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Effect of including liquid vinasse in the diet of rabbits on growth performance

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ABSTRACT - The effects of liquid vinasse (LV) in the diet for growing rabbits on performance, carcass yield and intestinal morphometry were assessed. Eighty New Zealand white rabbits were used in a randomized block design with five treatments (LV inclusion at 0, 25, 50, 75 and 100 g/kg diet) and four replications. There was no effect of the treatment on final weight, daily weight gain, mortality rate and carcass yield characteristics. The daily intakes of feed, dry matter, crude protein and energy and feed conversion decreased linearly with increase in LV in the diet. Including LV affected the duodenum crypt depth and the ilium villus perimeter and height linearly and affected the duodenum villus perimeter, height and the absorption surfaces and ilium crypt depth and absorption surface quadratically. There was no effect of including LV on jejunum morphometry. Vinasse can be used to feed growing rabbits at up to 87.8 g per kilogram of diet.

Key Words: alcohol production residue, animal feeding, vinasse

Introduction

Sugarcane and ethanol production in the state of Goias, Brazil, in the 2008/2009 growing season, was 29,486,508 tons and 1,726,080 liters, respectively (Unica, 2009a,b). Twelve to 20 liters of vinasse are produced per liter of ethanol produced (Cazetta & Celligoi, 2006). Vinasse has the greatest polluting load of the effluents produced by alcohol distilleries because it presents oxygen biochemistry demand ranging from 20,000 to 30,000 mg/L vinasse (Leite, 1999).

The word vinasse is derived from the Latin *vinacaeus*, originally known as fermented wine. Its use has been reported in various tropical countries and in Europe as an additive or food supplement for ruminants and non-ruminants. Vinasse has approximately 930 g/kg water and 17 g/kg solid compounds, dark color, sweet smell, acid pH and high salt levels. This effluent has high concentrations of potassium, calcium, magnesium, sulfur, nitrogen (Hidalgo et al., 2009) and also yeasts (403.56 mg/L) (Souza, 2007).

The average composition of vinasse, on a dry matter basis, is 121 g/kg crude protein, 66 g/kg mineral matter, 5 g/kg calcium, 2.4 g/kg phosphorus, 16 g/kg potassium, 0.8 g/kg sodium, and 1.08 g/100 mL lysine (Hidalgo et al., 2009) and according to Albers (2009), vinasse, *in natura,* presents the following characteristics: pH 4.4 to 4.6; 470 to 710 mg/L nitrogen, 9 to 200 mg/L phosphorus; 3340 to 4600 mg/L potassium; 1330 to 4570 mg/L calcium and 580 to 700 mg/L magnesium.

According to Hidalgo et al. (2009), vinasse has been used in the liquid form in animal feeding as an additive because of its probiotic properties, as a carrier in premixes, palatabilizer or promoter of sexual maturity and reproduction. Its use as an additive has generally resulted in better feed conversion and increased body weight and growth because of the presence of the organic acids, which improve nutrient use, digestion, vitamin D synthesis and vitamin C and mineral absorption, which facilitate the food metabolism.

Animal feeding accounts for almost 70% of production costs, so any alternative feedstuff that might reduce costs are useful to the producer because this increases his or her profit.

The objective of the present research was to assess the effects of including levels of vinasse (LV) in a diet for growing rabbits on the productive performance and intestinal morphometry.

Material and Methods

The experiment was carried out at the Rabbit Rearing Sector at the Universidade de Rio Verde, Goiás, Brazil.

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Eighty male and female New Zealand white rabbits, weaned at 35 days, were used. A randomized block design with five treatments and four replications was used. The treatments consisted of LV inclusion levels (Table 1) in the commercial rabbit diet (Table 1), based on weight. The inclusion levels were 0, 25, 50, 75 and 100 g/kg, based on the weight of the commercial diet ingested daily.

Each rabbit received 70, 90, 110, 130, 140 and 150 g diet per day, in the first, second, third, fourth, fifth and sixth weeks, respectively. The percentages relative to the LV were subtracted from this quantity. The vinasse was weighed, sprayed on the diet and then mixed into it for total incorporation in the pellets.

The rabbits were housed in a masonry barn, in metal cages with drinker and feeder. The water and pelleted commercial diet, mixed with LV, were supplied freely.

The animals were weighed at the beginning and end of the experimental period, when the rabbits reached 77 days of age, to obtain weight gain and the diets were weighed daily to calculate the diet intake and feed conversion. Mortality rate was also determined.

After the final weighing, the animals were feed-deprived for 12 hours and then weighed again to obtain the weight at slaughter, which served as reference to calculate the carcass yield. After evisceration, head and paws were removed, leaving only the carcass, which was then weighed. The edible viscera were also weighed and their relative weights were calculated in relation to the slaughter weight.

Small intestine samples of approximately 3 cm were collected from the duodenum (10 m from the stomach), the jejunum (in the middle region of the small intestine) and the ileum (at 10 cm from the ileocecal junction). The samples were fixed in 10% formaldehyde for 24 hours and fragments were processed for blocking in paraffin. Serial 5 µm sections were made in each fragment and stained with hematoxylin and eosin. Thirty readings were taken per fragment for villus height, perimeter and width, crypt depth, the thickness of the mucous and muscular tunics. The absorption surface (AS) is was calculated by the formula:

AS (mm^2) = villus height (mm) x width at 50% of the villus height (mm)

The economic viability was calculated considering the average sale values of live rabbits for slaughter, slaughtered rabbit and the price of the diet consumed. The gross margin was obtained by the difference between the gross profit (live animal weight × price kg live animal or carcass weight × price kg slaughtered animal) and the diet cost (weight of diet consumed × price kg diet) (Oliveira & Lui, 2006). The price of one kilogram of live animal was R$5.00, of slaughtered animal, R$12.00, and the diet cost R$0.92 kg. The rabbit prices were obtained from the Brazilian Scientific Association of Rabbit Breeders on 12/01/2012.

The results were subjected to analysis of variance and polynomial regression using software SAEG (System for Statistical Analyses, version 9.1) and $\alpha = 0.05$ was adopted.

Results and Discussion

There was no effect (P<0.05) of the treatments on final weight, daily weight gain and mortality rate but the daily intakes of feed, dry matter, crude protein and energy decreased linearly (P<0.05) with increased LV levels in the diet. Reduction in diet intake without reduction in weight gain resulted in better (P<0.05) feed conversion and these values decreased linearly with increase in LV (Table 2).

The reduction in feed and nutrient intake was probably due to the high water content in LV, which improved the action of the digestive enzymes and increased the quantity of nutrients available for absorption (Luis e Silva et al., 2011). Furthermore, the organic acids in LV, including fumaric acid (Ryznar-Luty et al., 2008), improved nutrient use in the small intestine, inhibited bacterial proliferation and decreased the host-bacteria nutrient competition (Roura & Javierre, 2008), which resulted in better feed conversion. Vinasse also kept the intestinal flora in balance, preventing propagation of intestinal pathogens that could negatively influence the total production (Hidalgo et al., 2009).

Table 1 - Nutritional levels of the commercial diet supplemented with increasing levels of liquid vinasse (LV) and LV based on dry matter

	LV level in the diets (g/kg)					
	0	25	50	75	100	Liquid vinasse
Dry matter (g/kg)	836	812	790	774	750	31.7
Crude protein (g/kg)	214	214	214	218	218	86.3
Crude energy (kcal/kg)	4032	4093	4049	4084	4063	3228
Ether extract (g/kg)	45.5	41.0	37.5	36.2	36.5	-
Mineral matter (g/kg)	64.5	66.5	63.3	71.6	73.5	211.4
Calcium (g/kg)	12.2	11.6	7.6	7.6	8.7	4.5
Phosphorus (g/kg)	8.5	9.2	6.8	6.0	6.0	1.4
pH	6.08	6.02	6.05	5.98	5.89	3.80

Similar results were reported by Maertens et al. (1994), who used vinasse obtained from beetroot in the proportion of 40 and 80 g/kg diet for 35 day-old rabbits and observed that the feed conversion was better at the highest inclusion level, and there was no effect on the other parameters.

There is little research on the use of vinasse in rabbit feeding, but according to Waliszewski et al. (1997), who used vinasse in chicken diet at the proportion of 40 and 60 g/kg, there was an improvement in the broiler performance with the dose of 40 g/kg. Similarly, Hidalgo et al. (2009) included 14 mL vinasse per bird/day in chicken diet, sprayed on the feed twice a day, and reported similar feed intake (3300 and 3309 g/bird) but with an extra 200 g gain in the bodyweight, better feed conversion (1.81 and 1.60) and a 2% increase in the survival rate and carcass weight.

Including LV in the diet did not affect ($P<0.05$) the carcass weight and yield and the edible viscera weight (Table 3).

Even with the presence of yeasts and nutrients in the vinasse such as vitamin C, minerals and organic acids, substances that beneficially alter the intestinal flora and increase nutrient digestibility and absorption, there was no effect of including LV on the carcass and edible viscera parameters. Hidalgo et al. (2009) assessed the supply of 5, 10 and 15 mL/d/bird in the initial, grower and finishing phases, respectively, for broiler chicks and reported that the carcass, breast and thigh yields increased along with vinasse use. According to the authors, LV can optimize nutrient use in poultry, improving the muscle disposition in the body, but this effect was not observed in the present experiment.

Including LV affected ($P<0.05$) the duodenum crypt depth (Table 4) and the ileum villus perimeter and height linearly (Table 5) and affected ($P<0.05$) the villus perimeter and height and duodenum absorption surface and ilium crypt depth and absorption surface quadratically. Including LV in the diet brought no effect to the jejunum morphometry (Table 6).

In the duodenum, the smallest villus perimeter, height and surface absorption values were obtained with 33.4, 38.8 and 38.5 g LV inclusion per kg of diet, respectively, and above these levels all the parameter values increased, showing a beneficial effect of LV on the intestinal morphometry.

In the ileum, the highest villus perimeter and height values were observed with 100 g/kg LV inclusion but the highest crypt depth and surface absorption values

Table 2 - Productive performance of rabbits at 77 days of age fed diets containing levels of vinasse

Parameters	Liquid vinasse levels (g/kg)					CV (%)	P-value			
	0	25	50	75	100		Linear	Quadratic	Cubic	Fourth level
Final weight (g)	2092	2081	2005	2111	1961	5.0	0.1888	0.3872	0.3804	0.2468
Daily weight gain (g/d)	37.17	36.82	34.83	37.49	33.84	5.9	0.1171	0.2755	0.2500	0.1125
Daily feed intake (g/d)[1]	133	131	111	111	108	4.3	0.0061	0.0560	0.0615	0.1011
Daily dry matter intake (g/d)[2]	112	106	88	86	81	5.5	0.0013	0.0714	0.0845	0.0981
Daily crude protein intake (g/d)[3]	23.90	22.85	18.75	18.75	17.75	4.5	0.0016	0.0523	0.0740	0.1170
Daily gross energy intake (kcal/d)[4]	450	436	355	352	331	4.1	0.0024	0.0609	0.0672	0.0874
Feed:gain ratio in natural matter[5]	3.58	3.55	3.19	2.98	3.21	5.0	0.0344	0.0694	0.0685	0.1418
Feed:gain ratio in dry matter[6]	2.99	2.88	2.52	2.30	2.41	5.9	0.0011	0.0533	0.0733	0.1521
Survival rate (%)	100.00	100.00	100.00	87.50	100.00	3.2	0.2472	0.2853	0.0891	0.1510

CV - coefficient of variation
[1] Linear effect ($\hat{Y} = 133.06 - 2.80x$, $r^2 = 0.35$).
[2] Linear effect ($\hat{Y} = 110.88 - 3.2405x$, $r^2 = 0.38$).
[3] Linear effect ($\hat{Y} = 23.68 - 0.6564x$, $r^2 = 0.34$).
[4] Linear effect ($\hat{Y} = 539.404 - 11.106x$, $r^2 = 0.33$).
[5] Linear effect ($\hat{Y} = 3.56 - 0.052x$, $r^2 = 0.64$).
[6] Linear effect ($\hat{Y} = 2.97 - 0.0698x$, $r^2 = 0.45$).

Table 3 - Carcass weight and yield for edible viscera from 77-day-old rabbits fed diets containing vinasse

Parameters	Liquid vinasse levels (g/kg)					CV (%)	P-value			
	0	25	50	75	100		Linear	Quadratic	Cubic	Fourth level
Carcass weight (g)	1130	1087	1125	1137	955	5.2	0.3800	0.4400	0.5000	0.4479
Carcass yield (g/kg body weight)	520.0	531.5	541.9	546.8	512.7	4.1	0.4241	0.4603	0.4441	0.4168
	Relative weight (g/kg body weight)									
Heart	4.1	4.1	4.4	4.2	4.4	4.6	0.1750	0.1321	0.1214	0.1162
Liver	48.8	42.9	42.6	45.9	45.2	6.3	0.3430	0.4832	0.4526	0.4199
Kidneys	12.9	12.9	11.5	12.8	12.5	4.2	0.1557	0.1028	0.0914	0.0891

CV - coefficient of variation.

Table 4 - Duodenum morphometry of 77-day-old rabbits fed diets containing levels of vinasse

Parameters	Liquid vinasse levels (g/kg)					CV (%)	P-value			
	0	25	50	75	100		Linear	Quadratic	Cubic	Fourth level
Villus perimeter (μm)[1]	1681	1481	1539	1799	1825	7.7	0.0270	0.0075	0.0518	0.0620
Villus height (μm)[2]	834	738	771	889	898	8.2	0.0420	0.0158	0.0553	0.0529
Crypt depth (μm)[3]	67	83	87	90	91	8.3	0.0046	0.05992	0.0643	0.0526
Villus height/crypt depth ratio	12.36	9.06	9.67	10.15	9.86	7.1	0.2020	0.1047	0.0611	0.0529
Absorption surface (mm^2)[4]	0.089	0.071	0.076	0.091	0.098	8.7	0.0435	0.0014	0.0572	0.0548

CV - coefficient of variation.
[1] Quadratic effect ($\hat{Y} = 1636.51 - 48.878x + 7.31x^2$. $R^2 = 0.44$).
[2] Quadratic effect ($\hat{Y} = 812.80 - 23.266x + 3.44x^2$. $R^2 = 0.38$).
[3] Linear effect ($\hat{Y} = 72.21 + 2.1766x$, $r^2 = 0.42$).
[4] Quadratic effect ($\hat{Y} = 0.086 - 0.00494x + 0.00064x^2$. $R^2 = 0.54$).

Table 5 - Jejunum morphometry of 77-day-old rabbits fed diets containing levels of vinasse

Parameters	Liquid vinasse levels (g/kg)					CV (%)	P-value			
	0	25	50	75	100		Linear	Quadratic	Cubic	Fourth level
Villus perimeter (μm)	1405	1503	1428	1546	1553	10.2	0.1178	0.1929	0.3342	0.2909
Villus height (μm)	683	746	659	751	774	9.9	0.0829	0.1449	0.2858	0.3124
Crypt depth (μm)	74	64	75	69	66	9.4	0.0735	0.0790	0.0887	0.1002
Villus height/crypt depth ratio	9.27	11.27	9.11	10.73	11.32	10.0	0.0810	0.1828	0.3273	0.2035
Absorption surface (mm^2)	0.072	0.078	0.074	0.085	0.078	8.7	0.1203	0.3017	0.3010	0.4104

CV - coefficient of variation.

Table 6 - Ileum morphometry of 77-day-old rabbits fed diets containing levels of vinasse

Parameters	Liquid vinasse levels (g/kg)					CV (%)	P-value			
	0	25	50	75	100		Linear	Quadratic	Cubic	Fourth level
Villus perimeter (μm)[1]	953	1120	1132	1217	1225	3.9	0.0045	0.0266	0.0583	0.0646
Villus height (μm)[2]	487	541	549	612	619	3.3	0.0008	0.0013	0.0611	0.1110
Crypt depth (μm)[3]	82	90	93	110	85	8.0	0.5000	0.5000	0.3265	0.1686
Villus height/crypt depth ratio	6.51	6.82	7.47	6.97	7.92	6.6	0.3255	0.5000	0.5000	0.5000
Absorption surface (mm^2)[4]	0.038	0.048	0.050	0.055	0.048	3.9	0.0075	0.0061	0.0775	0.0654

CV - coefficient of variation.
[1] Linear effect ($\hat{Y} = 1009.88 + 24.475x$, $r^2 = 0.72$).
[2] Linear effect ($\hat{Y} = 492.946 + 13.294x$, $r^2 = 0.80$).
[3] Quadratic effect ($\hat{Y} = 68.61 + 5.062x - 0.288x^2$. $R^2 = 0.42$).
[4] Quadratic effect ($\hat{Y} = 0.039 + 0.00316x - 0.0002x^2$. $R^2 = 0.49$).

were obtained with 87.8 and 79.0 g/kg LV inclusion, respectively.

The better development of the intestinal mucosa tissues in rabbits fed LV may have been due to the low pH value of the vinasse and the diets with LV levels over 75 g/kg (5.98 and 5.89). Low pH favors the presence of saprophytic intestinal flora and prevents the establishment of pathogenic bacteria that might damage the intestinal mucosa by colonization and toxin production. Acid substances, such as LV, have been used as additives in animal feeding also because they increase energy and crude protein digestibility, mineral absorption and retention (Diebold & Eidelsburger, 2006).

Furthermore, according to Hidalgo et al. (2009), the few fibers in LV are represented by glucan and mannan, present in the yeast walls, also having a beneficial effect on protecting the intestinal mucosa (Morales, 2007; Oliveira et al., 2008). These substances may be used as substrate by beneficial bacteria with later short-chain fatty-acid production that helps reduce the pH of the microenvironment of the brush border even further and decreases pathogen adhesion (Oliveira et al., 2008). Mannan fermentation may also increase the mucosa thickness, number of epithelial cells per crypt and the venous blood flow (Zafar et al., 2004).

Liquid vinasse has a good amino acid and mineral profile (Hidalgo et al., 2009) that may have collaborated with cell proliferation and integrity of the intestinal mucosa.

Considering the gross margin resulting from the sale of live rabbits to the slaughterhouse and rabbits already slaughtered for sale, the best values were obtained with inclusion of 75 LV g/kg, which were respectively 8.79 and 7.12% greater than the values obtained with the treatment without vinasse (Table 7).

Table 7 - Economic viability of the productive performance of growing rabbits fed diets containing vinasse

Vinasse level (g/kg)	Sale of live rabbits for slaughter			
	Body weight (kg)	Live animal price (R$)	Cost of diet ingested (R$)	Gross margin (R$)
0	2.092	10.46	5.14	5.32
25	2.081	10.40	5.06	5.34
50	2.005	10.02	4.28	5.74
75	2.111	10.55	4.28	6.27
100	1.961	9.80	4.17	5.52
	Sale of slaughtered rabbits			
	Carcass weight (kg)	Carcass price (R$)	Cost of diet ingested (R$)	Gross margin (R$)
0	1.130	13.56	5.14	8.42
25	1.087	13.04	5.06	7.98
50	1.125	13.50	4.28	9.22
75	1.137	13.64	4.28	9.36
100	0.955	11.46	4.17	7.29

Sarria & Preston (1992) used concentrated vinasse for finishing pigs and observed that its use resulted in 5% faster growth and reduced the diet price by 15%, as was also observed in the present experiment regarding the diet cost.

Conclusions

In natura liquid vinasse can be used to feed growing rabbits at 87.8 g per kilogram of diet.

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