fibre was shown to be better correlated to voluntary feed intake than the values obtained from the incubation of whole roughage. The use of various models for intake prediction was investigated and it currently appears that combination of gas volume measurements (4-8h) with concomitant determination of the amount of substrate degraded (> 24 h) is superior to the models based on kinetics of gas production only. The *in vitro* gas production from NDF explained more (82 % vs. 75 %) of the variation in dry matter intake than gas production from whole roughage.[13]

Interaction between dietary constituents

Gas measurement was also employed for evaluation of the interaction between basal and supplementary diets by incubating basal diet and supplementary diet separately as well as in combination and monitoring gas production at different hours of incubation using the pressure transducer system.[31]This will indicate the availability of readily fermentable material as a ready energy source, which will stimulate the activity of the rumen micro-organisms which in turn would accelerate the digestion of roughages. These workers, by incubating the basal diet and the supplement, observed a positive interaction in gas production in the early hours of incubation, which according to the authors can be an approach to study the synergetic effects of supplementation. However, it must be pointed out that measurement of gas only, could lead to misleading results. It is suggested to determine microbial mass production in addition to the gas measurement for such studies.

Organic matter digestibility

The digestibility of measured organic matter is closely correlated with that predicted from gas production and the crude protein and ash contents of feeds. Therefore, the method can be used to predict the extent of digestion for various feeds as below:

OMD (%) = 14.88 + 0.889 Gp + 0.45 CP + 0.0651 XA, R^2=0.92

Energy contents of feeds

The gas method has also been used successfully to predict the ME content of feeds. A regression equation has been developed with data generated by *in vivo* studies conducted with a variety of feeds and *in vitro* gas production. The gas measurement provides a better estimate of the ME level of feeds, when combined with some chemical constituents, compared with calculations based on chemical constituents only (see below).

ME (MJ / Kg DM) = 2.20 + 0.136 Gp + 0.057 CP, R^2= 0.94

Effects of added fat on feed degradation

Tallow and yellow grease (YG), both rendering byproducts, are typical fats used in the diets of lactating dairy cows. The gas technique was used at UC Davis to examine the effect of sources and levels of added fat on gas production and rumen fermentation of a total mixed ration.[32]Fatty acids in the form of triglyceride (YG) had no effect (when comprising up to 25% of the diet) on gas

production, but fatty acids in the form of potassium salts (YG soap) significantly depressed gas production. In the animal, however, there is a limit to the amount of fatty acids that can be successfully fed, and this is lower than *in vitro*. The fatty acids in potassium salts are quickly available to microbes as free fatty acids in ruminal fluid, and have detrimental effects on microbial growth.

In contrast, the fatty acids in the triglyceride form must be released through hydrolysis of the ester bond and therefore are available at a slower rate. Hydrolysis refers to breaking the chemical bond between the individual fatty acid and the glycerol backbone of the triglyceride.

The effects of fatty acids on rumen fermentation are important because feeds with high levels of residual fat, for example rice bran created in the production of white rice, are commonly fed to ruminants.

Anti-nutritive factors

The gas method can be used to measure how microbial activity lowers feed digestibility. Some feeds, such as forage legumes and cotton seed, contain phenolics, alkaloids and saponins that have negative biological effects on microbes and reduce microbial growth in rumen. Tannins are naturally occurring polyphenolic compounds found in plants, which form complexes with feed and microbial proteins and can depress feed digestibility in the rumen.

Fig 2: Effect of Yellow Grease (YG) and Yellow Grease Soap (YG Soap) on in vitro gas production (Getachew *et al*, 2001)

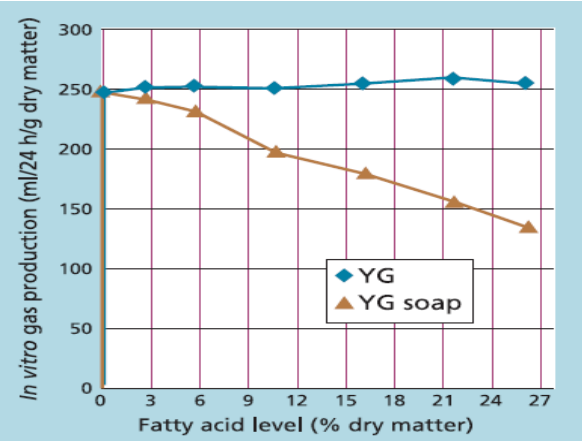
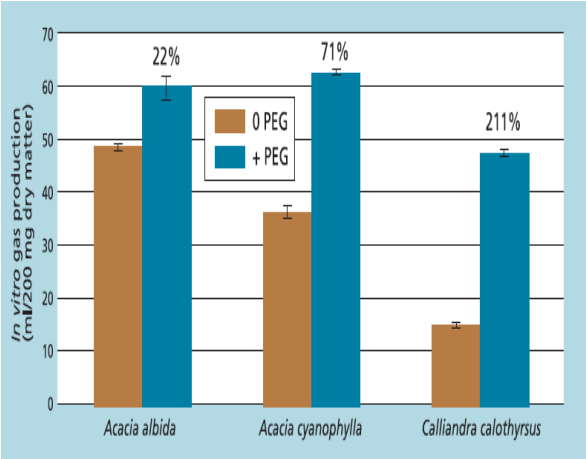


Fig 3: In vitro gas production of tannin-containing leaves in absence of PEG (0 PEG) and presence of PEG (+ PEG)



The effect of tannins on the nutritive value of feeds can be studied using tannin-binding agents, such as polyethylene glycol (PEG), which strongly binds to tannins and inhibits their biological effects. The percent increase in gas production when PEG is present indicates the rate at which tannins depress rumen fermentation of feeds.

After adding PEG to limit tannin effects, gas production increased by 22%, 71% and 211% in apple ring acacia *(Acacia albida)*, beach acacia *(Acacia cyanophylla)* and red calliandra *(Calliandra calothyrsus)*, respectively, which are browse plants.[33]

Rumen modifiers

The gas method is also utilized to study feed additives and rumen fermentation modifiers, such as monensin sodium, by incubating feeds in the presence or absence of these compounds. Rumen modifiers are compounds that are added to the diet to modify the populations of bacteria in the rumen. For example, some compounds are fed to reduce methanogenic bacteria to reduce methane production in the rumen. Previous studies have shown that the addition of saponins and tannins in an *in vitro* system increases microbial protein synthesis .[15] Yeast and yeast fermentation products are routinely added to the diets of lactating dairy cows, although their mode of action has not been clearly identified. By studying the impact

of various rumen modifiers on microbial fermentation, effects important to milk production in commercial dairy farms can be quantified.

Feed associative effects

The *in vitro* gas production method is currently being used to assess "associative" effects of feeds used in rations. Rations are mixtures of individual feeds, with a multitude of possible combinations. The energy value of a ration is generally calculated by adding up the energy values of the individual feeds in the ration, on the assumption that the individual energy value of any particular feed is the same in every possible combination with other feeds. However, this is not always true. For example, when poor quality forage — such as wheat straw — is fed to a ruminant, its digestibility is low, but by adding nitrogen in the form of urea or protein, the digestibility of the straw will be increased and in turn, the energy derived from straw organic matter in the diet will be increased. Recent studies indicate that positive associative effects on *in vitro* gas production occurred when rice straw was incubated in mixtures with hay or mulberry leaves.

Monitoring rumen microbial change

In addition to rates and extents of digestion, the gas production method can be used to study substrate related factors that influence microbial populations in the rumen. This enables manipulation of rumen microflora to increase the utilization of feeds through degradation of fiber and lignin, increasing the efficiency of nitrogen utilization or allowing the degradation of anti-nutritional and toxic components of feeds. Such controlled fermentation systems could potentially be used with genetic engineering of plants to solve animal productivity problems.

The technique is suitable for application of molecular-based assays, such as polymerase chain reaction (PCR) and ribonucleic acid (RNA)–targeted oligonucleotide probes, to study and measure rumen microbial growth, with the goal of increasing the efficient utilization of feeds and reducing environmental impacts. Recently, Muetzel and Becker (2003) used the gas technique, in combination with ribosomal RNA targeted probes, to measure the efficiency of microbial growth when barley straw was supplemented with legume leaves.

Nutrient synchronization

Carbohydrate and nitrogen sources must be available simultaneously in order to maximize microbial growth. Ruminal ammonia concentrations can be influenced by the degradation rates of carbohydrates and nitrogen-containing compounds. For a given level of dietary protein, an increased rate of protein degradation enhances the ruminal ammonia concentration while an increased rate of carbohydrate degradation decreases it. Increased carbohydrate availability for fermentation promotes microbial growth and as a result less nitrogen is lost from the rumen in the form of ammonia-nitrogen.[21] The gas method offers an opportunity to study microbial requirements for nitrogen and carbohydrate to enable efficient fermentative activity and accumulation in the rumen. Using this technique, studies have been conducted to assess rumen microbial requirements for nitrogen when different types of carbohydrate sources are incubated.

Plant breeding and biotechnology

We believe that animal nutritionists and plant geneticists should collaborate to select genetic materials that have better agronomic performance and superior nutritional qualities. Siaw *et al* (1993) used the gas technique to evaluate large numbers of browse species in order to select those high in feeding value. Browse is the edible parts of woody vegetation such as leaves, stems and twigs from bushes common on California foothills; they have been identified as integral to the development of fires that ravaged Southern California in the fall of 2003. Several forage and cereal crops have been genetically modified to increase yield, or produce chemical constituents normally deficient in a particular plant.

Plants have also been genetically engineered to produce human lysozyme, but it is unclear what effect lysozyme has on microbes in the rumen. Although many genetically engineered plants are intended for human consumption, their byproducts will be fed to animals as a means of disposal. The starch contained in cereals, including corn and milo, is found in granules surrounded by a tough protein matrix that reduces enzymatic degradation. There are new genetic varieties of these cereals with modified protein matrices. We are currently using the gas production method to explore whether these new varieties increase the extent and rate of starch digestion.

Environmental degradation

More than half of the nutrients consumed by ruminants leave the animal unutilized and undigested, and are excreted in feces, urine and gases. This increases animal production costs as well as environmental impacts, by contaminating surface and groundwater and contributing to air pollution. The nitrogenous and organic compounds excreted are further decomposed and can cause odors in residential areas. Increasing the efficiency of feed utilization reduces the amount of unutilized nutrients leaving the animal. Significant reductions in nitrogenous compounds and in methane can be achieved by manipulating animal diets. The *in vitro* gas method can be used to study the efficiency of feed utilization and to examine animal waste components that impact the environment in order to develop appropriate mitigation strategies.

Factors affecting the accuracy of the IVGPT

Many factors such as sample preparation and size, buffer and media, incubation conditions and time of reading, host animal management or material sampled, combine to influence activity of the microbial inoculum. That the prime purpose of the inoculum is to provide a suitable microflora with which to ferment or degrade a feed over time, and to use the outcome, for example, to provide an estimate of rate of in vivo digestibility, seems to have been overlooked. This has resulted in a series of statistically significant correlations, which are of little predictive use.

Considerable research has been conducted to reduce the requirement to surgically modify animals, and this is to be applauded. However, and while of no direct consequence, it should be recognised that faecal and rumen inocula are dissimilar. In addition, the methodologies used need to be fully described and appropriate conclusions drawn. It would appear that under certain conditions, for example, where long term in vitro end-point degradation assays are completed, that faeces have the potential to replace rumen fluid.

However, where precise fermentation kinetic data are required, the data suggests that fresh rumen fluid must be used. In vitro methodologies, in particular those such as gas production that are based on estimating the rate of fermentation, are highly adaptable and powerful research tools. To ensure their optimum application, it is vital that controls exist on the type and quality of inoculum. To this end, a series of research programs is required to address specific issues.

Considering the range of *in vitro* methodologies and equipment employed, it is unlikely that a particular system will be accepted as the 'standard' procedure. However, a great deal can be done to reduce variation among inocula by adopting an agreed upon set of guidelines relating to the host animal, sampling technique and inoculum preparation. A method to assess inoculum 'quality' needs to be developed with respect to its fermentative and/or degradative activity. This would allow the impact of preparation techniques to be accurately assessed. Equally a technique to store rumen fluid without loss of efficacy would allow use of rumen contents obtained following slaughter of known donor animals to be used, so obviating the requirement for surgically modified animals or use of faecal inocula. Finally, alternative approaches such as statistical mapping of fermentation profiles to generate estimates of degradation, need further investigation.

Advantages and limitations of the IGPT

The *in vitro* rumen fermentation method in which gas production and microbial mass

production are concomitantly measured has several major advantages:

It has the potential for screening a large number of feed resources, for example in breeding programmes for the development of varieties and cultivars of good nutritional value?it could also be of great value in the development of supplementation strategies using locally available conventional and non-conventional feed constituents to achieving maximum microbial efficiency in the rumen.

It has an important role to play in the study of rumen modulators for increasing efficiency of microbial protein synthesis and decreasing emission of methane, an environmental polluting gas. It provides a better insight into nutrient-anti-nutrient and anti-nutrient-anti-nutrient interactions. The method is also being used increasingly to screen plant-derived rumen modulators. These products have a lower toxicity to animals and humans, and are environmentally friendly. Consequently, they are becoming increasingly popular with consumers.

Further studies are required on:

- the development of simple approaches for identifying the incubation time in the *in vitro* gas system at which the PF (a measure of the proportion of fermented substrate which leads to microbial mass production) is maximum,
- the effect of nitrogen in the incubation medium on the PF,
- the *in vivo* significance of the PF so obtained.

The results of the limited experiments conducted so far have shown that simple models employing gas kinetic parameters and the PF are capable of predicting the dry matter intake of roughages and level of emission of methane by ruminants. Experiments also need to be done to test whether, for any given feed, the microbial protein synthesis as derived from digestion kinetic parameters (including PF) *in vitro* is sufficient to explain the observed microbial protein supply to the small intestine *in vivo*. At present, the simplest way of determining the latter parameter is to calculate it from the level of urinary purine derivatives.

This validation exercise should be conducted for a wide range of feed constituents and diets which should enable the above mentioned simple technique of measuring gas and microbial mass to be a routine and powerful tool for feed evaluation thus avoiding the need for timeconsuming, laborious and expensive feeding studies. Lately, much emphasis has been given to the development of statistical or mathematical models, which fit best the gas production profiles and describe the gas evolution with high accuracy.

Experiments must be designed to understand the biological significance of the various statistical and functional parameters being calculated using these models, and also to incorporate a measure of microbial mass into these mathematical descriptions.

Enhancement of the feeding value of tannin-rich feeds can be achieved by anaerobic storage in the presence or absence of urea, by the use of oxidising agents, by the treatment with white-rot fungi or by the use of PEG, preferably in a slow release form. PEG can be added to forages rich in tannins along with an energy supplements or to tannin-rich byproducts low in energy with the aim of synchronising nitrogen degradability and availability of energy and thus increasing the efficiency of microbial protein synthesis.

PEG is best given as an ingredient of nutrient blocks. so that not only will it enhance the incorporation of the feed nitrogen into microbial mass but will also allow the livestock to self-regulate the intake of PEG, thereby decreasing the cost of the treatment. The aim of future studies should be to explore the potential of these approaches for a wide range of tannin-containing feeds, and then to develop simple and economically viable detanninification approaches for use by farmers for feed resources such as foliage from trees and shrubs and for other available by-products.

Other techniques will be required for use by small-scale industry to treat agro-industrial and forestry by-products which are available in large quantities in one place. These approaches will help to alleviate the problems posed by the disposal of various agro-industrial byproducts and the shortages of conventional feeds.

Conclusions

The *in vitro* rumen fermentation method in which gas production and microbial mass production are concomitantly measured has several major advantages:

i) it has the potential for screening a large number of feed resources, for example in breeding programmes for the development of varieties and cultivars of good nutritional value,

ii) it could also be of great value in the development of supplementation strategies using locally available conventional and unconventional feed constituents to achieving maximum microbial efficiency in the rumen;

iii) it has an important role to play in the study of rumen modulators for increasing efficiency of microbial protein synthesis and decreasing emission of methane, an environmental polluting gas, and

iv) it provides a better insight into nutrient-antinutrient and antinutrient-antinutrient interactions, and into the roles of various nutrients (by changing the composition of the incubation medium) with respect to production of fermentative gases, SCFA and microbial mass. The method is also being used increasingly to screen plant-derived rumen modulators. These products have a lower toxicity to animals and humans, and are environmentally friendly. Consequently, they are becoming increasingly popular with consumers.

Further studies are required on:

i) the development of simple approaches for identifying the incubation time in the *in vitro* gas system at which the PF (a measure of the proportion of fermented substrate which leads to microbial mass production) is maximum,

ii) the effect of nitrogen in the incubation medium on the PF, and iii) the *in vivo* significance of the PF so obtained. The results of the limited experiments conducted so far have shown that simple models employing gas kinetic parameters and the PF are capable of predicting the dry matter intake of roughages and level of emission of methane by ruminants. Experiments also need to be done to test whether, for any given feed, the microbial protein synthesis as derived from digestion kinetic parameters (including PF) *in vitro* is sufficient to explain the observed microbial protein supply to the small intestine *in vivo*. At present, the simplest way of determining the latter parameter is to calculate it from the level of urinary purine derivatives. This validation excercise should be conducted for a wide range of feed constituents and diets which should enable the above mentioned simple technique of measuring gas and microbial mass to be a routine and powerful tool for feed evaluation thus avoiding the need for time-consuming, laborious and expensive feeding studies. Lately, much emphasis has been given to the development of statistical or mathematical models that best fit the gas production profiles and describe the gas evolution with high accuracy. Experiments must be designed to understand the biological significance of the various statistical and functional parameters being calculated using these models, and also to incorporate a measure of microbial mass into these mathematical descriptions.

Research and development efforts are required to establish a feed library for unconventional feedstuffs that includes information on nutritive values in addition to routine composition analysis. In the case of tannin-containing feedstuffs, there is a need to incorporate approach(s) measuring the

biological activities of tannins as well as measuring tannin levels by chemical methods.

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Nutritional Strategies to Combat the Effect of Heat Stress in Chicken

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Abstract

Heat stress reduces the production performance of chicken leads to immunosuppression and increases the mortality rate in chicken. All the nutritional supplements (e.g. vitamins, zinc, Kcl) have functions in relation to heat stress. Suitable mineral and vitamin premixes can be developed for heat stressed chickens for improved performance, welfare and reducing feed cost. Heat stress increase the serum concentration of ACTH which increase corticosteroid level and in turns reduces the production. Dietary supplementation of vitamin C (200 mg/kg) improved the egg production and egg shell quality in laying hen during summer stress. Ascorbic Acid supplementation (300 mg/kg of diet) improved body weight gain, feed conversion ratio and decreased the mortality in broilers. Dietary supplementation of anti-oxidant vitamins (vitamin E or vitamin C in combination) is helpful to maintain the growth performance, egg production and improvement in egg quality. Vitamin E (250 mg/kg diet) increased serum concentration of T_3, T_4 and decreased concentration of ACTH thereby production is maintained. Low protein diet (14%) with provision of additional methionine @ 0.44% maintained the production performance of laying hens. Supplementation of Dietary Electrolyte Balance like NaCl, $NaHCO_3$, $KHCO_3$ and NH_4Cl (360 m Eq/kg) in heat stress can improve eggshell quality of laying hens. Supplementation of 0.1% KCl with 400 mg vitamin C showed better performance for broilers reared under heat stress. Zinc in combination with vitamin A improves performance and carcass quality of broiler under heat stress.

Keywords: Immunosuppression; Acth; Electrolyte; Vitamin C; Vitamin E; Stress; KCl; Vitamin A.

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Introduction

Stress

The term stress is described as, "a physical or psychological stimulus that can produce mental tension or physiological reactions that may lead to illness."

Or

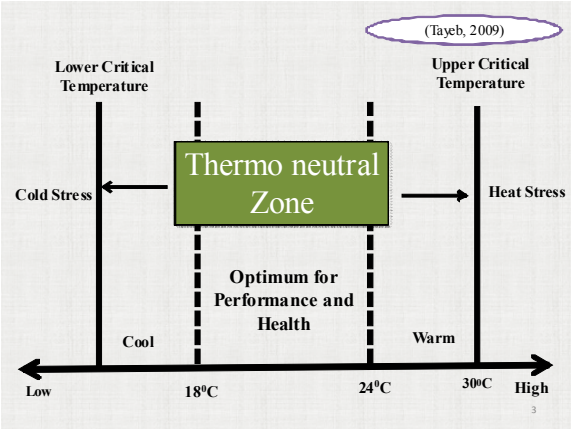
Deviation from Normal Physiological behaviour.

Or

The term "Stress" is used to describe the detrimental effects of variety of factors on the health and performance of poultry. Birds have limited body resources for growth, reproduction, response to environmental changes and defense mechanism.

Factor affecting stress

1. *Environmental:* Poor Ventilation, ammonia gas, Pollutants, wet liter, high light intensity
2. *Climatic:* Extreme heat / cold, humidity
3. *Physical:* Catching, Handling Transport, Injections, Ion mobilization etc.
4. *Nutritional:* Nutrient Shortages, Feed intake Problems, Adulterated feed, Toxic
5. *Physiological:* Rapid growth, high egg prod., Process of sexual Maturing, Molting etc.
6. *Social:* Over crowding various age/size, Grouping
7. *Psychological:* Fear, harsh care takers, abrupt changes etc.



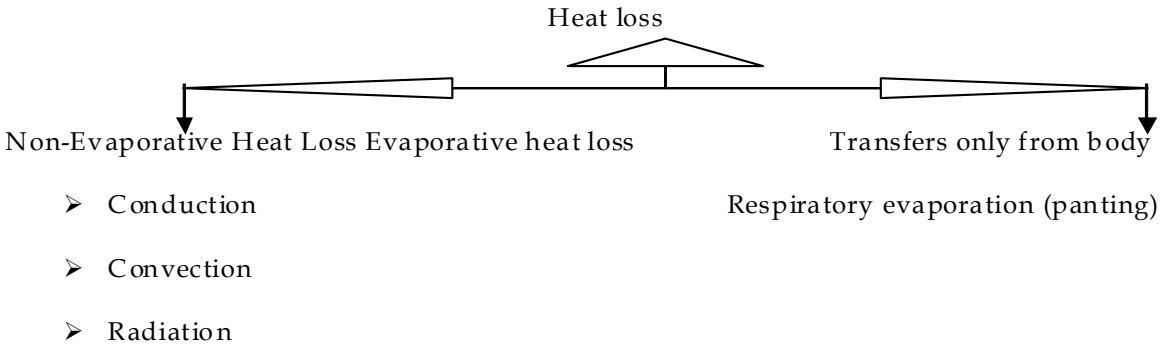
Exposure to high environmental temperature is of major concern for the poultry industry especially in hot regions of the world because of the poor performance and high mortality in chicken.[1]Birds can be reared in a thermoneutral zone (18 ^{0}C and 24 ^{0}C). When temperature increases beyond 30^{0}C it causes heat stress in poultry.[2]

Thermo Neutral Zone: The range of ambient temperature within which the animal doesn't need to expend any additional energy in order to regulate body temperature.[3]

Thermal balance: It is the sum of heat gain and heat loss**.**

Heat stress: It is a term commonly used to describe the birds response to elevated temperature and humidity, where abnormal response to increased heat dissipation such as, increase respiratory rate and panting. [3]

- High ambient temperature is of great concern in all types of poultry operations.



- Heat loss in poultry is limited due to feathering and the absence of sweat glands.
- When the temperature and relative humidity exceed the comfort level of a bird, it loses the ability to efficiency dissipate heat.
- High ambient temperatures compromise performance and productivity through reducing feed intake and decreasing nutrient utilization, growth rate and egg quality, which lead to economic losses in poultry.

Physiological mechanism

When animal first encounters a stressor, the neurogenic system is activated. It leads to release of neurotransmitters like NE and E. Stressors immediately results in the activation of the hypothalamic-pituitary-adrenal cortical system. When this system is activated, the hypothalamus produces corticotrophin-releasing factor (CRF), which in turn stimulates the pituitary to release adrenocorticotropic hormone (ACTH). Secretion of ACTH causes the cells of adrenal cortical tissue to proliferate and to secrete corticosteroids[4]

Why birds are more susceptible to high temperature?

- Lack of sweat glands
- Feather covered body
- Low surface area
- Higher Basal Metabolic Rate [5]

Consequences of heat stress

Effect of heat stress on feed intake and growth performance of chicken: At high environmental temperature feed intake of birds were decreased and ultimately decreased egg production and egg quality.[6]

High ambient temperature reduces the performance such as feed intake, live weight gain and feed efficiency in broilers.[7]

Effect of heat stress on nutrient retention: At high environmental temperature retention of dry matter, fat, protein and starch will be reduced and availability will lead to reduction in growth and production.[8]

Effect of heat stress on immunity: Heat stress causes release of corticosteroids and catecholamine's and causes lipid per oxidation of cell membrane mainly T-Lymphocytes, which have mainly immune-suppression action .[9]

Effect of heat stress on acid base balance: At high ambient temperature body temperature and respiratory rate of birds ultimately decrease PCO_2 level in blood plasma. In turn, bicarbonate buffer system decreases the concentration of carbonic acid and hydrogen ions and increase plasma p^H.[10]

In response to that, kidney increase bicarbonate ions excretion and decrease Hydrogen ion excretion as an attempt to keep birds in acid base balance. Excretion of more bicarbonate ion leads to respiratory alkalosis .[11]

Effect of heat stress on egg quality: In laying hens increase bicarbonate excretion ultimately decrease plasma concentration of bicarbonate ions causes limiting availability of anion required during formation of $CaCO_3$ crystals in the shell. So egg shell quality comprises .[12]

Nutritional strategies: Nutritional strategies include supplementation of:

I. Vitamins
II. Electrolytes
III. Amino acids
IV. Minerals[13]

Vitamins: Vita E, C and A used in poultry diet because of their anti stress effects and also because of their synthesis is reduced during the

Table 1: Effect of vitamins supplementation on feed intake and egg production

Vitamin C (mg/kg diet)	Feed intake (g/hen/day)	Feed efficiency (egg mass / feed)	Egg production (%)	Egg weight (g)
0	96.24	0.384[b]	74.79[b]	49.35
200	96.25	0.414[a]	80.24[a]	49.61
400	96.24	0.409[a]	79.60[a]	49.52
600	98.45	0.396[a b]	79.23[a]	49.32

Means with different superscripts within column differ significantly (P<0.05), n= 120 (4 Group), 46-54 wks, March – June, Ambient Temp. = 30 ^{0}C - 44 ^{0}C

heat stress.[14]

Why vitamins are necessary in heat stress: Vitamin E protect the lymphocytes and macrophages due to its anti oxidant property and enhanced proliferation and function of these cells.[15]

Vitamin A decrease Synthesis and secretion of corticosteroids which help to alleviate negative effect of heat stress.[16]

Vitamin C has been reported to enhance immune response by modifying corticosteroid synthesis in adrenal gland.[17]

Vitamin C plays a role in bone maturation by improving hydroxyproline production, which is required for collagen formation. Hence it is postulated that vitamin C stimulates 1, 25 dihydrocholecalciferol and increase calcium mobilization from bone, suggesting that vitamin C has important role in eggshell formation[8]

Effect of vitamin C supplementation on productive performance of WL

Panda *et al (*2007) carried out an experiment on 120 white Leghorn birds to found effect of vitamin C on feed intake and egg production and they reported that levels of Vitamin C supplementation did not influence food consumption but FCR was increased significantly due to supplementation of Vitamin C at either 200 or 400 mg/kg diet. Egg production increased significantly by supplementing 200 mg/kg Vita C as compare to control (Table 1).[17]

Ciftci et *al (*2005) conducted an experiment on 120 White Leghorn hens and reported that Hen day egg production was increased significantly and mortality percent decrease significantly in vitamin supplemented groups as compare to control group. [19]However, when both vitamin E and C were given in combination, the highest body weight was observed achieved, improved FCR and hen day egg production and decreased mortality up to 50% as compared to control group (Table 2).

Effect of vitamins supplementation on egg quality

Panda *et al (*2007) reported that egg quality parameters like specific gravity, shell breaking strength and haugh units were not influenced by Vitamin C supplementation. However, shell weight and shell thickness were improved significantly due to 200 mg/kg Vitamin C. No further benefits in these parameters could be observed by enhancing the level of supplementation beyond 200 mg/kg diet

Table 2: Effect of dietary vitamin E and C supplementation on growth and egg production performances in laying hens

Groups	Feed intake (g/hen/day)	Body weight (g)	Feed conversion ratio (g feed/ g egg)	Hen day egg production (%)	Mortality (%)
Control	92.02	1650.5[a]	2.21[a]	82.25[d]	6.25[a]
Vitamin E (125 mg/kg diet)	93.08	1680.0[a]	1.88[a]	84.25[c]	5.08[b]
Vitamin C (200 mg/kg diet)	93.09	1685.5[ab]	1.85[ab]	85.92[b]	4.06[c]
Vitamin E + Vitamin C	94.07	1697.0[b]	1.72[b]	88.29[a]	3.01[d]

Means with different superscripts within column differ significantly (P<0.05)
N= 120 Hyline-White leghorn hens, 150 days old (4 G), July 15th -sep.15th,Temp = 26 ^{0}C - 36 ^{0}C, R.H. = 50 % - 75 %

Table 3: Effect of Vitamin C supplementation on egg quality of White Leghorn layers

Vitamin C (mg/kg diet)	Specific gravity	Shell breaking Strength (Newton)	Shell weight (%)	Shell thickness (mm)	Haugh unit
0	1.074	28.05	8.99[b]	0.353[b]	64.24
200	1.075	27.36	9.26[a]	0.365[a]	63.82
400	1.074	29.14	9.24[a]	0.361[a]	64.28
600	1.073	27.04	9.28[a]	0.363[a]	65.06

Means with different superscripts within column differ significantly (P<0.05), n= 120 (4 G), 46-54 wks, March – June, Ambient Temp. = 30 0C - 44 0C

Table 4

Treatments	Shell thickness (mm)	Egg shell (%)	Egg yolk (%)	Hough unit
Control	0.2958	9.07	26.60[b]	82.70
Vitamin E (200 mg/kg diet)	0.3021	9.34	28.33[a]	84.25
Vitamin C (200 mg/kg diet)	0.2998	8.89	27.77[ab]	85.27

(Table 3). [17]

Maziar et *al (*2007) found the effect of Vitamin E and C on egg quality of White Langhorne. They showed that egg shell thickness was higher than control but did not significantly.[20] However yolk % was increased significantly (Table 4).

Effect of vitamins supplementation on Humoral immune response of heat stress in Layers

Maziar et *al (*2007) also found the effect of vitamins supplementation on antibody titer of 36 White Leghorn and concluded that vitamin supplemented group significantly higher antibody titer as compare to control group (Table 5).[20]

Effect of vitamins supplementation on feed intake and growth performance of broilers under heat stress

Onu (2009) reported that birds fed supplemental ascorbic acid achieved significantly higher weight gain than control.[21] The 300 mg of ascorbic acid supplementation in broiler starter diet gave significantly (P<0.05) the highest body weight gain. Birds fed 300 mg/kg ascorbic acid of feed recorded significantly (P<0.05) lower mortality (Table 6).

An experiment conducted by Sahin *et al* (2001) to know the effect of vitamin E and A on performance of 120 broilers. They concluded that supplementation of Vitamin A and E either considered separately or as a combination, increased feed intake and body weight gain significantly in broilers.[14] However feed efficiency remains similar in all treatment. (Table 7).

Effect of vitamins supplementation on serum concentration of T_3, T4, ACTH and anti oxidant status in poultry

Table 5: Effect of vitamin E and vitamin C on immune response of laying hens

Treatments	Antibody titer (log_2)
Control	6.33[b]
Vitamin E (200 mg/kg diet)	8.03[a]
Vitamin C (200 mg/kg diet)	7.83[ab]

Means with different superscripts within column differ significantly (P<0.05).
N= 36 (3 G) White leghorn (Hylinevariety), 42 days,
Temp = 33 ^{0}C - 35 ^{0}C, R.H. = 35 % - 55 %

Table 6: Effect of Ascorbic Acid supplementation on performance in broilers

Treatments	Body weight gain (g)	Feed intake (g)	Feed conversion ratio	Mortality (%)
Control	906.78[c]	3422.46	3.78[c]	13.33[c]
Vitamin C (150 mg/kg)	1081.21[ab]	3466.83	3.21[ab]	6.67[b]
Vitamin C (300 mg/kg)	1217.64[a]	3463.98	2.86[a]	3.33[a]
Vitamin C (450 mg/kg)	1057.00[b]	3492.60	3.30[b]	6.67[b]

Means with different superscripts within column differ significantly (P<0.05)
N= 120 Anak 2000, 4 G (7 day old) 35 days

Table 7: Effects of vitamin E (250mg/kg) and vitamin A (1500 IU/kg)supplementation on performance in broilers reared under heat stress

Treatments	Feed intake (g)	Body weight (g)	Feed efficiency (g body weight/g feed)
Control	3116.8[a]	1832.0[a]	0.58
Vitamin A	3151.8[b]	1890.14[b]	0.59
Vitamin E	3227.1[c]	1900.05[bc]	0.59
Vitamin A + Vitamin E	3314.8[d]	1985.78[c]	0.60

Means with different superscripts within column differ significantly (P<0.05)
N= 120 (Cobb 500 male broiler) 42 days, Temp = 32 ^{0}C, R.H. = 36-48 %

Sahin*et al* (2001) reported that Serum concentration of T3 and T4 were significantly higher and ACTH concentration in serum was lower in vitamins supplemented group as compared to the control group (Table 8).[14]

Table 8: Effects of vitamin E (250mg/kg) and vitamin A (1500 IU/kg) supplementation on serum concentration of T3, T4 and ACTH in broilers reared under heat stress

Treatments	T_3 (ng/ml)	T_4 (ng/ml)	ACTH (ng/ml)
Control	0.73[a]	4.11[a]	17.90[a]
Vitamin A	0.82[b]	4.42[b]	17.05[b]
Vitamin E	0.83[b]	4.45[b]	16.93[b]
Vitamin A + Vitamin E	0.88[c]	4.55[c]	16.13[c]

Means with different superscripts within column differ significantly (P<0.05)
N= 120 (Cobb 500 male broiler) 42 days, Temp = 32 ^{0}C, R.H. = 36-48 %

Table 9. Effect of vitamin C supplementation on anti oxidant status of Layers

Antioxidant enzymes	Supplemental vitamin C in diets (mg/kg)			
	0	200	400	600
Catalase, (K/g heamoglobin	284.71[c]	322.35[b]	361.01[a]	321.54[b]
Lipid peroxidation, (nmol MDA/mg protein)	286.26[a]	258.36[b]	243.67[c]	229.52[d]
Glutathione peroxidase, (unit/ml)	1.62	1.69	1.62	1.65

Means with different superscripts within column differ significantly (P<0.05)
n= 120 (4 G), 46-54 wks, March – June, Ambient Temp. = 30 ^{0}C 44 ^{0}C

Table 10: Effects of vitamin E supplementation on mineral concentration in broiler chicks

Vitamin E (mg/kg)	Ca (mg/dl)	P (mg/dl)
0	17.12	5.92
62.5	16.86	6.08
125	18.36*	6.68*
250	20.90*	7.04*
500	20.95*	7.09*

*= (P<0.01)
N= 150 male broiler chicks (5 G) 42 days, Temp = 30-35 ^{0}C, R.H. = 38-48 %

Panda *et al (*2007) found that Glutathione peroxidase activity was not influenced by supplementation of vitamin C in diet. However, Supplementation of vitamin C significantly reduced the activity of lipid peroxidase and increased activity of catalase (Table 9).[17]

Effect of vitamins supplementation on mineral concentration in poultry: Sahin*et al* (2002) observed that increasing dietary vitamin E supplementation caused linear increased in serum concentrations of Ca and P[22] (Table 10).

Panda *et al (*2007) studied the effect of vitamin supplementations on serum Ca and P concentration in laying hens. They reported that Supplemental Vitamin C did not influence serum inorganic phosphorus concentration.[17] However Concentration of Ca in serum increased significantly due to vitamin C supplement @ 400 mg/kg (Table 11).

Dietary electrolytes

- Supplementation of electrolytes in water

Table 11: Effect of vitamin C supplementation on mineral concentration parameters in laying hen

Parameters	Vitamin C (mg/kg diet)			
	0	200	400	600
Calcium (mg/dl)	12.31[b]	13.70[a]	14.24[a]	14.44[a]
Phosphorus (mg/dl)	5.08	5.99	5.02	5.26

Means with different superscripts within row differ significantly (P<0.05)
n= 120 (4 G), 46-54 wks, March – June, Ambient Temp. = 30 ^{0}C - 44 ^{0}C

Table 12: Effect of DEB on performance of laying commercial hens exposed to heat stress

Treatments DEB (m Eq/kg)	Feed intake (g/hen/day)	FCR (g feed/g egg)	Egg production (%)	Egg mass (g/hen/day)	Egg weight (g)
0	100.5	2.20	75.9	46.6	61.2
120	106	2.29	77.8	47.2	60.8
240	98.4	2.14	75.8	46.1	
360	104.7	2.40	73.6	44.8	60.9

N= 256 laying commercial hen, (4 G) (55-65 weeks), DEB (Dietary Electrolyte Balance) = NaCl + $NaHCo_3$ + $KHCO_3$ + NH_4Cl, Temp. = 30 ^{0}C -34 ^{0}C , R.H. = 70 %, Sept 23th – Dec 6th

enhance

- √ Water consumption.
- √ Increase tolerance to heat stress.
- √ Improve production performance.

- Supplementation of Potassium Chloride @ 300 or 600 mg improved body weight, FCR, oxidative stress profile and other welfare parameters during both hot & hot humid summer but effect were more beneficial during hot humid summer.

Table 13: Effect of dietary electrolyte balance on egg shell quality of laying commercial hens exposed to heat stress

Treatments DEB (m Eq/kg)	Egg Shell Weight (g)	Specific Gravity	Egg Shell Thickness (mm)
0	5.46^{ab}	1.069^{b}	0.320^{b}
120	5.20^{b}	1.063^{b}	0.321^{ab}
240	5.5^{a}	1.071^{b}	0.336^{ab}
360	5.6^{a}	1.08^{a}	0.340^{a}

Means with different superscripts within column differ significantly (P<0.05)

Table 14: Effect of potassium chloride supplementation on performance of broiler

Treatments	Feed intake (g)	Body weight gain (g)	Feed conversion ratio(g gain/g feed)
Control	3260.3	1584.3^{b}	2.06^{a}
0.3% KCl	3296.0	1604.3^{b}	2.05^{a}
0.6% KCl	3324.5	1709.3^{a}	1.94^{b}

Means with different superscripts within column differ significantly (P<0.05)
N=135 Hubbard broilers, (3 G),7-42 days in May-June, Temp = 28-38 ^{0}C , R.H.= 50-55 % , Control = Tap water without KCl 0.3 % and 0.6 % KCl (w/vl) by supplementing 3 and 6 g of KCl, respectively.

Hooge (1995) stated that Electrolytes are compounds which are dissolved and dissociated into positively and negatively ions in a suitable medium.[23]

Effect of Electrolytes supplementation on performance and egg quality of laying hen Under Heat Stress: Nobakht *et al (*2006) concluded that DEB levels did not significantly (P<0.05) affect egg production, FCR, Feed intake, Egg mass and Egg weight[24] (Table 12).

They also found DEB effect of egg shell quality and reported that Egg Shell weight and specific gravity in 240 and 360 level of DEB increased significantly (P<0.01). Egg shell thickness increased with increasing electrolyte balance (Table 13).

Effect of Electrolytes supplementation on performance of broiler: Ahmad *et al (*2008) carried out an experiment on broilers to know the effect of electrolytes supplementation on performance. They reported that water treatment with 0.6% KCl resulted in significantly higher BW gain.[A significantly improvement in FCR was noted in 0.6% KCl

Table 15: Effect of water supplements on the body weight gain (g) of broiler chicks

Weeks	Control	Acetic Acid	$NaHCO_3$	KCl
2	304.7^{c}	380.9^{a}	375.1^{b}	255.7^{d}
4	830.0^{d}	995.0^{a}	985.4^{b}	900.0^{c}
6	1601.7^{d}	1950.3^{a}	1868.6^{b}	1794.3^{c}

Means with different superscripts within row differ significantly (P<0.05)
N= 200 Hubbard Chicks (4 G), age= 0-6 week, Temp = 30-35^{0}C, Control = No Supp., Acetic Acid @1.5 ml/L water, $NaHCO_3$ @0.5%, KCl @0.15%

Table 16: Effect of potassium chloride supplementation on carcass weight of broilers

Treatments	Carcass weight (% of live weight)
Control	72.2
0.3% KCl	74.5
0.6% KCl	74.6

N=150 Hubbard broilers, (3 G), 42 days, Control = Tap water without KCl 0.3 % and 0.6 % KCl (w/vl) by supplementing 3 and 6 g of KCl, respectively.

Table 17: Effect of Ascorbic Acid and Potassium Chloride Supplementation on dressing percent of Broiler Chicks

Groups	Dressing Percent
Control	77.10^{ab}
T1	77^{b}
T2	78.15^{ab}
T3	78.24^{a}

n = 420 Rose Chick (4 G), 3-7 weeks, T1 = 0.1 % KCl/L + 200 mg/kg Ascorbic Acid, T2 = 0.1 % KCl/L + 400mg/kg Ascorbic Acid, T3 = 0.1 % KCl/L + 600mg/kg Ascorbic Acid

supplement group[25] (Table 14).

The three water supplements used by Hassan *et al (*2009) in this study, acetic acid, $NaHCO_3$ and KCl have improved weight gains in broiler chicks at 2, 4 and 6 wks of age except KCl treated groups at 2 wks of age which may be due to decreased water consumption at the first week[26] (Table 15).

Effect of electrolytes and vitamins supplementation on carcass quality: Ahmad *et al (*2008) found no significant effect of KCl on carcass quality of 150 broilers[25] (Table 16).

Ihsan et *al (*2011) stated that dressing percentage was significantly higher in T3

Table 19: Effect of Ascorbic Acid and Potassium Chloride Supplementation on Performance Broiler Chicks Reared under Summer Condition

Treatments	Body Weight Gain (g)				
	21-28 days	28-35 days	35-42 days	42-49 days	21-49 days
Control	471	459	463^{b}	461	1857^{b}
T1	405	457	448^{b}	441	1753^{c}
T2	461	456	520^{a}	496	1935^{a}
T3	420	459	479^{ab}	448	1807^{b}

Means with different superscripts within column differ significantly (P<0.05)
N= 420 Rose Chick (4 G), 3-7 weeks, T1 = 0.1 % KCl/L + 200 mg/kg Ascorbic Acid, T2 = 0.1 % KCl/L + 400mg/kg Ascorbic Acid, T3 = 0.1 % KCl/L + 600mg/kg

compared to T1[27] (Table 17).

Effect of combination of electrolytes and vitamins: Roussan et *al (*2008) reported that total feed consumption, FCR and mortality rate of birds in the HS-NON group were significantly greater than those in the HS-SUP group.[28] These clearly indicated that a significantly lowered mortality rate and FCR occurred under cyclic heat stress temperatures when ascorbic acid, ASA, KCl and $NaHCO_3$ were supplemented (Table 18).

Ihsan et *al (*2011) concluded that treatment had no effect on during the periods 21-28, 28-35 and 42-49 days old broilers.[27] In all experiment period T2 achieved significant mean highest gain (Table 19).

Amino acids

Protein requirement is decreased bz of

Table 18: Effect of supplementation of Ascorbic Acid, Acetylsalicylic Acid, Sodium Bicarbonate and Potassium Chloride on the Performance of Broiler (35 days age)

Groups	Total feed consumption (g/bird)	Live Body Weight Gain (g/bird)	Feed conversion ratio	Mortality Rate (%)
Control	910.2^{a}	592^{a}	1.538^{c}	1.33^{c}
HS-SUP	844.9^{b}	443^{b}	1.907^{b}	8.0^{b}
HS-NON	762.1^{c}	310^{c}	2.458^{a}	17.3^{a}

Means with different superscripts within column differ significantly (P<0.05)
N= 225 Female Ross broiler, (3 G) 7 days, Control = Thermo neutral, HS- SUP =Cyclic Temp. + Ascorbic Acid and ASA @ 62.5 mg/L, $NaHCO_3$ @ 75 mg/L and KCl @ 125 mg/L in water, HS-NON =Cyclic Tem. Without Suppl., Cyclic temp = 30-33 ^{0}C for 12 hrs, 21-23 ^{0}C for 12 hrs.

Table 20: Effect of methionine supplementation in the ration of commercial layers during summer

Methionine (%)	Feed Intake (g/hen/day)	Feed Conversion ratio (g feed/g egg)	Egg production (%)	Egg weight (g)	Broken eggs (%)	Respiratory rate no./minute
0.00	108.3[a]	122.5[a]	88.4[a]	53.5	2.7[a]	147
0.04	104.8[b]	114.8[b]	91.3[b]	53.7	1.7[a]	136
0.08	104.3[b]	113.8[b]	91.9[b]	54.4	1.3[b]	131

Means with different superscripts within column differ significantly (P<0.05)
N= 720 hens of commercial strain A of 28 wks age (3G) 6 wks, Temp. = 32 – 36 ^{0}C

suppression in Production performance. High protein diet during heat stress decrease growth rate & meat yield. Protein has high heat increment. Diets containing lower protein levels & supplemented with limited amino acids , methionine, lysine gave better results.

Ravikiran and Devegoeda, (1998) reported that significant (P<0.05) improvement in egg production and reduction in feed intake in both levels of methionine supplementation. However the differences between the two supplemented groups were non-significant, both with respect to egg production and feed intake. Feed efficiency improved significantly. Significantly decreased in broken eggs[29] (Table 20).

Bunchasak and Silapasorn, (2005) reported that the Low-CP diet with 0.26% methionine had significantly depressed feed consumption of hens compared to other experimental groups (P<0.01).[30]Methionine intake was significantly linearly increased as the supplemental levels increased. In addition, increased dietary Met intake significantly improved FCR and Feed consumption of hens. Adding Methionine at 0.38% or 0.44% diet better effect than other level (Table 21).

Table 21: Effects of additional methionine in low-protein diet on performance of laying hens from 24 to 44 weeks of age under tropical condition (35°C)

Methionine Level (%)	Group	Feed intake (g/day)	Feed Conversion Ratio (g feed/g egg)	Mortality (%)
0.38	Control (16% CP)	98.14[a]	2.33[c]	8.22[b]
0.26	Treatment (14% CP)	87.85[b]	2.63[a]	11.75[a]
0.30		98.21[a]	2.60[a]	6.07[b]
0.38		99.73[a]	2.47[b]	5.89[c]
0.44		99.98[a]	2.43[b]	5.87[c]

Means with different superscripts within column differ significantly (P<0.05)
N= 480 Commercial (Isa brown) laying hen, Temp = 24 – 36 ^{0}C, R. H. = 60-70 %

Minerals

- Addition of zinc @ 48 or 96 mg/kg of basal diets significantly improved body weight, FCR, immunoresponse, oxidative stress profile & other welfare parameters during hot & hot-humid summer. but results were more effective @ 96 mg/kg level that too during hot summer.
- Dietary supplementation of chromium (2.49 cr./kg diet) from 20 mg chromium picolinate was beneficial to reduce the adverse effect on growth performance, immunity and oxidative stress profile caused by higher ambient temperature during extreme summer.(Kulkarni, 2012)

Sahin*et al* (2002) stated that heat stress increases mineral excretion and also decreases concentrations in serum and liver.

Table 22: Effect of supplemental zinc and vitamin A on performance of broiler chicken reared under heat stress

Treatments	Feed intake (g)	Feed Conversion Efficiency	Live weight Gain (g)
Control	3101[c]	2.12[b]	1458[c]
Zn (30 mg/kg)	3150[b]	2.06[b]	1517[b]
Vit A (1500 IU/kg)	3162[b]	2.07[b]	1525[b]
Zn + Vit A	3200[a]	2.03[c]	1570[a]

Means with different superscripts within column differ significantly (P<0.05)
N= 120, ten day old male chick, (4 G), June 25– Aug 28

Table 23: Effect of supplemental zinc and vitamin A on carcass quality of broiler chicken reared under heat stress

Item	Control	Zn	Vitamin A	Zn+ vitamin A
Live weight (g)	1463^{c}	1522^{b}	1530^{b}	1591^{a}
Hot carcass yeild (%)	58.4^{c}	60.8^{b}	61.1^{b}	63.2^{a}
Chilled carcass yeild (%)	63.3^{c}	64.0^{b}	64.4^{b}	66.9^{a}
Heart wt. (%)	0.38^{c}	0.40^{b}	0.40^{b}	0.43^{a}
Liver wt. (%)	1.73^{c}	1.78^{b}	1.77^{b}	1.81^{a}
Abdominal fat wt. (%)	2.16^{a}	2.13^{b}	2.12^{b}	2.08^{c}

Mean values with different superscripts in a row with differ significantly (P<0.05)
N= 120 ten day old male chick 42 days

Kucuk *et al (*2003) concluded that live weight gain increased and feed efficiency improved greatly in chickens fed supplemented diets compared with the chicks fed the control diet.[31] However, a combination of zinc and vitamine A, rather than each separately, provided a greater performance (Table 22).

Kucuk *et al (*2003) also found effect on carcass quality and reported that combination of zinc and vitamine A, rather than each separately, provided a greater performance[31] (Table 23).

Other nutrients & feed additives

- Supplementation of probiotic *lactobacillus strains* may enrich diversity of micro flora in chicken.
- Restore microbial balance in jejunum & caeca of chicken.
- Reduce harmful effects of heat stress.

Dietary fat

It is recommended that the energy content of the diet be increased during hot weather. The use of supplemental fat is suggested. Dietary fat increases palatability of feeds and reduces the amount of heat increment that is produced during its utilization in the body.

- Inclusion of fats in the diet should be considered on hot days, particularly for broiler chickens, in order to maintain daily energy intake in line with the requirements for growth.
- But On many tropical farms, it is common practice to exclude fat from the diet during summer and include it during winter. It is though, that the energy requirement of broilers is less in summer than in winter. (Salah H. *et al., 2012)*
- Recent studies, however, have shown that the inclusion of fat in diets for heat-stressed broilers helps improve feed intake and performance, because of the lower heat increment of fat compared to other energy sources such as carbohydrates or proteins.
- Care must also be taken when selecting the fat source to be incorporated into the diet. Generally fat sources having large amounts of polyunsaturated fatty acids, such as soybean oil, canola oil, walnuts, flaxseed oil and fish oil, should all be avoided or be used at minimal levels in the diet.
- This is due to the fact that such sources have low levels of antioxidants and are especially susceptible to oxidative rancidity and destruction of vitamin A and E, with resulting changes in the flavour of poultry meats.

Water

- Water requirement increase during hot periods.
- 6% water intake increase per degree rise in temperature from that at 20 ^{0}C temperature.
- 25% more drinking space should be provided.
- Water below body temperature will certainly aid in heat dissipation.

Drug administration

- During heat stress need proper Care and Management.
- Medicines should be administered at the early morning when temperature is low.

- Drugs should be administered with cool, fresh & quality feed.

Disinfection & hygiene

- All-in & all-out system of poultry management in hot climates is most preferred.
- Appropriate disinfection programme is followed between the batches.
- In high temperature there may be rapid evaporation of disinfectant solution resulting in less contact time.

Conclusions

Heat stress reduces the production performance of chicken leads to immunosupression and increases the mortality rate in chicken. All the nutritional supplements (e.g. vitamins, zinc, Kcl) have functions in relation to heat stress. Suitable mineral & vitamin premixes can be developed for heat stressed chickens for improved performance, welfare & reducing feed cost. Heat stress increase the serum concentration of ACTH which increase corticosteroid level and in turns reduces the production. Dietary supplementation of vitamin C (200 mg/kg) improved the egg production and egg shell quality in laying hen during summer stress. Ascorbic Acid supplementation (300 mg/kg of diet) improved body weight gain, feed conversion ratio and decreased the mortality in broilers. Dietary supplementation of anti-oxidant vitamins (vitamin E or vitamin C in combination) is helpful to maintain the growth performance, egg production and improvement in egg quality. Vitamin E (250 mg/kg diet) increased serum concentration of T_3, T_4 and decreased concentration of ACTH thereby production is maintained. Low protein diet (14%) with provision of additional methionine @ 0.44% maintained the production performance of laying hens. Supplementation of Dietary Electrolyte Balance like NaCl, $NaHCO_3$, $KHCO_3$ and NH_4Cl (360 m Eq/kg) in heat stress can improve eggshell quality of laying hens. Supplementation of 0.1%KCl with 400 mg vitamin C showed better performance for broilers reared under heat stress. Zinc in combination with vitamin A improves performance and carcass quality of broiler under heat stress.

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