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Growth Response of New Zealand White Rabbits to Dietary Probiotic and Vitamin C under Tropical Humid Climate

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ABSTRACT

The study was conducted to evaluate the effect of supplementing Lactobacillus casei and vitamin C on growth performance of New Zealand White rabbits affected by heat stress during summer seasons between March and May in Kerala state, India. Twenty four two month old rabbits (average body weight 0.91kg) were distributed to four groups of six rabbits each. The treatments were control group (C) fed basal diet alone, L group fed basal diet along with Lactobacillus casei (106 colony forming units per g of feed), V group fed basal diet along with ascorbic acid (200 mg per kg feed), and LV group fed basal diet along with L. casei and ascorbic acid at the same rate as in V and L. The Temperature humidity index (THI) values in the rabbitry and rabbit fecal cortisol values (n=6 per group) were estimated to assess the heat stress level in rabbits. As per THI values, animals were exposed to stress (THI > 27.8) in the afternoon hours during the entire experimental period. The V, L and VL rabbits had lower mean cortisol level than control during the period of very severe and severe heat stress (1-9th experimental week- March and April). The groups did not differ for body weights during the period of very severe and severe heat stress (March and April). The V, L and VL rabbits had similar 21 week final weights but those of the L and VL rabbits were 25.52% and 32.29% higher than the control. The rabbits in the V, L and VL group showed 38.51%, 49.19% and 61.13% ($P<0.05$) higher overall mean daily body weight gain and 23.89%, 31.48%, 40.33% higher overall mean feed efficiency than the control. Dietary supplementation of the probiotic or ascorbic acid did not affect ($P>0.05$) the overall feed intake. Cost of production (Rupees) per kilogram live weight on feed basis was lowest for VL animals followed by L and V animals respectively.

Keywords: Ascorbic acid, Fecal cortisol, Heat stress, *Lactobacillus casei.*

Backyard rabbit rearing is an emerging enterprise in Kerala. One of the major threats to rabbit production in the state is the unpredictable economic loss encountered during summer season due to heat stress. A temperature of 21°C is known as the "Comfort Zone" for rabbits (Marai *et al.,* 1994). Temperature humidity index (THI) values above 27.8 results in heat induced physiological stress in rabbits (Marai *et al.,* 2002). In general, chronic exposure to extremes of heat leads to decomposition of normal physiological and biological mechanisms with a consequent damage of many organs (El- Sobhy, 2000). Alleviation of heat stress includes physical, physiological and nutritional techniques (Marai *et al.,* 1994). Dietary addition of the probiotic, *Lactobacillus* (Amber *et al,*. 2004) and antioxidant Vitamin C (Selim *et al.,* 2008) improved average daily gain and feed conversion ratio in New Zealand white rabbits. Supplementation with probiotics and ascorbic acid to alleviate heat stress has been tried in chicken and found to be successful (Chitra *et al*., 2008). Immune response of laying hens with dietary supplementation of multi strains probiotic and vitamin C was greater than control birds (Asli *et al.,* 2007). The present research work was aimed at evaluating the effect of supplementing ascorbic acid and probiotic, *Lactobacillus casei* on the growth performance of New Zealand White rabbits reared under hot summer in Kerala.

MATERIALS AND METHODS

The study was conducted at rabbit unit of Krishi Vigyan Kendra of Kerala Agricultural University, Vellanikkara located seven kilometers east to Thrissur district head quarters and geographically situated at longitude 76^{o}, 05" to 70^{o}, 45" E, at latitude 10^{o}, 20" to 10^{o}, 56" N and at an altitude of 22.25 m above mean sea level. The location of the study is endowed with humid tropical climate. Twenty four eight week old New Zealand White rabbits weighing 0.91 kg were randomly selected as uniformly as possible with respect to age and randomly allotted to four groups of six rabbits each. The rabbits were housed in individual cages (60X60X45 cm) placed in a semi open shed with wire mesh around the open area. The animals

Table 1: Proximate composition of rabbit feed (basal diet) in percent dry matter

Sl No.	Chemical composition	Grass	Concentrate pellets
1	Crude protein	9.74%	18.08%
2	Crude fiber	31.29%	5.23%
3	Ether extract	1.75%	2.59%
4	Ash	11.66%	13.20%
5	Nitrogen free extract	45.57%	60.89%
6	Acid Insoluble ash	6.18%	1.42%
7	Moisture	86.10%	5.40%

were reared for thirteen weeks which fell in the months of March, April and May. They were reared from 8 to 21 weeks of age. They were fed commercial pelleted feed (once a day), *ad libitum* fodder and water. The pellets were given at 10 AM every day. The left over feed was weighed the next morning to find the daily feed intake. Proximate composition of grass and concentrate pellets was estimated (AOAC, 1990) and presented in Table 1.

The treatments were control group (C) fed basal diet alone (n=6) probiotic group (L) fed basal diet along with *Lactobacillus casei* at the rate of 10^6 colony forming units per g of feed (n=6),vitamin group (V) fed basal diet along with ascorbic acid at the rate of 200 mg per kg feed (n=6), and probiotic and ascorbic acid group (LV) fed basal diet along with *L. casei* and ascorbic acid at the same rate as in V and L(n=6). The feed supplements were mixed with the pellet feed just before feeding.

Temperature humidity index (THI) values in the rabbitry were calculated as per Marai *et al*. (2001). The values obtained were classified as shown in Table 2.

Table 2: THI values and severity of heat stress as per Marai *et al.* (2001)

Sl No.	THI value	Severity of heat stress
1	<27.8	absence of heat stress
2	27.8–28.9	moderate heat stress
3	28.9–30.0	severe heat stress
4	30.0 and more	very severe heat stress

Collection and storage of fecal samples (Tesky- Gerstl *et al.,* 2000): Fecal samples were collected at monthly intervals from all the animals in the early morning. They were kept in polythene pouches and stored at -20°C till extracted for Radioimmuno assay (RIA)

Extraction of fecal cortisol for radioimmunoassay (Palme *et al.,* 1996)**:** The fecal samples stored at -20°C was crushed in the polythene pouch itself and thawed. Then 0.5 g of homogenized wet feces was extracted with 2 ml distilled water and 3 ml methanol after vortexing the mixture for 30 minutes. It was then centrifuged at 2500 rpm for 15 min. A 0.5 ml aliquot of the supernatant was decanted and the feces residue in the centrifuge tube was again extracted with 3 ml methanol same as before. Again 0.5 ml of the supernatant was taken and mixed with aliquot already taken in the screw capped vial. The fecal extracts were stored at -20°C until RIA analysis.

I^{125} labeled Cortisol Radio Immuno Assay was the method adopted for cortisol estimation from fecal extracts (Jasnow *et al.,* 2001).

The growth parameters recorded were weekly body weight, daily body weight gain, daily feed intake and feed efficiency. The cost of production on feed basis of the four treatments was determined. Data collected on various parameters were statistically analyzed by employing one way analysis of variance (ANOVA) (Snedecor and Cochran, 1994)

RESULTS AND DISCUSSION

Assessment of heat stress in rabbits

Temperature Humidity Index (THI): The animals were exposed to stress (THI > 27.8) in the afternoon hours during the entire experimental period (Fig. 1). Heat stress level was 'very severe' (THI > 30) in the afternoon hours of March and April, except for 5th and 9th week during which severe stress (THI =28.9-30) prevailed. Animals were exposed to moderate heat stress (THI =27.8-28.9) in May, except for 11th week during which severe heat stress prevailed (Figure 1).

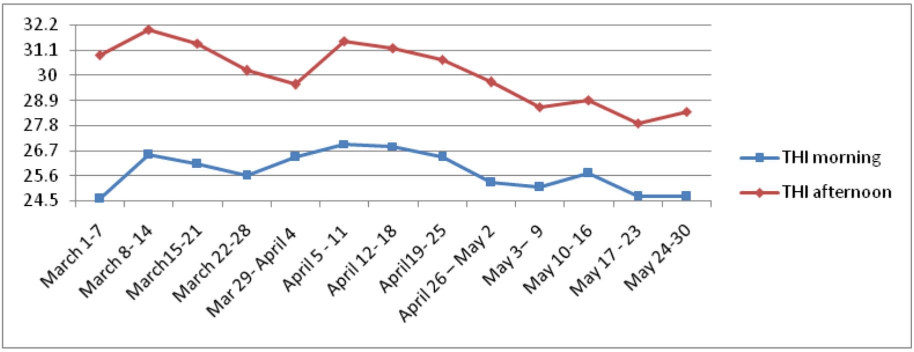

Figure 1: Mean weekly Temperature Humidity Index (THI) values in the rabbitry in March, April and May

Fecal cortisol**:** The V and VL animals had lower cortisol level (P≤0.05) than the control for March and April (very severe and severe heat stress) but the groups did not differ during May (moderate heat stress). The L rabbits did not differ for fecal cortisol during the experiment (Table 3). Hence it was concluded that supplementation of ascorbic acid or ascorbic acid in combination with *L. casei* was effective in reducing fecal cortisol level in rabbits during severe and very severe heat stress.

Growth performance

The body weights of rabbits were unaffected until the 9th week (end of April) of experimental period i.e., during severe and very severe heat stress but thereafter VL rabbits had 23.53% higher (P≤ 0.05) body weight and attained 32.29% higher

Table 3: Mean monthly fecal cortisol values of NZW rabbits (μg/dl)

Day	C	V	L	VL
Initial	5.95±1.91[a]	5.94±1.27[a]	6.18±1.45[a]	5.34±0.94[a]
March	6.12±1.85[b]	2.48±1.15[a]	5.88 ±1.33[b]	3.56 ±0.77[a]
April	7.58±0.15[b]	5.21±0.76[a]	5.97±2.13[ab]	5.49±1.80[a]
May	5.70±1.41[a]	4.31±2.99[a]	5.61±0.28[a]	3.62±0.87[a]
Mean±SE	6.34±0.33[b]	4.49±0.58[a]	5.91±0.61[b]	4.50±0.51[a]

Mean values bearing different superscript in a row differ significantly ($P \leq 0.05$)

21 week of age final body weight than the control rabbits. By the 11th week L rabbits had 24.43% higher ($P \leq 0.05$) body weight and attained 25.52% higher 21 week of age final body weight than the control rabbits (Table 4). Marai *et al.,* 2001. I.F.M. Marai, M.S. Ayyat and U.M. Abd El-Monem , Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *J. Trop. Animal Health Prod.* 33 (2001), pp. 1–12.Maraimmm According to Marai *et al.* (2001) the digestibility of dry matter, crude protein and crude fiber declined due to heat stress. Addition of probiotic to the diet caused reduction in the surface tension of cell membranes favoring better absorption of nutrients (Amber *et al*., 2004). The higher body weight in the VL and L group might be attributed to the better feed efficiency due to improved digestibility of nutrients. Marai *et al.,* 2001. I.F.M. Marai, M.S. Ayyat and U.M. Abd El-Monem , Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *J. Trop. Animal Health Prod.* 33 (2001), pp. 1–12.Maraimmm The rabbits in the VL group had higher daily body weight gain and feed efficiency than control from the 9th week onwards. Ascorbic acid also had a role in slightly improving the digestibility of nutrients (Yacout *et al.,* 2002). The rabbits in the V, L and VL groups showed 38.51%, 49.19% and 61.13% ($P<0.05$) higher over all mean daily body weight gain (Table 5) and 23.89%, 31.48%, 40.33% higher overall mean feed efficiency (Table 6) compared to the control rabbits. The reason might be improved digestibility of nutrients in V, L and VL rabbits. In this study supplementation of ascorbic acid alone did not have an effect on the body weight of rabbits. This observation was supported by the view of Konca *et al.* (2009).

Dietary supplementation of probiotic or ascorbic acid did not give any ($P>0.05$) difference in mean daily feed intake (g) between the four groups. This finding was supported by the observations of (Kamra *et al.,*1996). Studies had revealed that high summer temperature significantly reduced feed intake in rabbits (Chiericato *et al*., 1993). In this study, the THI values were low (<27.8) in the morning indicating absence of heat stress in the morning hours. The animals were fed pellets in the

Table 4: Body weight of NZW rabbits between 8 and 21 weeks of age (kg)

Week of study	C	V	L	VL
1	1.00±0.10	1.02±0.13	1.07±0.15	1.06±0.14
2	1.08±0.15	1.11±0.13	1.16±0.15	1.17±0.15
3	1.15±0.10	1.21±0.13	1.25±0.15	1.27±0.16
4	1.23±0.10	1.33±0.14	1.37±0.17	1.40±0.17
5	1.30±0.17	1.44±0.13	1.52±0.16	1.50±0.17
6	1.39±0.17	1.53±0.12	1.63±0.16	1.61±0.17
7	1.46±0.16	1.64±0.11	1.70±0.14	1.71±0.16
8	1.55±0.15	1.77±0.10	1.86±0.15	1.85±0.15
9	1.62±0.16	1.87±0.10	1.97±0.15	1.99±0.16
10	1.70±0.16[a]	1.96±0.10[ab]	2.07±0.13[ab]	2.10±0.16[b]
11	1.76±0.17[a]	2.06±0.10[ab]	2.19±0.13[b]	2.25±0.17[b]
12	1.86±0.18[a]	2.17±0.09[ab]	2.30±0.11[b]	2.39±0.18[b]
13	1.92±0.18[a]	2.30±0.10[ab]	2.41±0.11[b]	2.54±0.18[b]

Mean values bearing different superscript in a row differ significantly (P≤0.05)

Table 5: Daily Body Weight Gain of NZW rabbits between 8 and 21 weeks of age (g)

Week of study	C	V	L	VL
1	12.91±1.73	17.86±2.19	21.43±3.07	20.64±3.53
2	10.87±2.45	13.95±2.37	13.45±3.07	16.50±2.68
3	09.62±2.08	13.64±2.60	13.67±2.41	13.62±2.52
4	11.64±1.10	16.69±3.31	17.12±3.95	18.43±2.78
5	10.33±3.38[a]	15.79±3.26[ab]	21.36±2.94[b]	15.38±3.74[ab]
6	12.42±1.29	12.93±3.57	15.48±3.10	15.19±1.98
7	10.03±0.83	15.76±3.21	12.21±2.48	14.29±3.73
8	13.32±1.79[a]	19.12±3.52[ab]	21.64±2.27[b]	19.57±1.41[ab]
9	10.34±2.03[b]	14.29±1.27[ab]	16.33±1.77[ab]	18.88±2.67[a]
10	10.16±0.73[b]	12.43±1.33[ab]	14.95±3.18[ab]	17.77±2.22[a]
11	09.81±1.93[a]	14.26±1.10[ab]	15.93±2.15[b]	20.33±1.84[b]
12	11.90±2.52[a]	16.02±2.57[ab]	16.74±1.89[ab]	21.67±3.44[b]
13	11.48±3.13[a]	17.81±3.88[ab]	15.77±0.69[ab]	21.07±1.76[b]
1-13	11.14±0.34[c]	15.43±0.57[a]	16.62±0.86[ab]	17.95±0.75[b]

Mean values bearing different superscript in a row differ significantly (P≤0.05)

Table 6: Feed efficiency of NZW rabbits between 8 and 21 weeks of age

Week of study	C	V	L	VL
1	5.47±1.03	3.79±0.51	3.20±0.42	3.76±0.70
2	8.63±2.87	4.65±1.02	4.77±0.95	4.42±0.61
3	9.92±2.39^{b}	5.00±1.22^{a}	5.61±1.15^{ab}	4.78±0.56^{a}
4	7.27±1.35	6.49±2.65	5.88±1.75	4.23±0.58
5	8.07±2.68	6.35±2.11	3.89±0.76	4.83±4.19
6	5.57±0.82	5.49±4.99	4.48±2.29	4.44±0.57
7	7.19±0.94	6.02±1.80	6.09±3.42	5.61±3.63
8	5.59±0.58	4.71±1.84	4.16±0.56	4.45±0.56
9	9.93±3.79^{b}	6.76±0.87^{ab}	6.08±0.98^{ab}	5.32±0.82^{a}
10	8.81±1.14^{b}	6.98±0.96^{ab}	5.89±1.18^{ab}	4.28±0.24^{a}
11	9.18±1.19^{b}	7.06±0.51^{ab}	6.65±1.04^{ab}	4.90±0.49^{a}
12	9.91±1.72^{b}	7.41±1.40^{ab}	6.84±0.61^{ab}	4.56±0.47^{a}
13	9.25±5.13^{b}	7.60±2.84^{ab}	6.94±0.56^{ab}	5.09±0.72^{a}
1-13	7.91±0.44^{c}	6.02±0.33^{a}	5.42±0.33^{ab}	4.72±0.15^{b}

Mean values bearing different superscript in a row differ significantly ($P \leq 0.05$)

morning and green fodder was available *ad libitum*. The reduction in feed intake due to high summer temperature in the hotter parts of the day would have been compensated by increased feed intake during the cooler hours of the day ie, the night hours or morning hours.

Cost of production (Rupees) per kilo gram live weight on feed basis (Table. 7) for VL, L and V groups respectively were 33.49%, 30.54% and 16.23% lower than control group. In Kerala rabbits are usually slaughtered for meat at 4-5 months of age. Here, the VL rabbits could be marketed earlier than the control rabbits followed by L rabbits and V rabbits respectively.

Table 7: Cost of production per kg live weight of NZW rabbits (on feed basis)

Treatments	C	V	L	VL
No: of rabbits	6	6	6	6
Total feed intake (kg)	42.23	42.88	44.66	43.94
Total feed cost (₹)	612.34	684.21	656.5	709.9
Cost of 1 kg pellet feed (₹)	14.5	15.96	14.7	16.16
Feed efficiency	7.91	6.02	5.42	4.72
Cost of production per kg live weight on feed basis (₹)	114.7	96.08	79.67	76.28

CONCLUSION

The supplemented diets had only minor effects on growth parameters during very severe and severe heat stress (i.e. better feed efficiencies on week 3 in the V and VL rabbits and higher weight gains on weeks 5 and 8 in the L rabbits compared to the control rabbits). The combination of added vitamin C and *Lactobacillus casei* lowered mean fecal cortisol level and increased mean feed efficiency, body weight and daily body weight gain as compared to the control animals. The *L. casei* rabbits had higher daily weight gain and feed efficiency. The vitamin C diet resulted in lower overall mean fecal cortisol level, higher daily weight gain and feed efficiency than the control but final weight was unaffected. It was concluded that among the three diets, *Lactobacillus casei* in combination with ascorbic acid was most superior in economically improving the growth performance of New Zealand White rabbits under summer heat stress followed by *L. casei* alone and ascorbic acid alone respectively.

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