THE EFFECT OF JACKBEAN UREASE INJECTIONS ON PERFORMANCE, ANTIUREASE PRODUCTION AND PLASMA AMMONIA AND UREA LEVELS IN SHEEP AND SWINE

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INTRODUCTION

Urea is the metabolic end product of protein metabolism in most mammals. As a constituent of saliva and gastrointestinal secretions, urea may enter the gastrointestinal tract in large quantities. Urea may serve as a nitrogen source for ruminants since bacterial urease present in the rumen readily degrades urea to ammonia and carbon dioxide, thus making available ammonia nitrogen for amino acid synthesis by rumen bacteria (Gallup, 1956). The factors affecting the utilization of urea by ruminants have been extensively studied and reviewed by Reid (1953) and Gallup (1956). In view of the decreasing availability of protein supplements for use in ruminant diets, studies on the more efficient use of non-protein nitrogen sources such as urea are needed.

One problem in urea utilization is the rapid release of ammonia in the rumen, resulting in toxicity or poor utilization (Lewis, 1957). If the release rate could be slowed, it is possible that ruminal ammonia would be converted more efficiently into bacterial protein. Dang and Visek (1960) and Visek (1962) have shown in rats and chicks that the rate of release of ammonia from urea can be slowed with induced urease immunity, thereby increasing rate and efficiency of weight gain. The present experiments were designed to investigate the effect of jackbean urease injections on sheep and swine.

REVIEW OF LITERATURE

Some Properties of Urease

Urea amidohydrolase (urease, 3.5.1.5), which was isolated by Sumner (1926), was the first enzyme to be obtained in the crystalline form. It occurs in over 200 species of bacteria, in several species of yeast, in fungi, and in a large number of higher plants (Sumner and Somers, 1953).

Sumner and Somers (1953) described urease as a colorless crystalline octahedron that separates from 32 percent acetone. It is a globulin that is completely denatured and precipitated when its solution is boiled at pH 5.0, gives a positive nitroprusside test, and possesses sulfhydryl groups. Sumner et al. (1938) determined the molecular weight of urease to be 483,000, while Hand (1939) demonstrated that dissociated particles as small as 17,000 molecular weight may still be enzymatically active. Hand (1939) determined the isoelectric point of urease to be at pH 5.0.

The breakdown of urea by urease occurs via the following sequence:

$$\begin{array}{ccccc} NH_2CONH_2 & \xrightarrow{\text{Urease}} & H_2NCOOH & \longrightarrow & CO_2 \\ + & & + & & + \\ H_2O & & NH_3 & & NH_3 \end{array}$$

Evidence that carbamate is an intermediate was obtained by Gorin (1959) who observed the formation of carbamate during the urease reaction under conditions where recombination of ammonia and carbon dioxide could not occur.

Presence of Urease in Mammals

There is a divergence of opinion concerning the ability of mammals to produce urease. Salivary secretions taken from the oral cavity, gastric mucosal samples, and samples taken from cecum and colon scrapings readily exhibit urease activity. Davies and Kornberg (1950) and Kornberg and Davies (1952, 1955) have concluded that gastric urease is definitely of bacterial origin, and contend that workers who demonstrated the presence of mammalian urease were working with impure mucosal samples. Their conclusion is substantiated by Levenson et al. (1959), who observed that germfree rats injected intragastrically with ^{14}C-labeled urea expired only 1/100 as much $^{14}CO_2$ as controls during a six hour period. Dintzis and Hastings (1953) obtained near-germfree rats by repeated feeding of a mixture of antibiotics, and observed that formation of ammonia from urea in these rats was almost non-existent.

Conway et al. (1959) stated that the urease in the gastric mucosa is contained within the surface epithelial cells which contain no bacteria, and that the amount of urease in the gastric mucosa of the mouse is relatively large and would require so great a bulk of bacteria to supply it that a bacterial origin of all such urease appears untenable. The finding by Cardin (1933) of fetal gastric urease indicates that bacteria may not be concerned with its formation.

No extensive observations have been made on ruminant animals, although the tremendous numbers of urease-producing bacteria in the

rumen (Mackay and Oxford, 1954; Huhtanen and Gall, 1955) make it highly unlikely that any other source of urease, if any exists, could exert a significant effect on urea hydrolysis.

Inhibitors of Urease Activity

Groll (1917) ranked the following cations on their decreasing effect on urease activity: $NH_4^+ > K^+ > Na^+ > Sr^{++} > Ba^{++}$. Mapson (1946) noted that cuprous ions are more inhibitory to urease than cupric ions. Vandevelde (1947) screened the following heavy metals and cations: Pb++, Cd++, Fe++, Bi++, and Zn++ were clearly inhibitory, and Ni++, Mg++, Te++, Hg++, Co++, Cu++, and Ag++ greatly retarded the reaction of soybean urease. Shaw (1954) collected data from the literature on the relative toxicities of the common metal ions towards urease and arranged them in the following sequence: $Ag^{++} > Hg^{++} > Cu^{++} > Cd^{++} > Co^{++} > Ni^{++} > Mn^{++}$, with Pb++ and Zn++ unassigned but less inhibitory than Cu++.

Groll (1918) observed that with the same cation, the nature of the anion is of less influence on urease, but can be arranged in the following order: $SO_4^{=} > Cl^- > Br^- > NO_3^- > S^{=} > I^-$. Vandevelde (1947) observed that sulfate, chloride, and nitrite salts of both sodium and potassium were inhibitory, but that only the nitrite salt of ammonium was inhibitory at the same concentrations. Ambrose et al. (1949) also observed that sulfites and bisulfites are inhibitory. Van Slyke and Zacharias (1914) conducted experiments with isoionic phosphate solutions from pH 5.9 to

8.7, and observed that within these limits the velocity of the combination of urease and urea varies inversely with the hydrogen ion concentration. The velocity of decomposition of the urease-urea complex was most rapid in neutral solution and was retarded by either acidity or alkalinity. Harmon and Niemann (1949) concluded that the urease-catalyzed hydrolysis of urea was competitively inhibited by phosphate at pH 7.0 and 25° C. Fasman and Niemann (1951), however, reported that their initial results were in error and stated that the buffer anion apparently acts as an activator while the buffer cation acts as an inhibitor. The pH optimum for urease activity in phosphate buffer solutions has been found to be at neutrality (Sumner and Somers, 1953)

The inhibition of urease from Micrococcus pyogenes var. aureus by various sulfhydryl binding reagents appears to be dependent on the culture conditions. Yall and Green (1952) reported that when these organisms were grown in the presence of urea, they produced a urease which was inhibited by nitrofurazone, p-chloromercuribenzoate, and trivalent arsenicals. When the organisms were grown in the absence of urea, or when a nitrofurazone-resistant strain was grown in a medium that contained urea, urease was obtained that was not affected by any of the above inhibitors. Alloxan and several cyclic urea-related compounds, such as alloxanic acid, dialuric acid, alloxantin, and murexide, exert an inhibitory effect on urease activity (Siliprandi and Daghetta, 1950; Gray et al., 1959). Alloxan, murexide, and barbituric acid also inhibit gastrointestinal urease activity in chicks (Visek et al., 1961).

Thiourea is a competitive inhibitor of urease at pH 6.0, and inhibits both competitively and non-competitively at pH 7.6 (Kistiakowsky and Shaw, 1953). Wills and Wormall (1949) observed that the complex carbamide, suramin, competitively inhibits urease below but not above its isoelectric point. Deasy (1947) observed that high concentrations of the substrate urea are inhibitory under slightly alkaline conditions but not under acid conditions. An ammonia-urea complex is apparently formed under alkaline conditions that inhibits further urea hydrolysis.

The Control of Urease Activity by Antibacterial Agents

Slinger et al. (1952) noted that urea added to chick diets depressed rate and efficiency of gain, but that this depression was not as severe when penicillin was added. Prescott (1953) observed that penicillin as low as one mg./ml. markedly reduced urea hydrolysis in rumen fluid. In a study on the distribution of urease in the gastrointestinal tract of mice, Dintzis and Hastings (1953) found that feeding a mixture of sulfaguanidine, terramycin, and penicillin abolished all significant urease activity. They concluded that the marked reduction in ammonia production was due to the bactericidal activity of the antibiotics. Silen et al. (1955) observed that neomycin markedly reduced portal blood ammonia in dogs, while sulfasuxidine and achromycin were ineffective. Visek et al. (1959) demonstrated that rats fed a diet supplemented with penicillin, chlortetracycline, or arsanilic acid metabolized significantly less ^{14}C-urea than unsupplemented controls. Urea metabolism was

decreased to the greatest degree by chlortetracycline followed by penicillin and then arsanilic acid. Harbers et al. (1963a) and Alvares et al. (1964) obtained significant increases in growth rate in chicks fed diets supplemented with barbituric acid or chlortetracycline. In both cases, ureolytic activity of intestinal contents was markedly decreased when increases in rate of gain were obtained. Although none of the above studies clearly established the role of antibiotics as bactericidal, a profound effect on gastrointestinal urease activity was consistently obtained with antibiotic supplementation.

The major expression of the effect of chemotherapeutic agents may be bacterial death; however, Woods (1942) suggested that their primary effect may be that of interfering in some way with essential metabolites. Two ways in which Woods (1942) proposed that this may occur are: by formation of a definite compound between the antibacterial agent and essential metabolite, such as SH complexing; and by inhibition of an enzyme reaction involved in the synthesis or utilization of an essential metabolite. Turner et al. (1943) and Vargas and Escubos (1945) observed that highly purified preparations of penicillin markedly inhibited the activity of urease. Scudi and Jelinek (1944), however, were able to inhibit urease activity only with crude penicillin preparations. Murti and Shrivastava (1953) were able to inhibit urease activity with dihydrostreptomycin, chlortetracycline, and chlormycetin, while penicillin, neomycin, and terramycin were ineffective.

Antiurease

Kirk and Sumner (1931), found that serum of rabbits immunized

with crystalline urease contained an antibody which inhibited the in vitro and in vivo hydrolysis of urea by urease. Rabbits immunized with crystalline urease withstood 100 times the amount of urease fatal to normal animals and showed no rise in blood ammonia. Serum from immune rabbits was also used to confer passive immunity on normal rabbits and guinea pigs. Howell (1932), using hens, confirmed the above findings.

Kirk and Sumner (1934) observed that urease precipitated with urease antibody may retain 70 percent of its original activity. Marucci and Mayer (1955) confirmed the above results and concluded that the antibody exerts some influence on the active site without covering it. Pillemer et al. (1938) observed that urease and oxidized urease produced an immunologically similar antibody while irradiated urease did not.

Dang and Visek (1960) immunized growing rats and chicks with four successive weekly injections of urease during the first four weeks of experiments lasting eight weeks. During the post injection period, the immunized rats and chicks both gained weight at a faster rate and were more efficient than their controls. Visek (1962) observed that serum from immunized rats inhibited the ureolytic activity of a phosphate buffer extract of gastrointestinal contents of normal rats. He also reported that immunized animals injected with ^{14}C-labeled urea expired 40 percent less $^{14}CO_2$ than non-immunized animals during a four hour period. Weight gain and feed efficiency were also improved by urease immunity. Wagner et al. (1963), however, observed that urease injections failed to improve rate or efficiency of gain. Their results are questionable,

because crude urease preparations were used and they did not attempt to measure urease antibody titers. Kornegay et al. (1963) were successful in producing urease antibody with urease injections in swine, but urease immunity improved weight gain in only one out of three trials. Plasma urea levels were not affected, but high serum antiurease titer was associated with decreased ureolytic activity of intestinal contents.

Harbers et al. (1936b), in two experiments with vitamin A depleted rats, observed that urea hydrolysis in the large and small intestine was lowered when the vitamin A level was increased from 2.2 to 22.0 international units, and when the rats were immunized. Their data indicated that depression of ammonia production and urease activity was most beneficial to animals fed limited levels of vitamin A.

Visek et al. (1963) reported that 25 of 38 human subjects infected with Mycobacterium tuberculosis yielded sera capable of agglutinating jackbean urease sensitized red blood cells. Likewise, 43 of 47 sera from Brucella abortus infected or strain 19 vaccinated cattle agglutinated sensitized red blood cells. Unsensitized red blood cells were not agglutinated. The antibody produced in both cases was immunologically similar to that produced against jackbean urease. These results partly explain the known cross-resistance between tuberculosis and brucellosis, and become more intriguing when coupled with the fact that both organisms hydrolyze urea in vitro.

EXPERIMENT I

THE EFFECT OF JACKBEAN UREASE INJECTIONS AND CHLORTETRACYCLINE ON RATE OF GAIN AND FEED EFFICIENCY IN SWINE

The suppression of ammonia production, resulting from the breakdown of urea by urease, has often been suggested as an important function of antibacterial agents. Dang and Visek (1960) and Visek et al. (1961) have shown that antibodies to jackbean urease elicit a similar response in rats and chicks. These investigators further observed significant increases in gain and feed efficiencies in both species that were attributed to the increase in antiurease titer.

The purpose of this study was to investigate the effect of jackbean urease injections and chlortetracycline upon the performance of swine.

Experimental Procedure

Trial 1

Assay for urease activity. In general, the procedure described by Gorin et al. (1962) was used in the present trials; however, extensive modifications were made, thus the detailed procedure is reported herein: glass-distilled water was used in preparing all solutions. Solution A

contained 0.005 molar Na_2HPO_4 in 0.85% NaCl and solution B contained 28.0 gm. KH_2PO_4 plus 128.4 gm. Na_2HPO_4 per liter. Solution C contained 25 mg. bovine serum albumin plus 1.5 gm. urea made up to a volume of 50 ml. using solution B. M-Alka-Ver was the indicator.[1]

In the assay procedure, 1.5 ml. of solution A were diluted with 1.0 ml. of solution C and allowed to stand in a 20° C. water bath for 5 minutes. Two drops of indicator were added, and the mixture was titrated with 0.1 N HCl to the first purple color (V = ml. of acid used). To 0.5 ml. of solution A and 1.0 ml. of solution C was added 1.0 ml. of a dilute urease solution. The mixture was incubated exactly 5 minutes at 20° C., and V ml. of 0.1 N HCl were added immediately. Two drops of indicator were then added, and the mixture was titrated with 0.1 N HCl to the first purple color (V' = ml. of 0.1 N HCl used). The calculations are as follows:

$$(V' - V)(0.1)(14) = X \text{ Sumner units of urease}$$

Assay for antiurease. In the initial study, blood serum antibody titer was measured by the hemagglutination method described by Visek et al. (1962). Qualitative tests for the presence of antibody using the precipitin ring test were also periodically conducted throughout the trial. In addition, another assay procedure was developed in this laboratory as a modficiation of the method used by Kirk and Sumner (1931). The

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[1] M-Alka-Ver, Hach Chemical Company, Ames, Iowa.

details of the latter procedure were as follows: to each of two test tubes were added 0.2 ml. of blood serum. The first test tube received 1.3 ml. of solution A and 1.0 ml. of solution C, and the mixture was incubated 5 minutes at 20° C. Two drops of indicator were then added and the mixture was titrated with 0.1 N HCl ($\underline{v}$ = ml. of HCl used). To the second test tube was added 0.3 ml. of solution A and 1.0 ml. of dilute urease (2-4 Sumner units) of known activity. The mixture was incubated for 10 minutes in a 37° C. water bath, then removed and allowed to stand for 2 minutes at 20° C. One ml. of solution C was then added and the mixture was incubated exactly 5 minutes at 20° C., and $\underline{v}$ ml. of 0.1 N HCl were added immediately. Two drops of indicator were then added and the mixture was titrated to the first purple color ($\underline{v}'$ = ml. of HCl used). The calculations are as follows:

$$(\underline{v}' - \underline{v})\,(0.1)\,(14) = Y \text{ modified Sumner units of urease}$$

If the above procedure were quantitative, (X - Y) (5) would be equal to the units of antiurease per ml. blood serum; however, higher assays of urease were noted when $\underline{X}$ units were incubated with 0.2 ml. of blood serum, indicating that some factor in blood serum enhances urease activity. It has been further noted that, when serum proteins were denatured by heat, this increase in activity was not obtained. No satisfactory correction factor has been worked out to allow for this discrepancy; but as urease activity was markedly decreased in blood samples with a known high antibody titer when compared to samples with a low or no antibody

titer, the procedure was satisfactory for comparative purposes. As the specific precipitation of an enzyme by its antibody varies in efficiency with the amount of enzyme and antibody present, it is important for comparative tests that the same amount of urease be added to each sample. Day to day variations were minimized by blocking on days in the statistical analysis. The data were analyzed statistically by analysis of variance.

The hemagglutination assay (Visek et al., 1962) in our hands did not give reproducible results, thus the above method was developed. Although the above method is not absolutely quantitative, it has certain advantages over the hemagglutination assay method. The results are highly reproducible and readily subject to statistical analysis. Further, the urease assay method of measuring antibody titer measures only that antibody which destroys urease activity, in contrast to the total precipitable antibody titer measured by the hemagglutination assay. Kirk and Sumner (1934) and Marucci and Mayer (1955) have shown that the total activity of urease precipitated with rabbit serum antiurease is only partially lost.

Three-times recrystallized urease was isolated by the procedure described by Gorin et al. (1962) and used in this trial. Twenty-four Yorkshire X Poland China barrows weighing an average of 26 lb. were allotted by weight to the following factorially-arranged treatments: (1) basal ration; (2) basal ration plus the injection of 100 Sumner units of jackbean urease; (3) basal ration plus 100 ppm chlortetracycline; and

(4) basal ration plus 100 ppm chlortetracycline and the injection of 100 Sumner units of jackbean urease. The pigs were fed ad libitum in pens of two for a total of 12 weeks. All pigs on treatments 2 and 4 received an initial intraperitoneal injection of 10 Sumner units of crystalline urease dissolved in 1.0 ml. of physiological saline solution which was followed by successive weekly injections of 20, 30, and 40 units, respectively; pigs on treatments 1 and 3 received injections of 1.0 ml. of physiological saline solution at the same time. Composition of the basal ration is shown in Table 1.

Two pigs from each treatment were bled by jugular puncture at the beginning of the trial, and all pigs were bled 14 days after the last injection. Blood serum urease antibody titers were determined by the hemagglutination assay method (Visek et al., 1962) and by the urease assay method.

Trial 2

Thirty-two Yorkshire X Poland China barrows weighing an average of 67 lb. were allotted by weight to four treatments involving 0, 100, and 200 Sumner units of jackbean urease[2], which was injected intraperitoneally by the schedule shown in Table II. Composition of the ration is shown in Table I. The pigs were fed in pens of two to an average pen weight of 200 lb.

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[2]Jackbean urease (J3 preparation) obtained from Charles Pfizer and Company, Paul Lewis Laboratories, Milwaukee, Wisconsin.

TABLE I

PERCENTAGE COMPOSITION OF RATIONS

Trial	1	2
Ground sorghum grain	79.7	76.0
Soybean meal (44%)	14.5	16.0
Alfalfa meal (17%)	4.0	5.0
Dicalcium phosphate	1.0	1.0
Calcium carbonate		0.8
Trace-mineralized salt[a]	0.5	0.5
Premix[b]	0.3	0.3

[a]Percentage composition as follows: NaCl, 95.80; $CaCO_3$, 1.00; $CaHPO_4$, 1.70; $FeSO_4$, 0.680; $CuSO_4$, 0.083; $CoSO_4$, 0.026; $MnSO_4$, 0.690; $ZnSO_4$, 0.012; NaI, 0.008.

[b]Percentage composition as follows: ground sorghum grain, 74.85; $CuSO_4$, 10.37; $ZnSO_4$, 10.37; Vitamin A and D supplement containing 20,000 I.U. Vitamin A and 2,500 I.U. Vitamin D_2 per gram, 3.70; niacin, 0.204; pantothenic acid, 0.370; riboflavin, 0.089; thiamine, 0.037; Vitamin B_{12}, 0.008.

TABLE II

SCHEDULE OF SUMNER UNITS OF UREASE INJECTED, TRIAL 2

Sumner units urease			100	200	300
Injection No.	Week				
1	0	1 ml. P/SS[a]	10	20	30
2	1	"	20	40	60
3	2	"	30	60	90
4	3	"	40	80	120

[a]Physiological saline solution.

All pigs were bled by jugular puncture initially and at 14 days after the last injection. Blood serum urease antibody titers were determined by the urease assay method. All other details were as described in Trial 1.

Results and Discussion

Trial 1

Table III exhibits the daily gains and feed utilization results obtained in Trial 1. Chlortetracycline improved gains ($P < .01$) and feed efficiencies ($P < .05$). As no atrophic rhinitis was observed in pigs receiving the antibiotic, control of this disease could have been a factor in the improved growth response obtained when it was fed at this high level. Two severe cases of atrophic rhinitis were found in pigs on treatment 2 (confirmed

TABLE III

AVERAGE DAILY GAINS AND FEED EFFICIENCIES, TRIAL 1

Treatment	1	2	3	4
Number of pigs	6	6	6	6
Average daily gain, lb.				
First 4 weeks[a]	0.74±.04[c]	0.72±.04	0.99±.05	0.93±.05
Last 8 weeks[a]	1.27±.05	1.00±.13	1.54±.04	1.56±.08
Total[a]	1.09±.04	0.90±.15	1.35±.05	1.36±.07
Feed efficiency, feed/lb. gain				
First 4 weeks[b]	3.30±.06	3.51±.35	2.77±.05	2.87±.11
Last 8 weeks[b]	3.30±.07	3.93±.40	3.20±.04	3.21±.14
Total[b]	3.30±.07	3.79±.36	3.09±.04	3.13±.13

[a]Treatments 3 and 4 gained faster than treatments 1 and 2 ($P < .01$).

[b]Treatments 3 and 4 were more efficient than treatments 1 and 2 ($P < .05$).

[c]Standard error.

by postmortem examination) while two mild cases were observed on both treatments 1 and 2.

Antibody titers are shown in Table IV. As a hemagglutination titer of 1:50 is considered positive (Visek et al., 1962), the data indicate that the pigs had a titer against urease when the test was initiated. No significant differences ($P > .05$) between antiurease titers were found at any time after the trial was initiated. Likewise, no differences between urease activities after incubation with blood serum samples were significant. Since relative urease activities are reported, it should be noted that an inverse relationship exists between these values and antiurease titer determined by the hemagglutination method.

Trial 2

The growth and feed efficiency results of this trial are shown in Table V. Differences in rates of gains were not significant ($P > .05$) during any part of the feeding period. A significant quadratic response ($P < .01$) was found in feed efficiency when the data for the first 28 days and the entire trial were considered. As the pigs receiving 300 Sumner units were more efficient than those receiving the 100 or 200 unit levels, the data could indicate that the lower levels were not high enough. The greatest differences among treatments were noted during the very early stages of the trial when one could expect no beneficial effect of urease titer (Visek et al., 1962), thus the differences could be chance occurrences.

TABLE IV

INITIAL AND 35-DAY HEMAGGLUTINATION TITERS AND SUMNER UNITS OF UREASE ACTIVITY AFTER INCUBATION WITH BLOOD SERUM, TRIAL I

Treatment	Sample No.	Initial		Final	
		A[1]	B[2]	A[1]	B[2]
1	1	200-500	2.95	500-500	2.90
	2	100-500	2.93	200-500	2.95
2	7	500-500	2.93	500-500	2.88
	8	1000-200	2.85	5000-2000	2.60
3	17	50-200	3.05	500-1000	2.77
	18	500-500	2.77	5000-5000	2.57
4	13	1000-500	2.75	100-50	3.20
	14	100-100	3.00	200-50	2.98

[1]The two values represent the highest dilutions at which hemagglutination was observed on duplicates of the same sample. The hemagglutination assays were conducted by Dr. L. H. Harbers, Department of Pharmacology, University of Chicago, Chicago, Illinois.

[2]Urease assay for antibody titer determined by the method shown previously. The amount of urease added to the reaction medium was 2.75 Sumner units.

TABLE V

AVERAGE DAILY GAINS AND FEED EFFICIENCIES OF PIGS AND SUMNER UNITS OF UREASE ACTIVITY AFTER INCUBATION WITH BLOOD SERUM, TRIAL 2

Sumner units urease injected	0	100	200	300
Number of pigs	8	8	8	8
Average daily gain, lb.				
First 28 days	1.55±.06[3]	1.39±.03	1.37±.05	1.46±.06
28 days to 200 lb.	2.04±.05	1.92±.06	1.88±.04	1.91±.04
Total	1.85±.06	1.73±.06	1.69±.05	1.74±.03
Feed efficiency, feed/lb. gain				
First 28 days[1]	2.68±.03	3.01±.03	3.10±.04	2.81±.05
28 days to 200 lb.	3.23±.03	3.35±.05	3.45±.07	3.30±.07
Total[1]	3.05±.06	3.25±.01	3.34±.01	3.15±.06
Sumner units urease activity[2]				
Initial	2.91±.15	2.94±.13	2.88±.09	2.95±.11
35 days	2.80±.10	2.79±.14	2.76±.12	2.76±.08

[1] Significant quadratic response due to level of urease injected ($P < .01$).

[2] The standard amount of urease added was as follows: initial, 2.75; and 35 days, 2.65 Sumner units.

[3] Standard error.

Antiurease activity was not significantly affected ($P > .05$) by any urease injection level. Neither were there changes in antiurease activities when the initial levels of individual pigs were compared to those obtained 14 days after the last injection. These data support the idea that the pigs used in these studies had a high titer against urease at the beginning of the trial and that attempts to increase that titer were unsuccessful. Results by Visek et al. (1963), in which human patients infected with Mycobacterium tuberculosis and cattle infected with Brucella abortus yielded sera capable of agglutinating jackbean urease sensitized red blood cells, lend support to the idea. Both organisms hydrolyze urea in vitro and were shown to produce antigens which elicit production of antibodies which are immunologically similar to those which are produced against injected jackbean urease. Since pigs of both trials came from a herd infected with atrophic rhinitis, such a relationship could have existed in these trials.

Summary

Two trials, involving 55 Yorkshire X Poland China barrows, were conducted to study the effects of immunity to jackbean urease on rate of gain and feed efficiency in swine.

In the first trial, with factorially-arranged treatments including 100 ppm chlortetracycline in the diet and 100 Sumner units of urease injected intraperitoneally, chlortetracycline improved rate of gain and feed efficiency while the urease injections did not. A high initial serum titer

against urease was observed, and injections of jackbean urease failed to increase this titer. In the second trial, injections of up to 300 Sumner units of urease failed to increase the initial titer against urease and had no effect on feed efficiency and rate of gain.

EXPERIMENT II

THE EFFECT OF JACKBEAN UREASE INJECTIONS ON RATE OF GAIN AND FEED EFFICIENCY IN SHEEP

The purpose of this study was to investigate the effect of jackbean urease injections upon urease immunity and performance of sheep.

Experimental Procedure

Trial 1

Nine Rambouillet and nine Rambouillet X Suffolk wethers averaging seven months of age were randomly allotted within breed to three treatments involving 0, 100, and 200 Sumner units of jackbean urease injected subcutaneously in the left rear flank by the schedule shown in Table II. The lambs were fed ad libitum the ration shown in Table VI in individual concrete-floored pens. Feed troughs were cleaned and stale feed was discarded weekly. All lambs were weighed weekly.

The lambs were bled by jugular puncture initially, 14 days after the last injection, and at the end of the trial. Blood serum urease antibody titers were determined by the inhibition of urease activity method previously described in Experiment I. The data were analyzed statistically by analysis of variance, and orthogonal comparisons were made between treatment means.

TABLE VI

PERCENTAGE COMPOSITION OF RATIONS USED IN SHEEP TRIALS

Trial	1	2		3, 4
		A	B	
Soybean meal (50%)			22.00	
Starch	24.35	24.35	15.45	23.80
Cerelose	24.35	24.35	15.45	23.80
Cottonseed hulls	16.00	31.00	31.00	31.00
Mineral mix[a]	15.00	15.00	15.00	15.00
Solka floc	15.00			
Corn oil	1.00	1.00	1.00	1.00
Urea	4.20	4.20	4.20	5.30[c]
Choline chloride	0.10	0.10	0.10	0.10
Vitamin A and D[b]	10 gm.	10 gm.	10 gm.	10 gm.

[a]Oltjen et al. (1962), Table 2.

[b]NOPCO "Quadrex." Vitamin A, 20,000 U. S. P. units per gm. Vitamin D_2, 2,500 U. S. P. units per gm.

[c]Percent urea was lowered to 4.20 at four weeks. Starch and cerelose were increased accordingly.

Trial 2

Thirty Rambouillet X Suffolk lambs were randomly allotted to a 3 X 2 factorial arrangement of treatments with urea vs. soybean meal as nitrogen sources and 0, 100, and 200 Sumner units of jackbean urease injected subcutaneously on the same schedule as in Trial 1. Composition of the rations are shown in Table VI.

Feeding practices and other details were as described in Trial 1.

Trial 3

Thirty Rambouillet lambs, approximately seven months old and weighing an average of 56 lb. initially, were randomly allotted to three treatment groups which were, respectively, 0, 450, and 900 Sumner units of jackbean urease injected subcutaneously in the left rear flank on the schedule shown in Table VII. Composition of the ration is shown in Table VI. Feeding practices and other details were as previously described for Trial 1.

TABLE VII

SCHEDULE OF SUMNER UNITS OF UREASE INJECTED, TRIALS 3 AND 4

Sumner units urease		0	450	900
Injection No.	Day			
1	0	5 ml. P/SS[a]	30[b]	60
2	5	"	60	120
3	10	"	90	180
4	15	"	120	240
5	30	"	150	300

[a]Physiological saline solution.

[b]Jackbean urease was added to physiological saline solution and assay just prior to injection. Each animal received an injection of 5 ml.

Blood serum antiurease activity was determined on blood samples taken initially, at 45 days, and at the end of the trial by the procedure previously outlined.

At the end of Trial 3, four of the control lambs and eight lambs from each of the urease treatments were used in a 28-day study to determine the effect of a booster injection of 200 Sumner units of jackbean urease on rate of gain in lambs previously immunized against urease. Four lambs from those which had previously been injected with 450 and 900 Sumner units were given these booster injections, while the remaining four from each of the two treatments and the four controls were given injections of physiological saline solution. Other details were identical to those in Trial 3.

Trial 4

This was a companion trial to Trial 3 and differed only in that blood serum antiurease and blood urea levels were determined weekly. Fifteen lambs, five on each urease level, were used. All other details were as previously described in Trial 3.

Trial 5

Nine of the 15 lambs on Trial 4 were used in a study to determine the effect of urease immunity on jugular vein plasma urea levels at various intervals after infusion of the rumen with a urea-dextrose solution. Forty-two days after the first injection, or 12 days after the last injection of jackbean urease as shown in Table VII, three lambs from each of the

three treatments were selected at random and fasted for 18 hours. After an initial blood sample was taken by jugular puncture, each lamb was drenched with 100 ml. of a solution that contained 10 gm. urea and 50 gm. dextrose. Blood samples were taken from the jugular vein at 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after drenching.

Clotting of the blood samples was prevented by adding 0.5 ml. of a saturated sodium citrate solution to each tube prior to collection of the blood samples. The samples were refrigerated immediately after collection for approximately 24 hours, at which time they were centrifuged and the plasma was collected. Plasma samples were then frozen for future analysis. Plasma urea was determined by the method described by Conway (1957).

Trial 6

In another study, four lambs from Trial 3 were used to determine the effect of the above treatments on plasma urea and ammonia levels of blood taken from the posterior ruminal vein, jugular vein, and the cecal vein. Two of the lambs received 450 Sumner units of jackbean urease during Trial 3 and later received injections of 200 and 100 Sumner units, respectively, making a total of 750 Sumner units for the two lambs. The other two lambs were controls. The blood samples were taken 14 days after the last booster injection of jackbean urease.

The lambs were anesthetized by the slow intravenous injection of

a 10 percent solution of thialbarbitone sodium[3] to effect. Surgical anesthesia was maintained by the endotracheal inhalation of methoxyflurane[4] using an AVR positive-negative pressure anesthetic apparatus with bag attachment.[5]

A six-inch right paracostal laparotomy incision was made. After invasion of the peritoneal cavity, the posterior ruminal and cecal veins were exposed by blunt dissection, and polyethylene cannulae were inserted. The cannulae were attached to three-way stopcocks, through which blood samples were withdrawn. When blood samples were not being withdrawn, a 3 percent solution of sodium citrate was infused through these cannulae at the rate of approximately 100 ml. per hour to prevent clotting.

Blood samples were withdrawn at the time of cannulation and at 1, 2, 3, 4, 5, and 6 hours after intraruminal infusion of 100 ml. of the urea-dextrose solution previously described. Blood samples were handled as described in Trial 5 and plasma ammonia nitrogen (NH_3-N) was determined by the method described by Conway (1957).

Results

Trial 1

Average daily gains and feed efficiencies of lambs injected with 0, 100, or 200 Sumner units of urease are presented in Table VIII.

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[3]Kemithal, Fort Dodge Laboratories, Fort Dodge, Iowa.

[4]Metofane, Allied Laboratories, Indianapolis, Indiana.

[5]Chemetron Corporation, Chicago, Illinois.

TABLE VIII

AVERAGE DAILY GAINS AND FEED EFFICIENCIES OF LAMBS AND SUMNER UNITS OF UREASE ACTIVITY AFTER INCUBATION WITH BLOOD SERUM, TRIAL 1

Sumner units urease injected	0	100	200
Number of lambs	6	6	6
Average daily gain, lb.			
Total[a]	.20	.25	.25
First 4 weeks[b]	.50	.35	.38
Last 8 weeks[c]	.04	.20	.19
Feed efficiency, lb. gain/lb. feed			
Total	.074	.079	.092
First 4 weeks	.166	.124	.132
Last 8 weeks[c]	.019	.068	.075
Sumner units urease activity[d]			
Initial	2.86	2.84	2.84
35 days[e]	2.93	2.59	2.31
Final[f]	2.37	2.21	2.08

[a] 0 significantly different from 100 and 200 Sumner units ($P < .01$).

[b] 0 significantly different from 100 and 200 Sumner units ($P < .10$).

[c] 0 significantly different from 100 and 200 Sumner units ($P < .001$).

[d] The standard amount of urease added was as follows: initial, 2.80; 35 days, 2.88; final, 2.33 Sumner units. A decrease in urease activity indicates an increase in antiurease titer.

[e] Test for linearity significant ($P < .01$); test for quadratic effect, not significant.

[f] Test for linearity significant ($P < .05$); test for quadratic effect, not significant.

Lambs receiving injections of 100 and 200 Sumner units of jackbean urease gained at a faster rate over the last eight weeks of the trial ($P < .001$), and were more efficient in their gains during this period ($P < .001$). When results of the entire trial are considered, urease injections increased rate of gain ($P < .01$) while differences in feed efficiency were not significant ($P > .05$).

Antiurease activities, as measured by a decrease in urease activity after incubation of a known amount of urease with blood serum, are also shown in Table VIII. As noted previously in the swine trials, an increase in Sumner units of urease activity above the standard amount added was obtained at the initial bleeding of all lambs and in the control group throughout the trial; however, the standard urease activity was decreased with an increase in the amount of urease injected. This response did not significantly differ from linearity for the 35-day bleeding ($P < .001$) or the final bleeding ($P < .01$). The qualitative presence of antibodies against urease was also confirmed by intermittent use of the precipitin ring test. No positive tests for antiurease were obtained from control lamb serum samples.

These data indicate that antibodies against urease were produced in all treated lambs, and that an increase in rate and efficiency of gain was associated with the production of antiurease. It appeared that 100 Sumner units of jackbean urease injected subcutaneously was as effective as 200 Sumner units in improving rate and efficiency of gain.

Trial 2

Average daily gains, feed efficiencies, and relative antibody activities of the lambs injected with 0, 100, or 200 Sumner units are shown in Table IX. In contrast to Trial 1, injection of 100 or 200 Sumner units of jackbean urease produced no significant increases in rates or efficiencies of gains; however, the response of blood serum inhibition of urease activity, as in Trial 1, did not significantly differ from linearity when levels of urease were considered.

Lambs receiving soybean meal as the sole nitrogen source in the diet apparently gained at a faster rate than those receiving urea. Much of this difference, however, was due to their faster gains during the first four weeks of the trial ($P < .05$). Differences in feed efficiency were not significant ($P > .05$).

Trial 3

Treatment means for average daily gains, feed efficiencies, and relative antibody activities of the lambs injected with 0, 450, or 900 Sumner units are presented in Table X. A quadratic response ($P < .01$) in gains was obtained, indicating that 450 Sumner units injected subcutaneously increased rate of gain and the 900 Sumner unit level was too high, particularly during the early part of the trial. A similar response was obtained with feed efficiency during the first four weeks of the trial ($P < .05$).

As shown in Table VI, the urea level of the ration was decreased

TABLE IX

AVERAGE DAILY GAINS AND FEED EFFICIENCIES OF LAMBS AND SUMNER UNITS OF UREASE ACTIVITY AFTER INCUBATION WITH BLOOD SERUM, TRIAL 2

Nitrogen source	Soybean meal			Urea		
Sumner units urease	0	100	200	0	100	200
Number of lambs	5	5	5	5	5	5
Average daily gain, lb.						
Total[a]	.40±.04[f]	.41±.04	.40±.02	.35±.02	.38±.01	.36±.02
First 4 weeks[b]	.83±.01	.81±.08	.81±.08	.64±.06	.72±.05	.65±.08
Last 8 weeks	.20±.03	.22±.05	.20±.03	.21±.03	.21±.04	.23±.01
Feed efficiency, lb. gain/lb. feed						
Total	.11±.01	.12±.01	.13±.01	.10±.01	.11±.01	.11±.01
First 4 weeks	.22±.04	.23±.02	.23±.02	.21±.03	.22±.01	.18±.01
Last 8 weeks	.06±.01	.07±.02	.07±.01	.06±.01	.06±.01	.07±.01
Sumner units urease activity[c]						
Initial	3.60±.09	3.63±.10	3.69±.12	3.65±.11	3.64±.05	3.61±.15
35 days[d]	3.49±.03	3.32±.02	3.06±.08	3.50±.10	3.30±.02	3.06±.11
Final[e]	3.60±.04	3.48±.03	3.28±.05	3.59±.06	3.50±.04	3.30±.07

[a]Lambs receiving soybean meal gained at a faster rate ($P < .05$).

[b]Lambs receiving soybean meal gained at a faster rate ($P < .01$).

[c]Standard amount of urease added was as follows: initial, 3.60; 35 days, 3.42; and final, 3.58 Sumner units.

[d]Test for linearity significant ($P < .001$); test for quadratic effect, not significant.

[e]Test for linearity significant ($P < .05$); test for quadratic effect, not significant.

[f]Standard error.

TABLE X

AVERAGE DAILY GAINS AND FEED EFFICIENCIES OF LAMBS AND SUMNER UNITS OF UREASE ACTIVITY AFTER INCUBATION WITH BLOOD SERUM, TRIAL 3

Sumner units urease	0	450	900
Number of lambs	10	10	10
Average daily gain, lb.			
Total[a]	.16±.02[e]	.24±.03	.07±.04
First 4 weeks[a]	.01±.06	.10±.06	-.21±.05
Last 8 weeks[b]	.25±.03	.31±.03	.22±.05
Feed efficiency, lb. gain/lb. feed			
Total[b]	.09±.01	.11±.01	.03±.03
First 4 weeks[b]	-.03±.05	.01±.07	-.22±.06
Last 8 weeks	.13±.01	.15±.01	.13±.02
Sumner units urease activity[c]			
Initial	4.01±.03	4.04±.06	4.04±.03
45 days[d]	3.99±.03	3.63±.02	3.22±.07
Final[d]	4.02±.01	3.82±.02	3.49±.04

[a]Significant quadratic response to level of urease injected ($P < .01$).

[b]Significant quadratic response to level of urease injected ($P < .05$).

[c]Standard amount of urease added was as follows: initial, 3.78; 45 days, 3.80; and final, 3.83 Sumner units.

[d]Test for linearity significant ($P < .05$); test for quadratic effect, not significant.

[e]Standard error.

from 5.3 percent to 4.2 percent of the ration at four weeks. It is thought that the high level of urea might explain the poor average daily gains of all lambs during the first four weeks; gains increased after the urea level was lowered.

Six of the 10 lambs receiving 900 Sumner units of urease showed an apparent hypersensitivity against urease as demonstrated by severe muscular swelling and lameness in the left rear leg after the second and subsequent injections. All lambs showing lameness made less gains and utilized their feed less efficiently during the injection period than those on the same treatment that did not exhibit lameness. As extreme care was used in preparing fresh solutions for each injection and a separate sterile needle was used on each lamb, contamination was unlikely. The cause of this reaction is not known.

The effects of a booster injection of 200 Sumner units of jackbean urease on subsequent rate of gain of lambs from Trial 3 are shown in Table XI. Lambs which had received 450 Sumner units of urease but no booster injection gained faster than all others ($P < .01$). When compared to those animals which did not receive the booster, it would appear that the booster injection depressed rate of gain in the lambs that had previously received 450 Sumner units of urease; however, their gains were still faster than those of the control lambs ($P < .01$) and apparently (non-significant) faster than those which had received 900 Sumner units of urease. These data indicate that previous injection level was the

primary factor affecting rate of gain, and that 450 Sumner units of urease may be approaching the maximum injectable amount of urease for a desirable response. Treatment means of antiurease activities lend support to this idea.

TABLE XI

THE EFFECT OF A BOOSTER INJECTION OF 200 SUMNER UNITS OF JACKBEAN UREASE ON RATE OF GAIN OF LAMBS, TRIAL 3

Treatment	0	450	450+[a]	900	900+
No. of lambs	4	4	4	4	4
Average daily gain, lb.	.23±.08	.57±.08[b]	.37±.03[c]	.30±.02	.31±.02
Sumner units urease[d]	4.00±.04	3.85±.03	3.48±.02	3.54±.02	3.20±.04

[a]+ denotes lambs that received booster injections of 200 Sumner units.

[b]Lambs injected with 450 Sumner units but no booster injection gained at a faster rate than all others ($P < .01$).

[c]Lambs injected with 450 Sumner units plus a booster injection gained at a faster rate than the control lambs ($P < .01$).

[d]Standard amount of urease added to each blood sample was 3.95 Sumner units.

Trial 4

Average daily gains and feed efficiencies of the lambs injected with 0, 450, or 900 Sumner units are shown in Table XII. Although none of the differences among treatment means were significant, the trends in rate and efficiency of gain were quite similar to those observed in Trial

TABLE XII

AVERAGE DAILY GAINS AND FEED EFFICIENCIES OF LAMBS AND SUMNER UNITS OF UREASE ACTIVITY AFTER INCUBATION WITH BLOOD SERUM, TRIAL 4

Sumner units urease injected		0	450	900
	Standard			
Number of lambs		5	5	5
Average daily gain, lb.				
Total		.22±.05[d]	.28±.05	.20±.03
First 4 weeks		.23±.05	.37±.09	.20±.05
Last 8 weeks		.21±.06	.24±.07	.19±.03
Feed efficiency, lb. gain/lb. feed				
Total		.13±.02	.17±.04	.15±.02
First 4 weeks		.14±.02	.25±.05	.19±.05
Last 8 weeks		.12±.03	.15±.04	.13±.01
Sumner units urease activity				
Initial	3.06	3.39±.08	3.37±.08	3.42±.12
2 weeks	3.69	3.69±.09	3.58±.05	3.52±.05
4 weeks[a]	3.96	4.11±.15	3.82±.05	3.54±.12
6 weeks[b]	3.90	4.04±.02	3.64±.04	3.21±.06
8 weeks[c]	3.60	3.72±.09	3.10±.09	2.82±.02
10 weeks[b]	3.48	3.50±.02	3.40±.02	3.23±.05
12 weeks[a]	3.92	3.99±.04	3.79±.05	3.59±.09

[a]Test for linearity significant ($P < .05$); test for quadratic effect, not significant.

[b]Test for linearity significant ($P < .01$); test for quadratic effect, not significant.

[c]Test for linearity significant ($P < .001$); test for quadratic effect, not significant.

[d]Standard error.

3. Trends of the relative antiurease activities indicate that urease inhibition was present as early as two weeks after the initial injection, but differences were not significant until four weeks ($P < .05$). The magnitude of the differences was greatest at six weeks ($P < .01$) and eight weeks ($P < .001$). It is also interesting to note that at the end of the trial antiurease activity was still related to the amount of urease injected.

Trial 5

Plasma urea levels of the lambs injected with 0, 450, or 900 Sumner units of urease and used in Trial 5 are shown in Figure 1. The differences between treatment means were significant ($P < .05$) only at two points, 2 hours and 6 hours. However, certain interesting observations can be made about this trial. The immunized lambs tended to have higher plasma urea levels, while the control lambs appeared to reach their maximum level at an earlier time. Two control lambs reached a maximum level at 4 hours and the other control lamb at 3 hours, while all six of the immunized lambs reached their maximum plasma urea level at 5 hours after infusion of the urea-dextrose solution.

Trial 6

Plasma NH_3-N levels of jugular and ruminal vein blood from the four lambs injected with 0 or 750 Sumner units and used in Trial 6 are presented in Figure 2. Differences between ruminal blood plasma NH_3-N levels were approaching significance ($P < .10$) at 3, 5, and 6

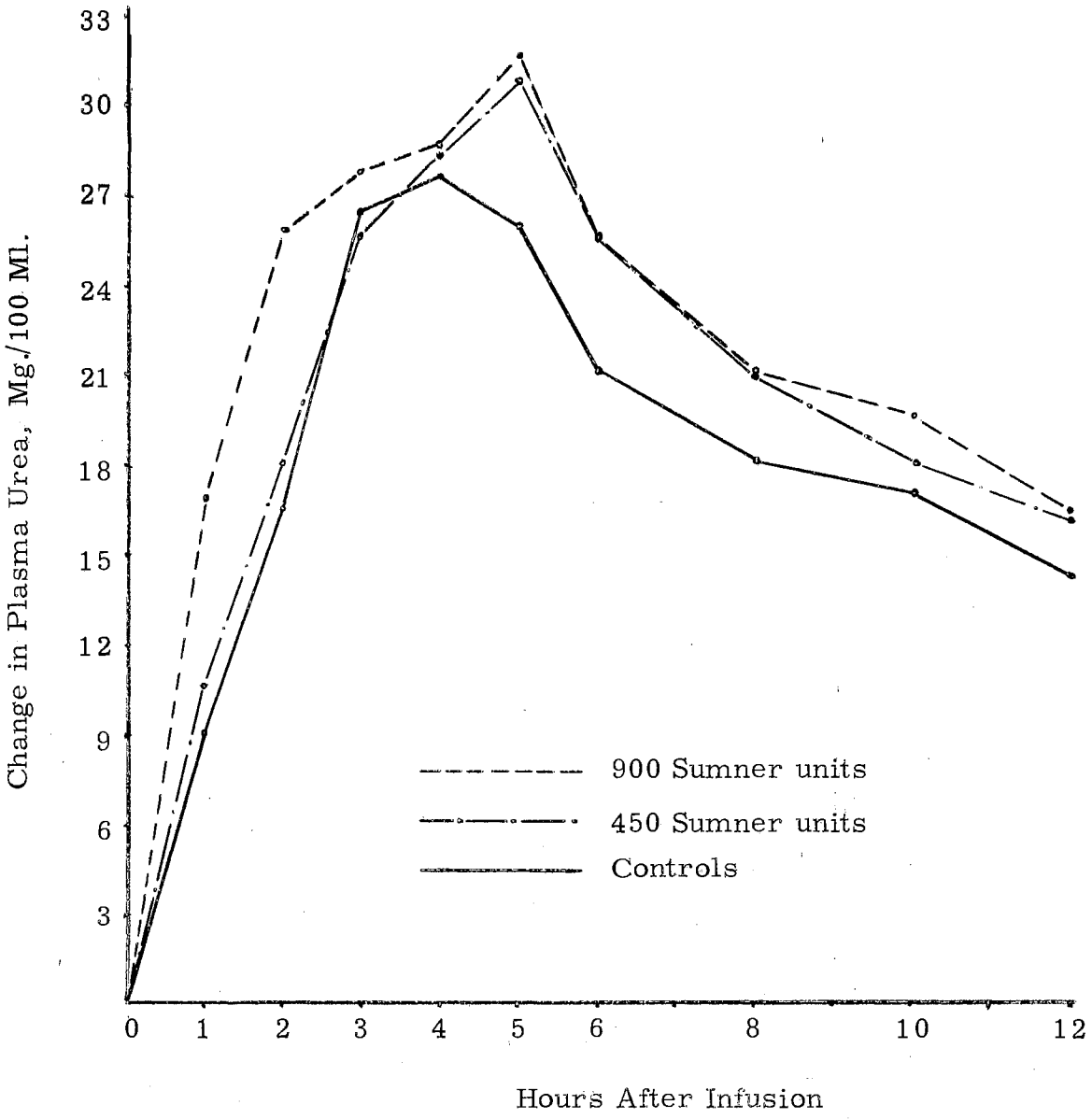

FIGURE 1. THE EFFECT OF JACKBEAN UREASE IMMUNITY ON JUGULAR VEIN PLASMA UREA LEVELS AFTER INFUSION OF A UREA-DEXTROSE SOLUTION, TRIAL 5 (3 LAMBS PER TREATMENT)

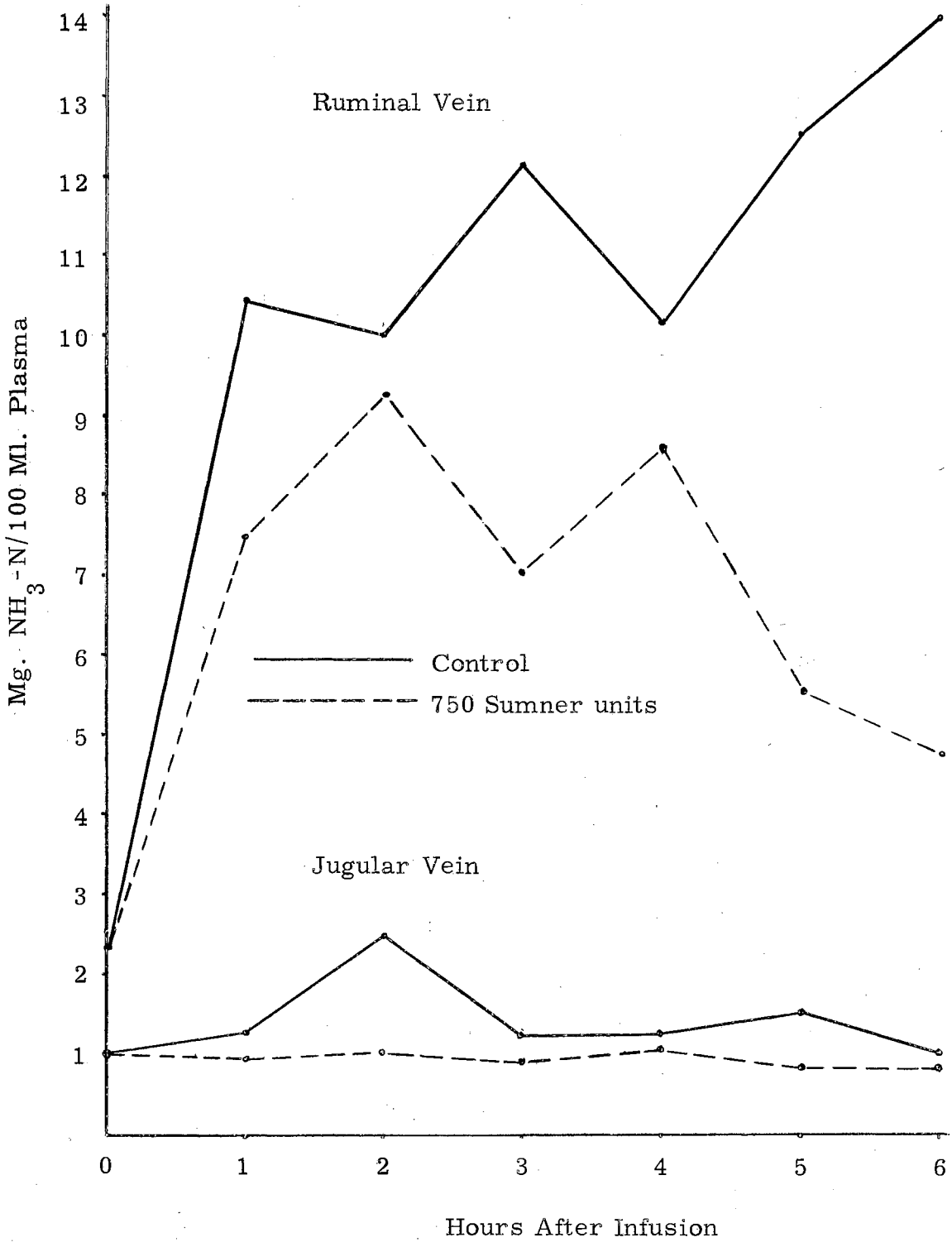

FIGURE 2. THE EFFECT OF IMMUNITY TO JACKBEAN UREASE ON PLASMA AMMONIA-NITROGEN LEVELS OF BLOOD TAKEN HOURLY FROM THE RUMINAL AND JUGULAR VEINS, TRIAL 6 (2 LAMBS PER TREATMENT)

hours after infusion of the urea-dextrose solution. Jugular blood NH_3-N levels were apparently different ($P < .10$) only at the 2-hour bleeding. Plasma NH_3-N levels were consistently lower in jugular blood than in ruminal blood.

Ruminal and jugular blood plasma urea levels for these lambs are shown in Figures 3 and 4, respectively. Differences between ruminal vein plasma urea levels were approaching significance ($P < .10$) at 5 hours and 6 hours after infusion of the urea-dextrose solution. The 2-hour bleeding was the only point at which jugular vein plasma urea levels were approaching a significant difference ($P < .10$). It should be recalled that the same results were obtained in the first study. Although plasma NH_3-N levels were consistently higher in the two control lambs, plasma urea levels were apparently higher in the immunized lambs during the latter stages of the 6-hour bleeding period.

Cecal vein plasma NH_3-N and urea levels generally reflected those of the jugular vein, indicating that the cecum does not play a major role in ruminant nitrogen metabolism.

Discussion

Circulating antibodies have been produced against injected jackbean urease in rats, chicks, rabbits, guinea pigs, humans, and swine (Dang and Visek, 1960; Thomson et al., 1962; Visek et al., 1962; Kornegay et al., 1963). Antibodies against injected urease were produced in all lambs used in this study. In trial 3, an apparent hypersensitivity was

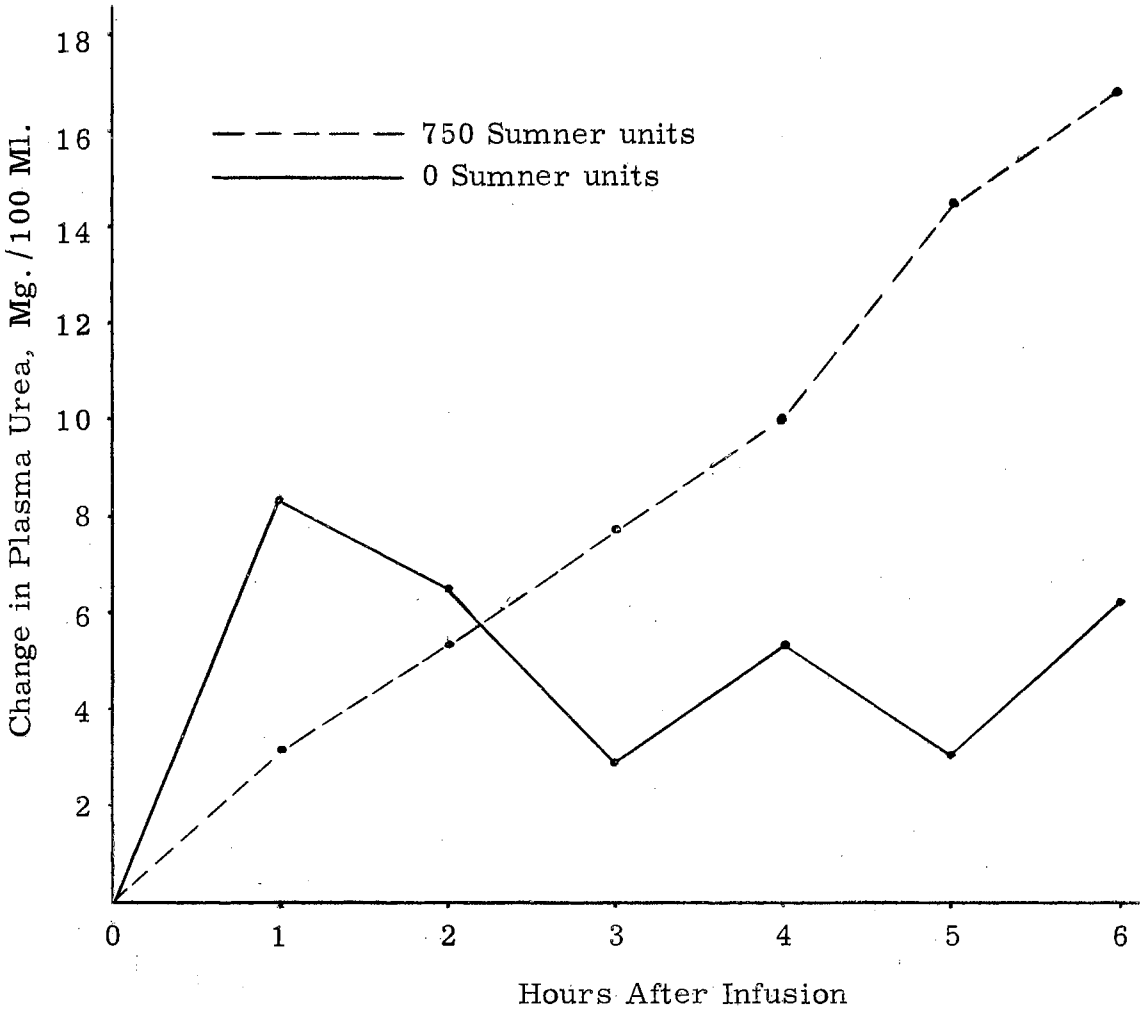

FIGURE 3. THE EFFECT OF JACKBEAN UREASE IMMUNITY ON RUMINAL VEIN PLASMA UREA LEVELS TAKEN HOURLY AFTER INFUSION OF A UREA-DEXTROSE SOLUTION (2 LAMBS PER TREATMENT)

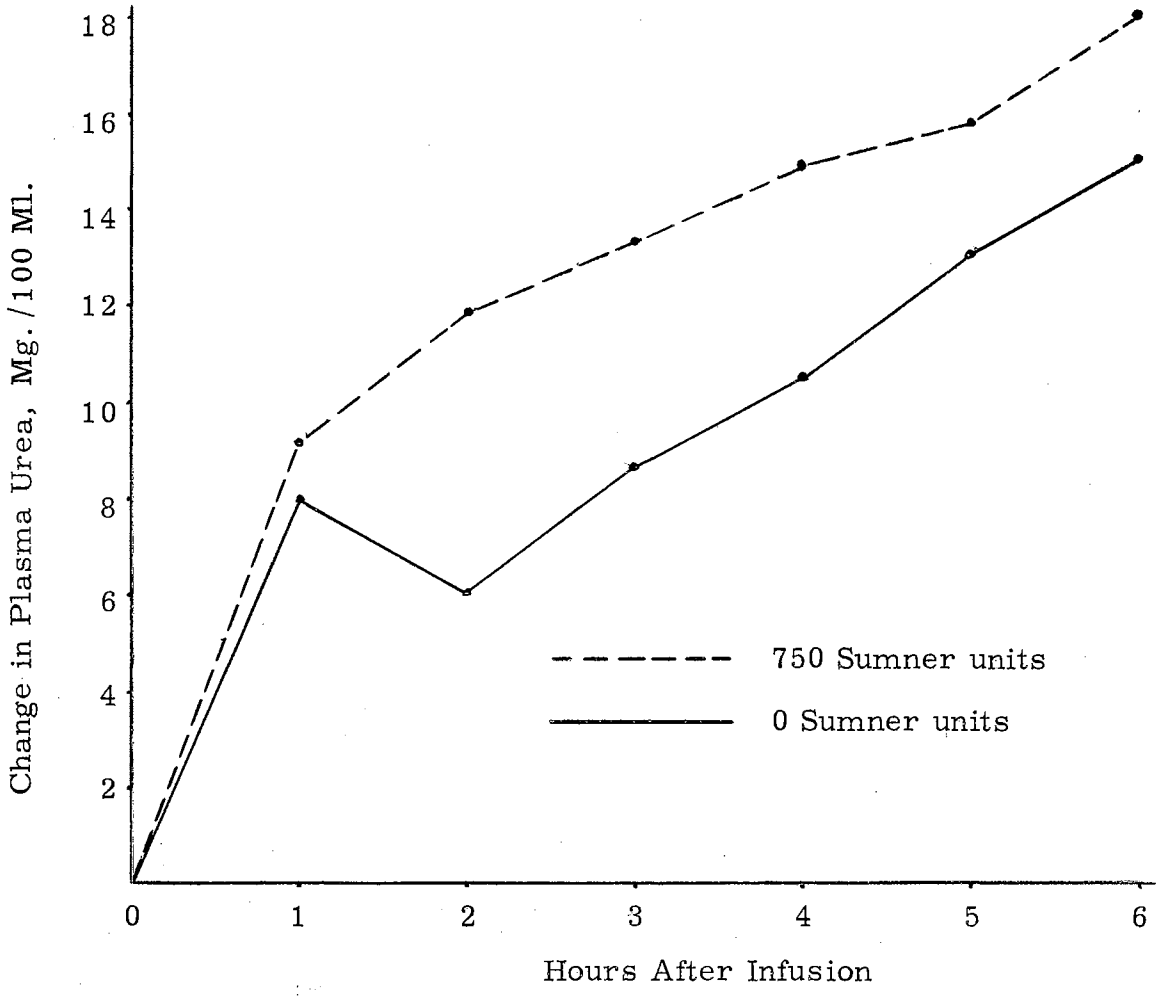

FIGURE 4. THE EFFECT OF JACKBEAN UREASE IMMUNITY ON JUGULAR VEIN PLASMA UREA LEVELS TAKEN HOURLY AFTER INFUSION OF A UREA-DEXTROSE SOLUTION (2 LAMBS PER TREATMENT)

obtained against the high levels injected on the 900 Sumner unit level. A different injection schedule or route of injection might have avoided this difficulty; however, no studies were conducted to confirm this possibility.

The titers obtained against urease were apparently responsible for increases in rate and efficiency of gain in three of the four trials conducted. These results with sheep confirm those of Dang and Visek (1960) and Harbers et al. (1963b) with rats and chicks, in which rate and efficiency of gain were improved during the post injection period in these species.

A possible explanation for the lack of response to urease injections of up to 200 Sumner units in Trial 2 concerns the younger lambs used in this trial. These lambs were 70 days of age while lambs used in all of the other trials were 210 days of age. Although the sheep may be immunologically competent at four to six weeks of age, increased antibody production would be expected from the older animals. Further studies are needed to determine the effect of age on antiurease production and the optimum level of urease injections to produce the optimum antiurease titer. Another possible explanation concerns the superior performance, as compared to all other lambs used in the sheep trials, of the lambs used in Trial 2. It has been the general observation of animal scientists that attempts to increase rate and efficiency of gain by imposing a treatment are most successful when the rates of gain are less than optimum. The rate and efficiency of gain obtained in Trial 2 were quite good, particularly during the first four weeks of the trial, while gains in the

other trials were considered less than optimum. The rate and efficiency of gain obtained by Dang and Visek (1960) with rats and chicks were sub-optimal for the respective species. The diets used in their studies were low quality diets for the species. It should be emphasized that a semi-purified diet containing urea as the only nitrogen source was used in these studies. Further studies are now needed to determine the response in rate and efficiency of gain to urease immunity when urea is fed as less than the total nitrogen fraction in a more practical-type diet.

Although Trials 5 and 6 were quite different in experimental approach, certain general observations can be made. In Trial 5, jugular blood urea levels were consistently higher in the immunized lambs. From Figure 4, it can be seen that the same was true in Trial 6. When these data are combined with the plasma NH_3-N levels, as shown in Figure 2, they are somewhat confusing. Higher ruminal vein ammonia levels are usually associated with higher jugular blood urea levels (Lewis, 1957). That the opposite was true in the present experiment lends strong support to the idea that a portion of the urea was being absorbed across the rumen wall of the immunized lambs, rather than being completely hydrolyzed in the rumen by bacterial urease and absorbed as ammonia, which apparently happened in the control lambs. Both the increased ammonia levels of the control lambs, as shown in Figure 2, and the increased ruminal blood urea levels of the immunized lambs during the latter stages of Trial 6, as shown in Figure 3, support this hypothesis. These findings with sheep suggest that urea hydrolysis

can be decreased in the rumen by antibodies against jackbean urease. Dang and Visek (1960) and Visek (1962) observed that immunized rats injected with ^{14}C-labeled urea expired 40 percent less $^{14}CO_2$ than non-immunized rats. Gastrointestinal ureolytic activity was lower in immunized rats as well. The above results with rats and chicks lend credence to the hypothesis that a decrease in intraruminal urease activity due to immunity against jackbean urease was the major reason for decreased ruminal vein ammonia levels in the immunized lambs in Trial 6. The data further suggest that intraruminal urease activity was inhibited to the extent that urea was being absorbed from the rumen before hydrolysis could occur. Under normal conditions, almost all of the urea is hydrolyzed intraruminally (Lewis, 1957).

Summary

Ninety-three lambs were used in four trials to determine the effect of varying levels of jackbean urease injections on antiurease production and rate and efficiency of weight gain of lambs. Urease immunity was produced in all sheep which received urease injections. Rate of gain and feed efficiency were increased in three of the four trials, indicating that the utilization of urea was improved.

Nine lambs were used in another study to determine the effect of several dosages of subcutaneously administered urease on jugular vein plasma urea level for 12 hours after drenching with 100 ml. of a solution

containing 10 gm. urea and 50 gm. dextrose. Four lambs, two controls and two which had been immunized with 750 Sumner units of jackbean urease, were used in a study to determine the effect of intraruminal infusion of the above solution on ruminal vein, jugular vein, and cecal vein plasma NH_3-N and urea levels. Jugular blood urea levels were higher in the immunized lambs in both trials. Rumen vein NH_3-N levels, however, were higher in the control lambs. These data indicate that the rate of breakdown of urea in the rumen was decreased by jackbean urease immunity.

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