# HEATING RAW VELVET BEANS (*Mucuna pruriens*) REVERSES SOME ANTI-NUTRITIONAL EFFECTS ON ORGAN GROWTH, BLOOD CHEMISTRY, AND ORGAN HISTOLOGY IN GROWING CHICKENS

**L.B. Carew[1,2]\*, D. Hardy[1], J. Weis[1,2], F. Alster[1], S. A. Mischler[1], A. Gernat[3] and E.I. Zakrzewska[1]**

[1]*Department of Animal Science*
[2]*Department of Nutrition and Food Sciences*
*University of Vermont, Burlington, VT 05405, USA. E-mail: lcarew@zoo.uvm.edu*
[3]*Departamento de Zootecnia, Escuela Agrícola Panamericana, Tegucigalpa, Honduras*
*\*Corresponding author*

## SUMMARY

Velvet beans (*Mucuna pruriens*) represent an interesting food and feed commodity because of the presence of harmful, but also potentially beneficial components that have been poorly studied. We developed a joint research project between the University of Vermont in the USA and the Escuela Agricola Panamericana (Zamorano) in Honduras to study the role of velvet beans (VB) in chicken nutrition and to determine methods for improving their usefulness and safety. The results of these studies, using the chicken as a research model, have application to all monogastric animals including humans with whom such research cannot be done. The two experiments described herein measured the effects of raw VB on the anatomy and blood chemistry of the chick, and the role that heating the velvet beans has on reversing any harmful effects inherent in the beans themselves.

Consumption of unprocessed, raw velvet beans by broiler chickens reduced body weight gain. Weights (relative to body weight) of the pancreas, gizzard, and proventriculus (stomach), as well as lengths of the small and large intestines, and ceca, increased in birds fed raw VB. Most of these changes were partially or wholly reversed by dry heating (i.e. toasting) the beans. It is likely that the effects of heating reflect, to some degree, partial destruction of anti-nutritional factors. Effects of VB on liver and heart weights were inconclusive. Significant changes were also found in blood components. Plasma creatinine was reduced to a similar degree in chicks fed both raw and heated VB, and most likely reflects changes in muscle mass or metabolism concurrent with feeding either form of VB. Plasma cholesterol levels were also consistently reduced in chicks fed raw VB compared to their pair-fed controls. These findings agree with our previous results and work done by others in a rat model. However, heating VB caused an even further reduction in plasma cholesterol. This may indicate the presence of a cholesterol-lowering substance in VB. Some anti-nutritional factors in VB may be heat-resistant and may explain such effects, although the effect of dietary fiber cannot be ruled out. Alanine aminotransferase was elevated in chicks fed both raw and heated VB compared to their pair-fed controls, and this is often an indication of liver damage. Intake of raw VB had inconsistent effects on plasma thyroxine and triiodothyronine, and little effect on the plasma content of sodium, glucose and alkaline phosphatase.

The microanatomy of the pancreas, liver, small intestine and kidney was altered in chickens fed raw VB. Increases in pancreatic necrosis, hepatic cellular degeneration and thinning of the mucosal muscular layer of the small intestine appeared to be at least partially a consequence of the reduced growth rate, as shown by pair feeding comparisons, and not to the VB themselves. However, kidney damage, as shown by invasion of blood into the renal tubules, was directly a result of feeding raw VB. Heating VB partially reversed this effect. Heating VB also partially reversed the thinning of the intestinal musculature. It is concluded that the many anti-nutritional factors in VB affect diverse parameters in growing chickens. Such anti-nutritional/toxic effects of raw VB may have similar effects on other monogastric species, including humans. Heating VB can reduce some of these deleterious effects, and our results suggest that heating can be an important adjunct to other processing methods to improve the nutritional value of VB. For greater accuracy, however, standardized methods of processing and analyzing VB must be developed to reduce observed variability.

## INTRODUCTION

There are numerous known anti-nutritional/toxic factors in velvet beans and probably some that remain

to be discovered. Among the known factors are antitrypsin factors, tannins and cyanide (Ravindran and Ravindran, 1988), anticoagulants (Houghton and Skari, 1994), analgesic, antipyretic and anti-inflammatory factors (Iauk *et al*., 1993), and others (Olaboro *et al*., 1991). Curiously, L-Dopa, a potentially neurotoxic agent used in the treatment of Parkinson's Disease, is found in large amounts (Bell and Jantzen, 1971; Daxenbichler *et al*., 1971) to the point where VB have been proposed as a medical source of L-Dopa (Bell and Jantzen, 1971) and even in the treatment of Parkinson's Disease (Hussain and Manyam, 1997). In order to use the seeds of the *Mucuna pruriens* plant (i.e. the velvet beans) in monogastric nutrition with humans, pigs, chickens and other animals, these anti-nutritional or toxic factors must be removed or destroyed. Some of these factors are either heat labile or water-soluble and can be diminished by proper heating or water extraction, while others may not respond to either treatment. The extent of successful processing on the quality and safety of velvet beans has not been sufficiently studied.

In humans, several investigators have reported toxic effects due to consumption of raw VB, including neurotoxicity and behavioral changes (Infante *et al*. 1990) and severe vomiting (Miller *et al*., 1925). In chickens, VB have been considered useless for many years. In the earliest work, Piper and Morse (1938) and Harms *et al*. (1961) reported poor growth and poor egg production, respectively, with their use. However, Del Carmen *et al*. (1999) recently showed that limited quantities of VB, if dry-heated, can be used successfully in broiler diets. Nevertheless, more research is needed to establish methods for reliably processing VB for use as an ingredient in poultry diets.

At the research conference "*Food and Feed from Mucuna: Current Uses and the Way Forward*" held in Tegucigalpa, Honduras, April 23-26, 2000, we reported that intake of raw, unprocessed VB by growing chickens has several deleterious effects (Carew, 2000; Carew *et al*., 2002). Growth rate and feed intake were markedly depressed. Physiological changes observed were decreases in blood plasma levels of triiodothyronine, cholesterol and creatinine, but an increase in plasma alanine aminotransferase (Carew *et al*., 1998a). Anatomical changes included increases in the weights of the pancreas, gizzard and proventriculus, but no change in liver weight. Lengths of the small and large intestines and ceca were increased (Carew *et al*., 1998b). We furthermore showed that, although L-Dopa has as dramatic and negative an effect on chick growth and feed intake as raw VB, most of the other physiological and anatomical effects of feeding raw VB, except for a decrease in plasma creatinine, did not occur when pure L-Dopa was added to chick diets (Valverde *et al*.,

2000). Thus, the L-Dopa content of raw VB cannot explain most of their negative effects when fed to chickens, and therefore, such changes must be due to other toxic/anti-nutritional factors or ordinary components such as fiber.

Our current research has two objectives. One is to study means for increasing VB use in poultry diets through various processing techniques, especially heating. The second is to use the chicken as a model to study the effects of raw and heated VB on the anatomy and physiology of monogastric animals; such studies with humans would be impossible. Unlike other animals, the use of chickens in research has several advantages: they are readily available, economical to use, have a relatively short life cycle, grow at a rapid rate and are an important food animal.

The purpose of the two experiments reported herein was to determine if heating raw VB alters their deleterious effects on the anatomy and physiology of the chicken, and to measure the effect of raw and heated VB on the microanatomy of selected organs in the chicken.

## MATERIALS AND METHODS

Two similar experiments of three weeks duration were done using male chicks 1-21 days of age. The effect of heating raw VB was studied. Organ size and blood chemistry were studied in Experiment 1, whereas the focus of Experiment 2 was on organ size and histological examination of the tissues.

### Animal management

Day-old Arbor Acres (Glastonbury, CT) male broiler chicks were housed in Petersime, multi-tier, battery brooder cages with raised wired floors, and with internal fluorescent lights and heaters in each pen. Triplicate groups of seven chicks each were used per treatment. They were distributed at one day of age based on body weight. A randomized complete block design was used in both experiments. Water was given *ad libitum*. Feed was also given *ad libitum* except where pair feeding was indicated. Pair feeding was accomplished by matching a pen of seven chicks fed the control diet with one of the pens fed a VB diet and feeding it once daily, at 0800 hr, the same amount of feed that the chicks fed VB ate the previous day. From previous experience, it was expected that feeding VB would reduce feed intake. Pair feeding control chicks with VB-treated chicks thus allowed us to take into consideration the effect of changes in feed intake *per se,* apart from the direct effect of VB, on observed responses. Behavior was observed daily and birds weighed weekly.

**Diets**

Both raw (unprocessed) and heated velvet beans (*Mucuna pruriens*) were obtained from the Escuela Agricola Panamericana in Honduras.  The whole beans were heated by rotating inside a metal drum for 30 min over a gas flame at a temperature of about 130$^{\circ}$ C and cooled immediately.   The beans were analyzed for amino acid, mineral, protein, fiber, ash and fat contents and had compositions similar to that reported previously (Del Carmen *et al*., 1999). In each experiment, diets containing 20% ground beans (10 mm sieve openings) were used. They were added to a

commercial-type broiler starter diet at the expense of soybean oil meal and corn contents such that the diets remained nutritionally balanced.  Amino acid, protein and energy levels were similar for all diets. Fortunately, VB have an amino acid profile similar to soybeans, and the substitution described does not alter the balance of essential amino acids.  All diets were adequate in vitamins, minerals and fat.  As reported previously (Del Carmen *et al*., 1999), a true metabolizable energy value of 2,370 kcal kg$^{-1}$ was used for the beans.  Composition of diets is shown in Table 1.

Table 1. Broiler starter rations.

| Ingredient | Control (%) | Raw VB (%) | Heated VB (%) |
|---|---|---|---|
| Corn, ground yellow | 48.2 | 36.6 | 37.1 |
| Soybean oil meal | 36.2 | 28.0 | 27.5 |
| Soybean oil | 4.3 | 5.6 | 5.6 |
| Poultry blend | 3.0 | 3.0 | 3.0 |
| Distiller's grains | 2.0 | 2.0 | 2.0 |
| Dicalcium phosphate | 1.5 | 1.5 | 1.5 |
| Limestone | 1.1 | 1.1 | 1.1 |
| Alfalfa meal | 1.0 | 1.0 | 1.0 |
| Salt | 0.4 | 0.4 | 0.4 |
| Vitamin premix[1] | 0.5 | 0.5 | 0.5 |
| Mineral premix[2] | 0.1 | 0.1 | 0.1 |
| DL-methionine | 0.2 | 0.2 | 0.2 |
| Sand | 1.5 | 0.0 | 0.0 |
| Velvet beans, raw | 0.0 | 20.0 | 0.0 |
| Velvet beans, heated | 0.0 | 0.0 | 20.0 |

[1] Per kg diet: 5 mg thiamin, 5 mg riboflavin, 10 mg Ca pantothenate, 20 mg niacin, 10 mg  pyridoxine, 2 mg folacin, 0.5 mg biotin, 300 mg choline, 0.1 mg vitamin B$_{12}$, 10,000 USP vitamin A, 1000 IU vitamin D$_3$, 2 mg menadione, 25 IU vitamin E, 125 mg ethoxyquin.
[2] Per kg of diet: 40 mg Fe, 4 mg Cu, 0.86 mg I, 75 mg Mn, 60 mg Zn, 0.05 mg Se.

**Blood and tissue samples**

Blood samples were taken only in Experiment 1.  At three weeks birds were rendered unconscious with a blunt cranial blow. Blood was removed by cardiac puncture and the birds were euthanized.  Blood was expressed into heparinized syringes, transferred to heparinized test tubes and centrifuged at 1,800 x g for 15 min.  Plasma was removed and frozen for later analyses.  At the end of both experiments, chicks were euthanized by cervical dislocation and the organs were removed and weighed or measured.  Liver, heart, pancreas, proventriculus, gizzard, small and large intestines and ceca were collected.

In Experiment 2, about 1-cm samples of the same tissues were removed and placed in buffered 10% formalin for fixing prior to histological preparation. A sample of kidney but not its weight was also taken. Samples were imbedded in paraffin, cut,  mounted on glass slides, and stained with hematoxylin and eosin. Preparation of slides was done in the histology laboratory of the Fletcher Allen Hospital, Burlington, Vermont.

**Statistics**

Data were analyzed using the General Linear Model analysis of variance with p$\leq$0.05 considered significant (SAS, 1990).  Means were compared using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

The effects of treatment on chicken performance, organ weights, intestinal and cecal lengths, blood chemistry, and organ histology will be described. Differences were considered statistically significant at p≤0.05.

### Chicken performance

In both experiments, weight gain was significantly less (-16 and -40%, respectively) in chicks fed raw VB compared to the *ad libitum*-fed controls. Heating the beans totally reversed this loss in Experiment 1, but only partially, yet significantly so, in Experiment 2. Feed intake for the 3-week period was not significantly altered by any treatment in Experiment 1 while there was a marked reduction (-31%) in feed intake in Experiment 2 that was not reversed by heating the VB. The net result was that feeding of raw VB significantly reduced efficiency of feed conversion by 11% in both experiments and heating the beans partially and significantly reversed this. The differing results in feed intake between the two experiments is probably due to variability in processing the beans or the effects of different harvest years. The beans in the two experiments were obtained from two different harvests a year apart. Even though identical equipment was used for processing in this and our previous work, this is not a closely standardized system.

### Organ weights

In Experiment 1, liver size increased significantly in chicks fed raw VB (+ 7%) compared to their pair-fed controls, and this difference disappeared on heating the VB. However, the opposite occurred in Experiment 2; restricted or pair feeding of the control diet resulted in significantly increased liver size compared to chicks fed either form of VB, and heating had no effect. We earlier reported no effect of raw VB on liver size (Carew *et al*., 1998b). Thus, the effect of VB on liver size apart from the effects of the reduction in feed intake is inconclusive. Liver size is known to increase in response to several factors, especially deficiencies of protein and amino acids, and is usually due to accumulation of fat (Velu *et al*., 1971). Thus, it is possible that the availability of protein and/or amino acids differed between lots of VB and thus between experiments. Availability of these nutrients is greatly influenced by heat treatment, and this may have been a factor in the results obtained between treatments.

Relative heart weights were little changed in Experiment 1, except the heated VB and pair-fed treatments differed significantly. However, heart weight was significantly heavier (+26%) in chicks fed raw VB in Experiment 2 compared to either the *ad*

*libitum* or pair-fed control groups, and heating the VB did not reverse this. In an earlier study, we also noted that consumption of raw VB increased relative heart weights (Carew *et al*., 1998b). Thus, enlargement of the heart muscle is not a consistent change in chicks fed VB. If it does occur, it may represent extra workload imposed by stress or disease.

In both experiments, pancreatic weights were markedly increased in chicks fed raw VB (+ 103 and 91%, respectively, compared to the *ad libitum*-fed control) but only in Experiment 1 was this partially reversed by heating the VB; no change occurred in Experiment 2. Pair feeding of the control diet in itself resulted in significant increases in pancreatic weights, but these were much smaller than with the raw VB. This finding confirms earlier results (Carew *et al*., 1998a). The consistent effect of raw VB on pancreatic weights is most likely a consequence of the well-known presence of anti-trypsin factors in the beans that prevent adequate function of pancreatic enzymes, thus forcing the pancreas to work harder. We, however, have no data on the amount of the anti-trypsin factors in either raw or heated VB in these two experiments. Nevertheless, under similar experimental conditions, we reported earlier that with our method of heating, the antitrypsin factor in VB produced in Honduras was reduced from 4.41 trypsin inhibitor units (TIU) to zero (Del Carmen *et al*., 1999).

Feeding raw VB increased gizzard weights in both experiments (+ 23 and 49%, respectively) compared to *ad libitum*-fed controls, and large differences persisted when compared to pair-fed controls, which is consistent with our earlier results (Carew *et al*., 1998b). This increase was partially reversed by heating the beans. In Experiment 2, pair feeding the control diet also increased gizzard weights but to a much lesser extent than feeding either form of VB. A similar situation occurred with proventricular weights except that heating the VB reversed the increase in weights only in Experiment 1. Pair feeding of the control diet in Experiment 2 also increased proventricular weight slightly but not to the extent that feeding either the raw or heated VB did. Increases in gizzard and proventricular weights may reflect the extra muscular or secretory work required to process VB, which are higher in fiber than the soybean oil meal and corn in the control diet. It is unknown why heating may partially reverse this effect, although alteration in fiber structure is a possibility, as well as destruction of other factors in the raw VB. Increases in organ size in pair-fed controls may simply reflect differential rates of growth of organs in chicks and result from some organs growing at different rates in relation to the growth of general muscle mass. Therefore, the observation that these two organs were significantly larger in chicks fed VB than in their pair-fed controls may mean that the beans had an effect

separate from that due to the reduction in body weight alone. These particular comparisons show well the importance of using matched, pair-fed controls in studies where reductions in feed intake and hence growth are expected due to treatments imposed on the animals.

Thyroid weights were measured only in Experiment 1. None of the differences was statistically significant between the bean treatments and any of the control groups.

**Intestinal and cecal lengths**

Feeding raw VB to growing chicks significantly increased the length of the small intestine in both experiments (+25 and 63%, respectively) compared to any controls. This was either fully (Experiment 1) or partially (Experiment 2) reversed by heating the beans. This is perhaps the most consistent observation in all our studies (Carew *et al*., 1998b). We do not have a definite explanation for this. Various fibers in feed ingredients, such as oligofructoses (Yusrizal *et al*., 2002) and other non-starch polysaccharides (Jorgensen *et al*., 1996; Iji, 1999), are known to increase the size of the small intestine and in some cases, this effect can be reversed with enzyme treatments that disrupt dietary fibers (Brenes *et al*., 2002). However, the possible presence of a specific growth promoter in VB cannot be excluded without further studies. That mild heating of VB reverses this effect suggests the possible presence of a heat-labile growth factor.

The large intestine was also longer in both experiments in chicks fed raw VB (+ 18 and 61%, respectively); heating VB partially reversed this only in Experiment 1. Furthermore, the process of restricted feeding in pair-fed chicks also increased the length of the large intestine in Experiment 2, although this effect was significantly less compared to chicks fed heated VB. The same factors that affect size of the small intestine may also affect the large intestine.

Feeding raw VB also increased the length of the ceca in both studies (+ 29 and 50%, respectively) and heating reversed this completely in Experiment 1 and partially in Experiment 2. This also agrees with our earlier work (Carew *et al*., 1998b). In Experiment 2, part but not all of this effect was attributable to the process of pair feeding. This is similar to the effects observed with the small intestine and may be due to the same factors discussed above.

**Blood chemistry (Experiment 1 only)**

Plasma creatinine was markedly reduced (- 43%) by consumption of raw VB as well as by restricted, pair-feeding (- 27%), but the effect due to intake of VB was statistically more severe. Heating did not reverse this

effect. Decreases in plasma creatinine may reflect either damaged (or reduced) muscle tissue or changes in the glomerular filtration rate in kidneys (Finco, 1997). We later show evidence of kidney damage in chicks fed raw VB. However, there is an inverse correlation between plasma creatinine and renal glomerular filtration rate, and with kidney damage, filtration rate diminishes and plasma creatinine increases (Finco, 1997). Thus, muscle damage is the more likely explanation for reduced plasma creatinine in chicks fed VB as well as in the pair-fed controls. The lower amount of plasma creatinine in chicks fed VB compared to their pair-fed controls suggests that VB have a more damaging effect on muscle metabolism than can be attributed to the reduction in growth rate alone. Much of the plasma creatinine is derived directly from muscle creatine, and where muscle mass is low, less release of creatine may be expected.

Compared to their pair-fed controls but not to the *ad libitum*-fed control, both types of VB produced lower plasma cholesterol levels (-12 and -18%, raw and heated, respectively). The greater reduction in plasma cholesterol with heated VB was significantly different from all controls and raw VB. Pair feeding significantly increased plasma cholesterol. The reduction in blood cholesterol on feeding raw VB agrees with our earlier results (Carew *et al*., 1998a), which are the first such observations in avian species fed VB. This also confirms results reported with rats (Pant *et al*., 1968; Iauk *et al*., 1989). That heated beans reduce cholesterol further may be of importance. This effect may be due to adsorption of intestinal cholesterol by dietary fiber and rapid excretion or to a more specific effect of some other component in VB. The observation that restricted feeding in itself elevated plasma cholesterol shows the importance of the pair feeding technique. Because of the importance of plasma cholesterol in the etiology of human heart disease, the effect of VB on lowering blood cholesterol deserves further study.

Thyroxine ($T_4$) levels of chicks fed VB were not significantly different from the *ad libitum* control. However, plasma $T_4$ levels in chicks fed raw VB were significantly lower (-14%) than in their pair-fed controls. Heating the VB partially reversed this. We observed no decrease in plasma triiodothyronine ($T_3$) as reported previously (Carew *et al*., 1998a) in chicks fed raw VB. $T_4$ is the metabolic precursor of $T_3$. Therefore, these results suggest a possible effect of VB on thyroid hormone metabolism and deserve further study. Usually, reductions in feed intake depress plasma levels of $T_3$ (May, 1978; Keagy *et al*., 1987). Why this common effect did not occur in this study is not known. Nevertheless, changes in thyroid hormone metabolism are known to affect both growth and

energy metabolism and may explain some of the depressive effects of VB on growth rate.

With alanine aminotransferase (ALT), chicks fed either raw or heated VB had significantly higher plasma levels (+32% in both) than their pair-fed controls, but not when compared to the *ad libitum*-fed controls. In general, this agrees with our earlier conclusions (Carew *et al.*, 1998a). Elevated plasma levels of ALT are often used as an index of liver damage (Tennant, 1997). Although our results on the effect of VB on liver size were inconclusive, these results with ALT suggest that there was liver damage in chicks fed either raw or heated VB compared to controls.

Results with other blood analyses were inconclusive. There were no effects of feeding VB on plasma alkaline phosphatase and sodium levels. Plasma protein was significantly lower in chicks fed raw VB compared to their pair-fed controls but not different from other groups. Plasma calcium was significantly reduced in chicks fed both raw VB and their pair-fed controls compared to *ad libitum*-fed controls, but not in those fed heated VB. The increase in plasma calcium with heating was not statistically significant. With both plasma glucose and potassium, the only significant effects were higher levels in chicks fed heated VB compared to their pair-fed controls. We had reported earlier that 20% dietary raw VB had no effect on levels of plasma alkaline phosphatase, sodium, potassium, protein, glucose, and calcium results (Carew *et al.*, 1998a).

**Organ histology (Experiment 2 only)**

There was a marked increase in pancreatic necrosis in all chicks fed VB as well as their pair-fed controls compared to *ad libitum*-fed control chicks. Therefore, necrosis of the pancreas observed in birds fed either raw or heated VB appeared to be primarily a consequence of the reduction in growth rate and not a specific effect of the VB. We, however, only measured the incidence of necrosis and did not evaluate the severity. Chicks fed heated VB had significantly more pancreatic necrosis than either their pair-fed controls or chicks fed raw VB (36 and 11% more, respectively). This is a confounding result since there should be less anti-trypsin factor in heated beans, which is believed to be the primary factor in VB that affects the pancreas. These results demonstrate the importance of using pair-fed controls. The pancreatic necrosis was evident primarily as a substantial loss of the normal structure of the secretory tubules. Pathology of the pancreas is usually thought to reflect the presence of anti-trypsin factors in raw VB, but the current observations that restricted feeding (i.e. pair feeding) of the control diet also increased pancreatic necrosis confound these conclusions.

Cellular degeneration of the liver followed an unusual pattern. Degeneration was observed as loose cell structure and apparent fatty infiltration. Both pair feeding and intake of heated VB increased hepatic cellular degeneration compared to the *ad libitum*-fed controls. However, there was no negative effect due to feeding raw VB; hepatic cellular structure appeared normal. The interpretation of this finding is unclear. Based on the results obtained with elevated ALT in chicks fed either form of VB, histological evidence of hepatic degeneration was expected in these chicks. Using only hematoxylin and eosin staining, it is difficult to determine exactly the cause of tissue pathology. This would be possible with differential staining such as for lipids, but we have not yet done that.

The mucosal muscular layer of the small intestine was significantly thinner in chicks fed raw VB compared to the *ad libitum*-fed control but not compared to the pair-fed control. This loss of mucosal lining was returned to normal by heating the beans. Height of the villi did not differ significantly between any treatments. However, when muscle thickness to villus height ratios were calculated, chicks fed raw VB showed a decrease, and again, heating reversed this phenomenon. This suggests that some heat-labile factor and not dietary fiber affects growth of the intestinal musculature

Kidney damage was very evident in chicks fed raw VB. There were marked increases (about 240% greater than controls) in blood in the kidney tubules of chicks fed raw VB as well as a decrease in the apparent presence of lymphocytes compared to either set of control chicks. Heating VB partially reduced the incidence of bloody tubules (to about 134%) and restored the presence of lymphocytes to *ad libitum*-fed control levels. We believe this is the first report of a renal toxic factor in raw VB. The significance of the changes in lymphocyte contents is unclear. One might expect more lymphocytes in damaged kidney tissue rather than the opposite. Only further research can determine the meaning of this.

## CONCLUSIONS

1. Chicks fed 20% raw velvet beans (VB) showed a marked reduction in weight gain. This can be partially or fully corrected by heating the beans. The degree of reversal probably depends on the condition under which the beans were heated and also the content of other anti-nutritional factors in the beans.

2. Feed intake is not always reduced in chicks fed raw velvet beans, and where it is, heating may not be able to reverse this. This suggests that some anorexic factors in raw VB may not be heat labile.

3. Consumption of raw VB may increase heart and liver weights but this is not a consistent finding. Where this occurs, heating VB does not appear to reverse it.

4. Chicks fed raw VB showed consistent increases in pancreatic weights, and heat treatment diminished this effect. This may reflect the presence of heat-labile anti-trypsin factors in VB.

5. Thyroid weights were unaffected by any treatment.

6. Both gizzard and proventricular weights relative to body weights were heavier in chicks fed raw VB, and these are partially reversed by heating. Part of this increased weight may be due to the higher fiber content of diets containing VB compared to soybean oil meal, which would increase the muscular work of digestive organs. However, the presence of a heat-labile growth factor in raw VB cannot be excluded.

7. Most consistent with earlier experiments, chicks fed raw VB had longer small intestines and ceca, and these effects were partially reversed by heating the VB.

8. Consumption of raw VB increased the length of the large intestine, but heating VB reversed this only in one experiment. The higher fiber content of diets containing VB may play a role in this effect.

9. Consistent with earlier observations, plasma creatinine was markedly reduced in chicks fed raw VB, but this was not reversed by heating the beans. This is most likely related to muscle damage as the opposite effect would be expected if the kidneys and hence glomerular filtration rate were the problem.

10. Compared to pair-fed controls, plasma cholesterol was lower in chicks fed raw or heated VB, and the lowering effect was stronger with the heated VB. In general, this agrees with our earlier results and results reported with rats.

11. The effects of raw VB on plasma thyroxine, glucose, alanine aminotransferase and calcium were indeterminate. They were not different from the *ad libitum*-fed control but in some cases did differ from the pair-feed controls.

12. Other blood components such as triiodothyronine, alkaline phosphatase and sodium were not affected by feeding VB.

13. An increase in pancreatic necrosis was attributed solely to the decreased growth rate and not a result of anti-nutritional/toxic properties in the VB themselves.

14. Effects on liver necrosis were unclear. Both restricted feeding and use of heated but not raw VB increased this.

15. Width of the mucosal muscular layer of the small intestine as well as the ratio of muscle thickness to villi length were decreased in chicks fed raw VB as well as in pair-fed controls. This was restored to normal by heating the beans.

16. Chicks fed raw VB showed a marked increase in blood in kidney tubules (200% +> control) as well as a significant reduction in what appeared to be tissue lymphocytes. This was separate from any effect due to reduced feed intake, and was completely reversed by heating.

In summary, consumption of raw VB by growing chickens causes several changes in organ growth, blood chemistry and tissue histology. Dry heating the VB reverses in part or completely some of these effects, but not all of them. VB, therefore, contain anti-nutritional, anti-physiological factors that are heat labile, and heating them is a useful adjunct to other methods of processing for improving their nutritional value. However, there is a need for standardization and further study of techniques for heat processing VB.

## ACKNOWLEDGMENTS

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