INFLUENCE OF DEODORASE IN COMBINATION WITH DIFFERENT LEVELS OF PROTEIN ON RABBIT FEED INTAKE, BODY WEIGHT, AND UTILIZATION OF UREA

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Abstract - Forty-eight New Zealand White rabbits about 35 days of age were assigned to eight groups. Treatments consisted of four levels of crude protein (CP) as follow : high, medium, low and low dietary crude protein + urea. Each treatment was tested with and without Deodorase at 250 mg/kg diet. Body weight and feed intake were recorded weekly. At the end of the experimental period, six animals from each group were sacrificed to measure blood, cecal ammonia, urea nitrogen, cecal pH and cecal VFA's. Due to protein level and Deodorase, respectively body weight increased significantly ($P < 0.01$). The same trend was observed for total body gain. Feed intake was improved significantly ($P < 0.01$) due to protein level but not significantly due to Deodorase. The result showed improvement in the feed/gain ratio due to the high level of protein and the inclusion of Deodorase. Deodorase decreased insignificantly the cecal pH though the cecal pH was affected significantly ($P < 0.05$) by the protein level. A significant reductions ($P < 0.05$) in the cecal ammonia in the Deodorase and low protein level groups were observed. The high and medium protein groups showed significant ($P < 0.0001$) increase in blood ammonia and urea nitrogen.

INTRODUCTION

Rabbits are susceptible to enteritis, especially when fed concentrates (CHEEKE, 1987). Prevention of enteritis and disease control are most important factors for rabbit producers. MORISSE *et al.* (1989) have shown that diarrhea in rabbits is associated with a drop in the level of volatile fatty acids (VFA's) in the cecum and a subsequent increase of NH_3 level, leading to a high pH and proliferation of Clostridia and Colibacillus bacteria. The concept of using Deodorase (glucoproteins) to reduce ammonia and other noxious gases in animal housing has well been studied (PAUZENGA, 1991 ; AL-BAR *et al.*, 1992). Currant research by AL-BAR *et al.* (1993) indicates that Deodorase has ammonia binding properties in the cecum, and thereby reduces cecal pH, stimulates growth, improves feed/gain (F/G) ratio, increases cecal VFA's and modifies blood and cecal ammonia and urea nitrogen concentrations in rabbits. According to previous results, it is possible to use concentrates in rabbit feeding without any diarrhea or enteritis problems.

The purpose of this study was to insure the following questions : (1) Does Deodorase affect utilization of NPN such as urea ?, (2) Is there a difference in effects of Deodorase on N metabolism depending upon dietary protein level ?, and (3) Via effects on cecal ammonia, does Deodorase affect cecal pH and VFA's production ?

MATERIALS AND METHODS

Forty-eight New Zealand White rabbits about 5 weeks of age and 650 g average body weight were randomly assigned to eight treatments. The animals were kept in individual wire cages. Treatments consisted of four levels of crude protein (CP), each tested with and without Deodorase at 250 mg per kg diet, as follows : (1) High dietary crude protein with Deodorase and Deodorase-free (HPD and HPDF, respectively) ; (2) Meduim dietary crude protein with Deodorase and Deodorase-free (MPD and MPDF, respectively) ; (3) Low dietary crude protein with Deodorase and Deodorase-free (LPD and LPDF, respectively) ; and (4) Low dietary crude protein + urea, with Deodorase and Deodorase-free (LPUD and LPUDF, respectively). The experimental diets were prepared at Oregon State University Laboratory Animal Research center and were tested for five weeks. Ingredients and chemical composition of the diets are shown in Table 1. Feed and water were offered ad libitum.

Table 1 : Ingredients and chemical composition (% kg)

Ingredients	High (CP)	Medium (CP)	Low (CP)	Low (CP) + Urea
Alfalfa meal	54	54	54	54
Soybean meal	21	10	-	-
Ground corn	-	11	21	19
Wheat mill run	20	20	20	20
Vegetable oil	1.25	1.25	1.25	1.25
Molasses	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25
Dicalcium phosphate	0.25	0.25	0.25	0.25
Vitamin	0.25	0.25	0.25	0.25
Urea	-	-	-	2.00

Analysis of feed samples fed to rabbits (% kg)

Components	Dry Matter	Crude Protein	Average ash
High Protein	90.16	23.41	8.73
High Protein + Deodorase	90.35	22.41	9.02
Medium protein	90.28	18.79	8.56
Medium protein + Deodorase	90.15	19.09	8.37
Low protein	90.81	16.63	9.20
Low protein + Deodorase	90.70	16.34	8.70
Low protein + Urea	91.17	22.13	9.01
Low protein + Urea + Deodorase	90.12	20.33	7.80

Body weight and feed intake were recorded weekly. After five weeks of feeding, six animals belonging to each group were sacrificed to measure blood and cecal ammonia and urea nitrogen, cecal pH and cecal VFA's. Blood and cecal ammonia were determined by diagnostic kits (Sigma ; quantitative, enzymatic determination) and measured spectrophotometrically at 340 nm. Blood and cecal urea were assayed by quantitative, calorimetric method (Sigma kits) and measured spectrophotometrically at 525 nm. VFA's were measured by gas chromatography (HP 5890). Means and standard errors of all parameters were estimated and Tukey's test (SAS institute 1990) was used to detect significant differences among the means of the experimental groups.

RESULTS AND DISCUSSION

Body Weight, Body Gain and Feed Intake

Means ± SE for final body weight, total body gain and total feed intake are presented in Table 2. ANOVA revealed significant ($P < 0.01$) increases in body weight due to level of protein and Deodorase, but the interaction effect between protein level and Deodorase was not significant. The differences in body weight between high, medium, and low levels of protein were not significant, but these three levels of protein with or without Deodorase were increased significantly ($P < 0.01$) than the low protein + urea level.

The same trend was observed for total body gain. The body gains were high with high levels of protein, and decreased gradually with decreasing levels of protein, regardless of the effect of Deodorase. The effect of Deodorase on total body gain, regardless of protein level, was so clear and increased total body gain. Deodorase was most effective in the low crude protein group, where total body gain increased from 365.6 g in the LPDF group to 465.6 g in the LPD group.

The effect of protein level on feed intake was clear, where ANOVA and Tukey's test revealed significant ($P < 0.01$) increases in feed consumption in the high (HP) and medium protein (MP) groups compared to the low protein (LP) and low protein with urea (LPU) groups. Similarly, significant differences were found between the LP and LPU groups regardless of Deodorase. However, no significant differences were detected either between HP and MP or HP and LP groups. Feed intake was not significantly increased in all Deodorase-supplemented groups compared to Deodorase-free groups, Table 2. The interaction between Deodorase and protein level was

not significant. The effect of Deodorase on feed intake was great in the LP group, where the value increased from 1309 g in the LPDF group to 1462 g in the LPD group. Thus, in accordance with previous results, use of Deodorase is suggested for a low protein diet, to increase total body gain and feed intake. In general, Deodorase improved feed intake regardless of protein level.

Table 2 : Means (± SE) for final body weight, total feed intake and total body gain

Items	High (CP)	Medium (CP)	Low (CP)	Low (CP) + Urea
Final Body Weight (g)				
No Deodorase	1112 ± 17[a]	1119 ± 17.7[a]	1052 ± 17.7[a]	956 ± 17.7[b]
Deodorase	1260 ± 17[a]	1139 ±17.7[a]	1150 ± 17.7[a]	994 ± 17.7[b]
Total body gain				
No Deodorase	436.67	450.00	365.670	292.50
Deodorase	573.00	491.00	465.660	307.17
Total feed intake				
No Deodorase	1445 ± 27[ba]	1546 ± 27.3[a]	1309 ± 27.3 [bc]	1210 ± 27.3[c]
Deodorase	1484 ± 27[ba]	1562 ± 27.3[a]	1462 ± 27.3 [ba]	1260 ± 27.3[c]

[a, b, c] Means within a row with no common superscripts differ significantly (P < 0.01)

The average daily gain is shown in Table 3. Regardless of the Deodorase addition the average daily gain of HP level was more significant (P < 0.001) than LP + urea group. Similarly, there was a significant different (P < 0.001) in the average daily feed intake between HP and LP + urea group. The greatest improvement in feed / gain ratio due to Deodorase was with the high level of protein, where the value improved from 3.31 in the HPDF group to 2.59 in the HPD group, followed by the medium protein level where the value improved from 3.44 in the MPDF group to 3.18 in the MPD group. So regardless of protein level, Deodorase improved the feed/gain ratio. Thus Deodorase was not markedly effective when used with urea (as a source of nitrogen), although it did increase body weight, total body gain and feed intake and improved the feed/gain ratio slightly from 4.13 to 4.10.

These results are in agreement with NIEDZWIEDEK *et al.* (1975) and SINGH *et al.* (1990), who reported that urea up to a level of 1 % in the diet had no adverse effect on performance. However , 2% urea in the diet as in the present study lowered weight gain and increased feed intake/kg gain. MATHIUS *et al.* (1988) reported very poor reproductive results and very high mortality of pre-weaning kits when 1 % urea was fed to breeding dose.

Table 3 : Average daily gain, feed intake and feed/gain ratio

Items	High (CP)	Medium (CP)	Low (CP)	Low (CP) + Urea
Average daily gain (g)				
No Deodorase	31.19[a]	32.14[a]	26.12[a]	20.90[b]
Deodorase	40.93[a]	35.10[a]	33.26[a]	21.94[b]
Average daily feed intake (g)				
No Deodorase	103.21[a]	110.49[a]	98.49[a]	86.42[b]
Deodorase	106.04[a]	111.55[a]	104.39[a]	90.02[b]
Feed / gain ratio				
No Deodorase	3.31	3.44	3.58	4.13
Deodorase	2.59	3.18	3.14	4.10

[a,b] Means within a row with no common superscripts differ significantly (P < 0.001)

Cecal Parameters

Means (± SE) for cecal pH, ammonia and urea nitrogen are given in Table 4. Cecal pH showed insignificant differences between groups due to Deodorase, but lower pH was recorded in the Deodorase groups as compared to the Deodorase-free groups. Tukey's test revealed significant differences (P < 0.05) in cecal pH due to protein level, where group LPU recorded the highest value of 6.63, followed by groups LP, MP, and HP respectively. This result is in agreement with AL-BAR et al. (1993). There were significant differences between HP group and each of LPU and LP groups but without significant differences between the HP and MP groups, nor between the MP and LP or LP and LPU groups. Results indicated that cecal pH might be affected by dietary protein levels, source and quality.

The effects of Deodorase and protein levels and their interaction on cecal ammonia were significant ($P < 0.05$). Cecal ammonia was high and very low with LP and LPU groups with significant differences. According to previous works, Deodorase combines with ammonia in the cecum. This effect was evident only in the HPD group compared to the HPFD group, where values were 32.90 *vs.* 30.95, respectively. In contrast to the present result show that Deodorase with the LPU group decreased cecal ammonia. The values 27 for the LPUDF group *vs.* 17.9 for the LPUD group, indicate the marked interaction between protein and Deodorase levels. Hence, the effect of Deodorase is modified by diet component and the nature and level of protein. However, generally, there is no contraction between the present and previous results, regarding the binding effect of Deodorase on cecal ammonia.

Results for cecal urea nitrogen were similar. The effect of Deodorase with HP was contradictory to its effect with other protein levels. Deodorase increased cecal urea nitrogen in the HPD group as compared to the HPDF group (16.2 *vs.* 15.5, respectively) and decreased cecal nitrogen in the other three levels.

Table 4 : Means (± SE) for cecal urea nitrogen, ammonia and pH

Items	High (CP)	Medium (CP)	Low (CP)	Low (CP) + Urea
Cecal pH				
No Deodorase	5.9 ± 0.05^{c}	6.2 ± 0.05^{bc}	6.5 ± 0.05^{ab}	6.61 ± 0.05^{a}
Deodorase	5.9 ± 0.05^{c}	6.2 ± 0.05^{bc}	6.4 ± 0.05^{ab}	6.63 ± 0.05^{a}
Cecal Ammonia				
No Deodorase	30.9 ± 4.4^{a}	28.5 ± 7.8^{a}	23.7 ± 3.3^{b}	27.0 ± 6.8^{a}
Deodorase	32.9 ± 1.4^{a}	28.7 ± 3.3^{a}	23.8 ± 0.9^{b}	17.9 ± 4.1^{c}
Cecal Urea Nitrogen				
No Deodorase	15.5 ± 1^{ab}	15.6 ± 0.12^{ab}	14.9 ± 0.12^{b}	15.1 ± 0.12^{ab}
Deodorase	16.2 ± 0.1^{a}	15.0 ± 0.12^{ab}	14.7 ± 0.12^{b}	14.6 ± 0.12^{b}

^{a,b,c} Means within a row with no common superscripts differ significantly ($P < 0.05$)

The effects of Deodorase on cecal VFA's were observed by interactions with the source and level of protein. Deodorase increased insignificant in acetate, propionate butyrate and, subsequently, total VFA's Table 5. The interaction between Deodorase and HP resulted in reduced acetate and butyrate and some increase in propionate, with subsequently decreased total VFA's. These results were different from the interaction between Deodorase and MP, LP and LPU, which resulted in increases acetate, propionate, butyrate and, subsequently, total VFA's. The effect of Deodorase on VFA's production was greatest in the LPD group, in which VFA's increased by about 25 % compared to the LPDF group. The level of protein greatly affected VFA's production, with acetate, propionate and butyrate increasing gradually with increasing protein level.

Using urea as source of NPN with the low protein diet (LPD group) seemed to improve VFA's production as indicated by the high level of acetate, propionate and butyrate compared to VFA's in the low protein (LP) group. Use of urea with Deodorase had antagonistic effect, with the decrease in all VFA's in the LPUD group as compared to the LPD group. Thus, urea may inhibit or decrease the beneficial effects of Deodorase. Urea has a favorable effect when used alone with a low protein diet, but in combination with Deodorase its effect may decrease or reverse, as mentioned previously.

Table 5 : Means for acetate, propionate, butyrate and total VFA's

VFA's	High (CP)	Medium (CP)	Low (CP)	Low (CP) + Urea
Acetate				
No Deodorase	80.17	60.17	44.58	46.95
Deodorase	64.73	61.20	54.29	53.17
Propionate				
No Deodorase	4.44	3.26	2.02	3.68
Deodorase	5.22	3.43	4.25	3.79
Butyrate				
No Deodorase	14.16	12.00	9.07	11.09
Deodorase	12.85	13.05	11.35	10.37
Total VFA's				
No Deodorase	98.77	75.43	55.67	61.72
Deodorase	82.80	77.68	69.58	67.33

Means ± SE for blood ammonia and urea nitrogen are presented in Table 6. There were no significant differences in blood ammonia between the Deodorase-free and Deodorase groups ; however, a decrease in blood ammonia was noted with all the protein levels (MPD, LPD and LPUD) except HP, where blood ammonia increased to 7.76 in the HPD compared to 6.14 in the HPDF group. The greatest effect of Deodorase in decreasing blood ammonia was in the MP group, where blood ammonia decreased from 6.09 to 5.12 in the MPDF and MPD groups, respectively.

Table 6 : Means (± SE) for blood ammonia and urea nitrogen

Items	High (CP)	Medium (CP)	Low (CP)	Low (CP) + Urea
Blood ammonia				
No Deodorase	6.14 ± 0.2^{a}	6.09 ± 0.2^{ab}	4.58 ± 0.21^{b}	4.62 ± 0.21^{b}
Deodorase	7.76 ± 0.2^{a}	5.12 ± 0.2^{ab}	4.26 ± 0.21^{b}	4.49 ± 0.21^{b}
Blood urea nitrogen				
No Deodorase	29.12 ± 5^{a}	22.24 ± 4.3^{b}	15.61 ± 2.4^{c}	30.9 ± 4.5^{a}
Deodorase	28.03 ± 1^{a}	20.60 ± 3.2^{b}	14.62 ± 1.3^{c}	21.5 ± 6.1^{b}

$^{a, b, c}$ Means within a row with no common superscripts differ significantly ($P < 0.0001$)

In this study, protein level had a significant ($P < 0.0001$) effect on blood ammonia. Tukey's test revealed a significant increase in blood ammonia in HP and MP groups, but with no significant difference between these two levels. There was a significant difference between the HP group compared to the LP and LPU groups, but no significant differences either between these two levels or between them and the MP level. Thus, blood a ammonia increased with increasing protein level and decreased gradually with decreasing protein level. Urea showed a slight increase in blood ammonia when used as a NPN source. These results are in agreement with MAKKAR *et al.* (1990), who reported that ammonia levels were statistically similar in rabbits on urea diets.

Deodorase decreased blood urea nitrogen with all protein levels. Its greatest effect was in the LPUD group, in which blood urea nitrogen was 21.46 in comparison to 30.95 (the highest value) for the LPUDF group. Level of protein had a significant ($P < 0.0001$) differences on blood urea nitrogen. Values were higher in the HP and LPUDF groups, with no significant differences between these levels, lower in the MP group, and much lower in the LP group, with significant differences between the MP and LP levels. Differences were significant both with the HP group compared to the MP and LP groups, and with LPUDF group compared to the MP and LP groups. The level and source of protein seemed to have a great effect on blood urea nitrogen. Urea as a NPN source dramatically increased blood urea nitrogen. Using Deodorase with urea reduced the dangerous levels of blood urea nitrogen resulting from use of urea alone. There was a significant ($P < 0.0001$) interaction between Deodorase and protein level with regard to blood urea nitrogen, which increased as protein level decreased. The best interaction was in the LPU group, where blood area nitrogen was low in the Deodorase group as compared to the Deodorase-free group. In general, these are in agreement with MAKKAR *et al.* (1990), who reported a significant increase in blood urea nitrogen ($P < 0.05$) in rabbits on urea diets.

In conclusion, final body weight and total gain were increased with dietary Deodorase. The increase was greatest with the high protein diet and the diet with added urea, suggesting that Deodorase improved utilization of nitrogen with the high medium and low protein diets. Dietary Deodorase had little effect on blood and cecal ammonia or urea nitrogen. Cecal and blood ammonia and urea nitrogen increased with increasing dietary protein level with the urea containing diet. Addition of Deodorase reduced cecal and blood ammonia and urea nitrogen, suggesting the binding of cecal nitrogen to Deodorase. There were no consistent trend in VFA's with Deodorase. However, total VFA's production increased with increasing dietary protein level.

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