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Time Interval and Intervening Quantity of Cooked Rice Prediction to Induce Ruminal Acidosis

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ABSTRACT

Background: Small holding farmers feed surplus cooked rice as the only source of concentrated feed to cattle leading to onset of acidosis. In order to plan preventive measures at appropriate time, determination of time interval required to induce acidosis for respective quantity of cooked rice fed is essential.

Methods: An experiment with graded quantity cooked rice was tested *in vitro* to determine the respective time interval required to induce acidosis. Since varying quantity of cooked rice are fed to dairy cattle by farmers, a prediction equation was developed to determine the precise duration required to induce acidosis for the respective quantity of cooked rice. Thirty-two cooked rice samples, each incubated with 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0g in six replications were taken.

Result: The onset of acidosis (pH <5.8) occurred at 9 hours of incubation with 0.5 and 1.0 g of cooked rice, at 6 hours of incubation with 1.5 2.0 and 2.5 g of cooked rice and at 3 hours of incubation with 3.0, 3.5 and 4.0 g of cooked rice, respectively. The results obtained were fitted in regression equation *viz.* $Y = \alpha + \beta_1 x_1 + \beta_2 x_2 + \mu$ Where Y = pH; x_1 = Quantity of cooked rice; x_2 = Incubation period; α = Intercept; $\beta_{1,}$ β_2 = Slope and μ = Error turn. There was a close relationship between regressive calculated pH (X,) and *in vitro* determined pH $Y = 7.79 - (W \times 0.421) - (t \times 0.184)$ r^2 = 0:61; p<0.001. Hence, it is possible to predict the fall of pH based on the quantity and duration of incubation for any intervening quantity of cooked rice or period of incubation. Thus, appropriate timely intervention or correction in feeding management on intervening quantity of cooked rice to prevent onset of acidosis is possible wherever farmers feed surplus cooked rice to cattle.

Key words: Acidosis, Cattle, Cooked rice, pH, Time.

INTRODUCTION

In Tamil Nadu, most dairy farms belong to the unorganized sector and consequently, they utilize locally available feed resources for feeding their cows (20th Livestock census, 2019). Attempts to improve the feeding practices have met with little success due to the widespread nature of the problem and adoption of region-oriented traditional feeding practices (Rao *et al*., 1995). Further farmers share their staple food (cooked rice) with cows due to special religious sentiment, besides in Tamil Nadu, rice is available at a very subsidized cost to economically weaker sections, which also largely depend on cows for their survival. Cooked rice fed to cattle in 71.5% of the unorganized dairy farmers of Tamil Nadu. Cooked rice is fed alone or along with other feed ingredients, namely, other cereals, oil cakes and rice bran which was amply reflected in this survey (Murugeswari *et al*., 2018).

The survey (42.87% of dairy cattle produce <5 litre/day of milk) correlated with the observation recorded in the unorganized farms, which indicated that majority of cattle are reared with unbalanced feeding regimen. It is a well-established fact that feeding cereal grains or cereal flours or boiled cereal flour in excess to cattle leads to acidosis, ruminal bloat, loose dung, laminitis and reproductive problems (Mirzad *et al*., 2021). The overall incidence of acidosis (37.76%) in cross-bred dairy cattle fed with cooked rice was recorded in an earlier study by author. The incidence of ruminal acidosis varied due to the intake of various quantity of cooked rice and rice gruel. Rumen acidosis is associated with the feeding of diets with higher amounts of grain. Consumption of a large amount of rapidly fermentable carbohydrates, primarily starches and sugars lead to ruminal acidosis. Since varying quantity of cooked rice are fed to dairy cattle by farmers, it is imperative to determine corresponding duration required to induce acidosis so as to accordingly plan cost-effective preventive measure. Hence, the study was conducted for determination of time interval required to induce acidosis for respective quantity of cooked rice fed to plan preventive measures at appropriate time for preventing the ruminal acidosis.

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MATERIALS AND METHODS

Study design

An experiment was conducted for establishment of the ill effects (drop in pH) due to over feeding of cooked rice under *in vitro* conditions during 2016 to 2017 at Institute of Animal Nutrition Kattupakkam, Chengalpattu district, Tamil Nadu. Thirty-two rice samples (500 g of each) were collected at Tamil Nadu, India from dairy farmers of each district who adopted rice-based feeding regimen. Rice samples (100 g) prior to analysis were cooked with water in the ratio of 1:10, in boiling water bath to a gel like consistency (Bhattacharjee *et al*., 2020). The cooking of rice samples was similar to cooked rice fed by farmers of Tamil Nadu and hitherto rice would be mentioned as cooked rice.

Experimental design

The rumen liquor needed for the experiment was collected in a slaughter house from six cows immediately prior to slaughter and brought to the laboratory by maintaining the temperature of rumen liquor at 39°C, under anaerobic conditions during the transit. Ruminal fluid was filtered through four layers of muslin cloth and stored in pre-warmed thermos container at 39°C till its use. For each replication, different inoculums were used to account for the variability in inoculums, while judging the effect on pH (Murugeswari *et al*., 2023).

The cooked rice samples were weighed (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0g) in six replications for each of 32 samples. The cooked rice samples were transferred into 100 ml closed plastic containers fitted with one-way bicycle valves. Mc Dougall Buffer (80 ml) as per Mickdam *et al.* (2016) and 20 ml of strained rumen liquor were added and incubated. Incubation for 0, 3, 6, 9 and 12 hours was performed at 39°C with 120 rpm shaking (Sung *et al*., 2006). A shaking incubator was used for incubating the containers, which was maintained under oxygen free condition. At the end of incubation period, pH was measured in all the containers to document the changes in pH (Neubauer *et al*., 2018). In this study pH ≤5.8 was considered as acidosis (Beauchemin *et al*., 2007).

Development of regression equation to predict ruminal pH on variable quantity of cooked rice and incubation period

Though graded quantities of cooked rice were tested for its effect on pH. The quantity of cooked rice fed to cattle by farmers varies very widely and may fall in the intervening quantity between graded values tested. Hence, the data on the effect of graded quantities of cooked rice at varied incubation periods on pH was processed to evolve prediction equitation and extrapolate it.

Data on pH changes documented with varying incubation periods (0, 3, 6, 9 and 12 hours) for each of the quantity of cooked rice experimented (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g) were fed in IBM® SPSS® Statistics version 20.0 for Windows® software. The data was analyzed using linear regression analysis. Weight of cooked rice and incubation period was considered as independent variables and pH was considered as dependent variable. The regression equation of the following form was fitted.

$$Y = \alpha + \beta_1 x_1 + \beta_2 x_2 + \mu$$

Where,

Y= pH.

x_1= Quantity of cooked rice.

x_2= Incubation period.

α= Intercept

μ= Error turn.

The quantity of rice and incubation periods were considered as independent variables and *in vitro* ruminal pH values were considered as dependent variables. The regression coefficient was determined between the independent variables and as dependent variable. Using this regression coefficient, a predictive equation was evolved that could predict pH with varied quantity of cooked rice at different incubation period.

This equation was used to arrive at derived values for pH when the same quantity of cooked rice (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g) at the same incubation periods (0, 3, 6, 9 and 12 hours) were fitted in the evolved regression equation. These pH values were designated as derived values. The derived pH values were compared to the actual pH values to check the validity of the evolved prediction equation.

Statistical analysis

Obtained data was analysed with analysis of variance (ANOVA) and linear regression analysis using IBM® SPSS® Statistics version 20.0 for Windows® software as per the Snedecor and Cochran (1989). The critical difference between the groups was analyzed by Duncan's multiple range test. Data is presented as means ± SE.

RESULTS AND DISCUSSION

Effect of graded level cooked rice on ruminal pH at different incubation period

Ruminal acidosis is defined as a fermentation disorder in the rumen characterized by a lower than normal ruminal pH, reflecting an imbalance between microbial production, microbial utilization and ruminal absorption of volatile fatty acids (Castillo *et al*., 2012). In current study, the effect of graded level of cooked rice (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g) and incubation period (0, 3, 6, 9 and12 hours) on *in vitro* ruminal pH is presented in Table 1. Ruminal acidosis has been defined as pH ranging <5.5 to ≤5.8 for diagnosis of sub-acute ruminal acidosis (Beauchemin *et al*., 2007). Hence, the pH 5.8 was considered as baseline to decide onset of acidosis for this study.

There was an increase in pH with increase in incubation time when cooked rice was not included in the incubation. Nonetheless, a significant drop in pH ($p<0.01$) was observed with increase in incubation time for the respective weight of cooked rice incubated. Similarly declining trend in pH was also observed with increase in weight of cooked rice incubated within any given incubation period. Similarly, irrespective of the quantum of cooked rice tested at 0 hour of incubation, no significant ($p>0.01$) variation was observed in pH. Increasing quantum of cooked rice caused a highly significant ($p<0.01$) drop in pH. Garrett *et al*. (1999) have reported ruminal pH less than equal to 5.5 and Beauchemin *et al.* (2007) reported ruminal pH less than or equal to 5.8 for diagnosis of acidosis. In our study, pH below 5.8 was documented at three hours of incubation itself when higher quantity of cooked rice (3.0, 3.5 and 4 g) was incubated.

Acidosis was set (pH $\leq$5.8) at 9 hours of incubation in 0.5 and 1.0 g of cooked rice, at 6 hours in 1.5, 2.0 and 2.5 g of cooked rice and at 3 hours of incubation in 3.0, 3.5 and 4.0 g of cooked rice, respectively. The onset of *in vitro* acidosis was at pH $\leq$5.8 that was significantly ($p<0.01$) lower than the pH (6.0) documented at the respective previous hour of incubation. Fermentation of carbohydrates releases organic acids that readily dissociate to decreased pH (Stefańska *et al*., 2017). Fermentation of nutrient leads to proton release and, thereby, decreases pH as a consequence; fermentation usually proceeds at pH less than 7. Excess feeding of cereal grains or cereal flours or boiled cereal flour leads to acidosis and to prevent onset of acidosis synchronized release of nitrogen is required to divert carbon and hydrogen present in fermentable organic matter to synthesize microbial protein (Murugeswari *et al.,* 2013). When only cooked rice is fermented *in vitro* there is a mismatch between available energy and protein, which indicates that a source of nitrogen needs to be supplemented for energy protein synchrony to augment microbial biomass production (NRC, 2021).

Similar findings were documented by Yury *et al.* (2015), who reported that when four different ratios of concentrate to roughage: 30:70, 40:60, 60:40 and 80:20 were tested, apparent digestibility of organic matter and crude protein showed a linear association with concentrate proportion (p = 0.01). However, the lowest ruminal pH values were observed in animals fed with diet having 80:20 concentrate to roughage ratio the pH of which remained below pH 6.0 from 6 hours after feeding, while in the other diets, the ruminal pH was below 6.0 but not before 12 hours after feeding. The results of this experiment indicate that acidosis (pH below 5.8) set at 9 hours of post incubation up to 1.0 g of cooked rice, at 6 hours for 1.1 to.2.5 g of cooked rice and at 3 hours for 2.6 to 4.0 g of cooked rice, which in turn, reveals that acidosis set at varied hour depending upon the quantum of cooked rice incubated stand justified.

Development of Regression equation on changes of rumen pH

The results of the linear regression model to estimate the regression co-efficient for graded level of cooked rice (g) and varied incubation period (hour) are presented in Table 2.

A predictive equation that could predict pH with varied quantity of cooked rice at different incubation period was evolved and is given below:

$$pH = 7.79 - (W \times 0.421) - (t \times 0.184)$$

Where,
W= Weight of cooked rice in grams.
t= Incubation time in hours.

Using the evolved equation, fitted values (derived values) for pH to cooked rice (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g) at incubation periods (3, 6, 9 and12 hours) were calculated and the same is presented in Table 3 along with the observed values for the respective treatment to judge the efficacy of prediction.

The derived values were well within the range of observed pH across weight of cooked rice and incubation period. Hence, it is possible to predict the fall of pH based on the quantity and duration of incubation for any intervening quantity of cooked rice or period of incubation.

It was evident from the survey that the quantity of cooked rice feeding differs considerably among farmers and it is

Table 1: Effect of graded level of cooked rice* (g) and incubation period (hour) on *in vitro* ruminal pH (Mean ± SE).

Weight of cooked rice* (g)	Incubation period (hours) 0	3	6	9	12
0.0	$7.2^{g1}\pm0.07$	$7.4^{g2}\pm0.08$	$7.5^{g2}\pm0.05$	$7.8^{g2}\pm0.08$	$7.9^{g2}\pm0.09$
0.5	$7.2^{g4}\pm0.08$	$6.5^{f3}\pm0.06$	$6.2^{f3}\pm0.09$	$5.7^{cd2}\pm0.07$	$5.4^{bc1}\pm0.06$
1.0	$7.2^{g5}\pm0.07$	$6.2^{f4}\pm0.05$	$6.0^{e3}\pm0.07$	$5.4^{bc2}\pm0.07$	$5.0^{a1}\pm0.08$
1.5	$7.2^{g4}\pm0.09$	$6.2^{f3}\pm0.07$	$5.7^{cd2}\pm0.08$	$5.1^{a2}\pm0.07$	$4.9^{a1}\pm0.08$
2.0	$7.2^{g4}\pm0.07$	$6.0^{e3}\pm0.08$	$5.5^{c2}\pm0.09$	$5.0^{a1}\pm0.06$	$4.6^{a1}\pm0.07$
2.5	$7.2^{g4}\pm0.06$	$5.9^{e3}\pm0.07$	$5.4^{bc2}\pm0.06$	$4.7^{a1}\pm0.07$	$4.5^{a1}\pm0.06$
3.0	$7.2^{g4}\pm0.09$	$5.7^{cd3}\pm0.08$	$5.3^{ab2}\pm0.09$	$4.7^{a1}\pm0.10$	$4.3^{a1}\pm0.11$
3.5	$7.2^{g3}\pm0.10$	$5.6^{c2}\pm0.11$	$5.1^{a1}\pm0.09$	$4.5^{a1}\pm0.09$	$4.2^{a1}\pm0.09$
4.0	$7.2^{g3}\pm0.11$	$5.5^{c2}\pm0.12$	$5.1^{a1}\pm0.11$	$4.3^{a1}\pm0.10$	$4.0^{a1}\pm0.11$

*On dry matter basis; Values are mean of 32 sample.

Means bearing different alphabets as superscripts differ significantly ($p<0.01$).

Means bearing different numerical as superscripts within row differ significantly ($p<0.01$).

Table 2: Linear regression model to estimate the regression co-efficient for graded level of cooked rice* (g) and varied incubation period (hour).

Coefficients[a]					
Model	Un standardized coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		
1 (Constant)	7.790	0.017		500.368	0.000
Weight of cooked rice*	-0.421	0.002	-0.479	-74.967	0.000
Incubation period	-0.184	0.003	-0.623	-128.569	0.000

a. Dependent variable: pH.

*On dry matter basis.

Table 3: Effect of graded level of cooked rice* (g) and incubation period (hour) on observed *in vitro* ruminal pH and fitted *in vitro* ruminal pH using evolved regression equation.

Weight of cooked rice*(g)	Incubation period (hours)							
	3		6		9		12	
pH	Observed	Derived	Observed	Derived	Observed	Derived	Observed	Derived
0.5	6.3-7.2	7.1	5.9-6.6	6.5	5.2-6.1	5.9	4.9-6.0	5.5
1.0	5.9-6.9	6.8	5.7-6.3	6.3	5.3-5.7	5.7	4.6-5.4	5.2
1.5	6.0-6.6	6.6	5.5-6.1	6.1	4.9-5.5	5.5	4.5-5.3	5.0
2.0	5.7-6.4	6.4	5.3-5.8	5.8	4.8-5.3	5.3	4.2-5.1	4.7
2.5	5.7-6.2	6.2	5.0-5.6	5.6	4.3-5.1	5.1	4.4-4.9	4.5
3.0	5.6-6.0	6.0	4.8-5.5	5.4	4.3-5.1	4.9	4.0-4.6	4.3
3.5	5.3-5.8	5.8	4.7-5.4	5.2	4.2-4.9	4.7	3.8-4.8	4.1
4.0	5.2-5.7	5.6	4.8-5.3	5.1	3.9-4.7	4.5	3.7-4.3	3.9

*On dry matter basis.

imperative to assess its impact on rumen pH to plan feeding regimen accordingly. A predictive equation that could predict pH with varied quantity of cooked rice at different incubation period was hence evolved. It reflected the changes in the acidosis condition of the animal from mild to severe due to changes in the rumen pH. The validity of the evolved equation was checked by comparing the derived values for the same quantity of cooked rice (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g) at the same incubation periods (0, 3, 6, 9 and 12 hours) against observed values. The tested equation was found valid within the range of cooked rice and incubation period tested. Thus, this equation, when extrapolated can serve to predict ruminal pH of dairy cattle when fed with various quantity of cooked rice.

CONCLUSION

A prediction equation that could predict the fall of pH with varied quantity of cooked rice at different incubation period was evolved and as follows:

$$pH = 7.79 - (W \times 0.421) - (t \times 0.184)$$

Where,
W= Weight of cooked rice in grams.
t= Incubation time in hours.

Hence, it is possible to predict the fall of pH based on the quantity and duration of incubation for any intervening quantity of cooked rice or period of incubation. Thus, appropriate timely intervention or correction in feeding management on intervening quantity of cooked rice to prevent onset of acidosis is possible wherever farmers feed surplus cooked rice to cattle.

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Conflict of interest

The authors declare that they have no competing interests.

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