Selected Hemocytological Effects of Vitamin B6 
Deficiency in Chicks 1,2

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ABSTRACT One hundred and eight chicks were allotted at random to 3 treatments, 
with 3 replicates of each treatment and 12 chicks per replicate pen. Treatments were:
1) vitamin B6-deficient diet (1.22 mg/kg) consumed ad libitum; 2) vitamin B6-adequate 
diet (2.86 mg/kg) consumed ad libitum; and 3) vitamin B6-adequate diet pair-fed to the 
amount consumed by the B6-deficient chicks. During the 4-week experimental period, 
response was measured by hemoglobin, packed cell volume values, mortality, weight 
gains, and feed conversion ratios. Pair-fed chicks had significantly higher hemoglobin 
values than the vitamin B6-deficient chicks in the second, third, and fourth weeks of 
the trial. Packed cell volume values of the pair-fed chicks were significantly higher 
than those of the vitamin B6-deficient chicks in the second and fourth weeks of the trial. 
Chicks consuming the vitamin B6-adequate diet ad libitum had hemoglobin and packed 
cell volume values which remained fairly constant throughout the 4-week experiment, 
at a level lower than those of the pair-fed chicks consuming the same vitamin B6- 
adequate diet. Deaths observed in pair-fed chicks indicated that vitamin B6 deficiency 
in chicks depressed appetite sufficiently that starvation was a factor in deaths observed 
in deficient chicks. Feed conversion ratios in deficient and pair-fed chicks were 
significantly poorer than those of the groups receiving adequate vitamin B6 on an ad 
libitum feeding regimen. Weight gains of the deficient chicks and the pair-fed chicks 
were similar.

The essentiality of vitamin B6 for chicks was recognized in 1939 when Hegsted et al. 
(1) and Jukes (2) reported on vitamin B6 studies with chicks. These reports were 
followed by others detailing various aspects of vitamin B6 metabolism in chicks. Jukes (2) 
reported no departures from the normal range in differential blood cell counts of 
chicks fed either a vitamin B6-deficient basal diet or the basal diet supplemented 
with pyridoxine hydrochloride. These cell counts were made on the seventeenth day 
of the experimental period. Luckey et al. (3) reported that vitamin B6 deficiency in 
chicks was accompanied by a definite anemia. Hegsted and Rao (4) reported microcytic 
anemia as one of the symptoms of vitamin B6 deficiency in chicks. The present study was made to investigate further the relationship between vitamin B6 
and hemoglobin level and packed cell volume in chicks.

EXPERIMENTAL PROCEDURE

Broiler-type cokerels were used in the 4-week experiment. All chicks were housed in 
electrically heated battery-brooders for the duration of the experiment. After a 
one-week pre-trial period, during which all chicks consumed a commercial starter diet, 
experimental birds were allotted at random to treatments. The treatments were: 1) 
adequate vitamin B6, consumption ad libitum, 2) deficient vitamin B6, consumption 
ad libitum, and 3) adequate vitamin B6, consumption restricted to that amount 
consumed by the deficient vitamin B6 group within the same replication. The 
treatments are hereafter referred to as 1) Ad AL, 2) D AL, and 3) Ad R.

The diet contained the following ingredients: (in per cent) dextrose, 58.85; purified 
soybean protein, 28.00; soybean oil, 2.00; non-nutritive fiber, 3.00; vitamin premix, 
1.00; mineral premix, 5.30; choline chloride (25%), 1.00; DL-methionine, 0.70; 
glycine, 0.10; and DL-tryptophan, 0.05. The vitamin premix contributed the following 
per kilogram of complete ration: vitamin A, 10,000 IU; vitamin D3, 1,500 ICU; vitamin 
E, 22 IU; menadione, 4.4 mg; thiamine-HCl, 4.4 mg; niacin, 132 mg; 
B-aminobenzoic acid, 66 mg; niacin, 88

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mg; ascorbic acid, 220 mg; vitamin B₁₂, 22 μg; and biotin, 220 μg.

The mineral premix added the following per kilogram of complete ration: (in grams) calcium, 11.7; phosphorus, 5.8; potassium, 2.16; sodium, 1.87; chlorine, 2.9 (and in milligrams) manganese, 286; magnesium, 0.9 g; zinc, 94.5; iron, 95; copper, 9.5; cobalt, 0.9; and iodine, 3.5. The adequate vitamin B₆ rations contained sufficient added pyridoxine-HCl to provide a level of 2.86 mg of vitamin B₆/kg of complete ration. This level had been shown previously to be an adequate vitamin B₆ level for broiler chicks (5). No pyridoxine-HCl was added to the deficient vitamin B₆ ration. The calculated vitamin B₆ level of this ration was 1.22 mg/kg. After mixing, all diets were stored in a walk-in cooler at 10° ± 2°.

The amount of feed consumed the previous day by each vitamin B₆-deficient group was determined each morning. This amount was then fed to the pair-fed group which received the Ad R ration. When mortality occurred in an experimental pen, mean feed consumption was determined for the D AL group, and appropriate adjustment made in the amount fed to the pair-fed group.

A completely random design was used for the experiment. Each treatment was applied to 3 replicate groups of 12 chicks per replicate. At the start of the trial, 6 chicks in each experimental pen were selected at random for use in hemoglobin (Hb) and packed cell volume (PCV) determinations. The same 6 chicks from each pen were used throughout the trial. If a death occurred, a replacement was selected at random from the other chicks in the respective pen.

Chicks were weighed and blood samples were obtained at weekly intervals. Free-flowing blood samples were obtained from wing vein for the Hb and PCV determinations. Hemoglobin determinations were made by using the technique of Bankowski (6) as modified by Denington and Lucas (7). Packed cell volume determinations were made by a microcapillary method similar to that described by Natelson (8).

Statistical analyses of these data included analyses of variance tests on weekly Hb level, weekly PCV values, experimental period weight gains, and experimental period feed conversion ratios and determination of the correlations between Hb and PCV values within treatments. Two planned comparisons were made on the data in each analysis of variance test. First, data from chicks consuming the Ad R diet were compared with that of chicks consuming the D AL diet. Second, data from chicks consuming the Ad AL diet were compared with the combined data from chicks consuming the other 2 rations.

All statements concerning statistical significance are made at probability of 0.05 or less.

RESULTS AND DISCUSSION

Results of the analysis of variance tests on hemoglobin values indicated that chicks fed the vitamin B₆-deficient diet (1.22 mg/kg) had hemoglobin values which were significantly depressed compared with those of the pair-fed groups in the second, third, and fourth weeks of the trial (table 1, comparison 1 and fig. 1). When the Hb values for the these 2 groups were combined and compared with the groups receiving adequate vitamin B₆ on an ad libitum feeding regimen, the mean hemoglobin value for the combined groups was significantly higher at the end of the first and third weeks (table 1, comparison 2). The data from the second week approached significance. Luckey et al. (3) had observed a lower Hb level in vitamin B₆-deficient chicks than in chicks receiving a complete ration.

These results indicate that vitamin B₆-deficient chicks become anemic due to a metabolic failure in their normal pathway for hemoglobin synthesis and degradation. Since the deficient chicks had significantly depressed values over the pair-fed group, this failure is not due to the reduced feed intake per se, but results from the insufficient intake of vitamin B₆. The reduced feed intake (Ad R) resulted in hemoglobin values higher than those observed in chicks receiving adequate vitamin B₆ on an ad libitum feeding regimen (fig. 1). The noticeable increase in Hb level observed in chicks fed the Ad R ration over the level of those consuming the Ad AL ration may have been related to a Hb concentration occurring in the chicks whose growth was
VITAMIN B6 DEFICIENCY IN CHICKS

Fig. 1 Effect of feeding regimen on hemoglobin level.

TABLE 1
Summary of mean squares from analyses of variance tests on weekly hemoglobin and packed cell volume values

<table>
<thead>
<tr>
<th>Determination and source of variation</th>
<th>df</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison 1</td>
<td>1</td>
<td>0.0541</td>
<td>4.9504*</td>
<td>4.7171**</td>
<td>14.2913**</td>
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<tr>
<td>Comparison 2</td>
<td>1</td>
<td>0.6087*</td>
<td>4.6309</td>
<td>4.7432**</td>
<td>0.0242</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.0952</td>
<td>0.8176</td>
<td>0.2673</td>
<td>1.0388</td>
</tr>
<tr>
<td>Packed cell volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison 1</td>
<td>1</td>
<td>3.53</td>
<td>37.50*</td>
<td>32.67</td>
<td>150.00*</td>
</tr>
<tr>
<td>Comparison 2</td>
<td>1</td>
<td>13.52*</td>
<td>44.18**</td>
<td>50.67</td>
<td>6.97</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>1.37</td>
<td>2.96</td>
<td>10.84</td>
<td>15.39</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.
** Significant at P < 0.01.
1 Comparison made between groups fed deficient vitamin B6, consumption ad libitum (D AL) and groups fed adequate vitamin B6, with consumption restricted to that amount consumed by the deficient vitamin B6 group within the same replication (Ad R).
2 Comparison between group receiving adequate vitamin B6, consumption ad libitum (Ad AL) and combined value of groups fed Ad R and D AL.

severely restricted by the limited amount of feed they were allowed to consume. The individual PCV determinations within a treatment were more variable than the Hb determinations. However, the analysis of variance tests showed a generally similar pattern (table 1). The PCV values of chicks consuming the D AL ration exhibited a nearly linear decrease with time over the period studied in this experiment (fig. 2). A decrease was also noted in chicks fed the Ad AL ration, but this decrease was smaller and appeared to have reached a plateau at the terminus of the trial.

Restricting the feed intake of the pair-fed chicks resulted in feed conversion values similar to those of chicks consuming the D AL ration (table 2). Whereas differences existed, these were not statistically significant. The group receiving adequate vitamin B6 with ad libitum intake had a significantly better feed conversion than that of the other 2 groups.
Fig. 2 Effect of feeding regimen on packed cell volume.

### Table 2

**Average weight gains, feed conversion ratios, and mortality**

<table>
<thead>
<tr>
<th>Feeding regimen</th>
<th>4-Week wt gains of survivors</th>
<th>Feed conversion ratios (F/G)</th>
<th>4-Week mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient vitamin B6, ad libitum</td>
<td>55.7 ± 6.39</td>
<td>4.02 ± 0.153</td>
<td>80.5 ± 4.79</td>
</tr>
<tr>
<td>Adequate vitamin B6, restricted intake</td>
<td>62.6 ± 5.67</td>
<td>4.45 ± 0.207</td>
<td>27.8 ± 9.58</td>
</tr>
<tr>
<td>Adequate vitamin B6, ad libitum</td>
<td>415.9 ± 5.24</td>
<td>2.03 ± 0.038</td>
<td>2.8 ± 4.79</td>
</tr>
</tbody>
</table>

1 All pens contained 12 chicks initially.
2 Mean ± se of mean.

Weight gains of chicks receiving the same amount of feed were similar (table 2), indicating that a major cause of the greatly reduced rate of gain in vitamin B6-deficient chicks is the loss of appetite, associated with a vitamin B6 deficiency, and consequent reduced feed intake. Most researchers report loss of appetite as one of the vitamin B6-deficiency symptoms in chicks. Weight gains of chicks receiving the Ad AL diet were about 7 times greater than gains of chicks consuming either the Ad R or D AL diets.

Mortality data (table 2) indicate that some deaths observed in vitamin B6-deficient chicks were due to starvation mediated through loss of appetite rather than to direct metabolic effects such as loss of co-enzyme activity. Most reports indicate that deaths of chicks receiving vitamin B6-deficient diets occur at about 10 to 14 days after the chicks start to eat the vitamin B6-deficient diet. In this trial, nearly one-third of the deaths of chicks receiving deficient diets and one-fifth of the deaths of chicks receiving adequate vitamin B6 at a restricted intake occurred in the tenth to fourteenth day of depletion. The one death recorded in the Ad AL groups occurred in this period. Postmortem observations of deficient chicks showed varying degrees of wing feather follicle hemorrhage, whereas
none was observed in chicks fed diets containing adequate vitamin B₆. Daghir and Balloun (5) reported wing feather follicle hemorrhage as one of the symptoms of vitamin B₆-deficient chicks.

Correlation coefficients obtained between PCV and Hb within the different treatments were 0.914, 0.768, and 0.752 for D AL, Ad R, and Ad AL, respectively. The test for homogeneity of these coefficients indicated that they were homogeneous, and a pooled correlation coefficient was determined. This value of 0.829 was statistically significant.

The extent to which weight gains of chicks receiving the adequate vitamin B₆ diet in restricted amounts were depressed and the number of deaths observed in these chicks were unexpected. These results show the importance of vitamin B₆ in stimulating chicks to eat. The means through which vitamin B₆ enhances appetite is unknown.

LITERATURE CITED