INFLUENCE OF TIME BETWEEN RUMINAL GLUCOSE CHALLENGES ON RUMEN FUNCTION

[INFLUENCIA DEL LAPSO ENTRE DESAFIOS RUMINALES CON GLUCOSA EN LA FUNCIÓN RUMINAL]

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SUMMARY

Ruminal lactic acidosis is one of the most important metabolic problems in feedlot cattle. Gradually transitioning cattle to finishing-feedlot diets may reduce the risk for ruminal acidosis by providing sufficient time for adaptation. This adaptation of feedlot cattle to high-concentrate diets may causes marked changes in the ruminal environment, and time is required to establish stable ruminal conditions. However, few studies have evaluated the ruminal adaptation in steers. A metabolism trial was conducted to evaluate the effects of two consecutive glucose challenges on rumen function in steers fed a high-energy finishing diet. Four Holstein steers (320 kg LW) with cannula in the rumen were used in a 4 x 4 Latin square design. Four treatments were used and consisted of the time elapsed between both challenges of glucose (2, 4, 6 or 8 d). Ruminal fluid samples were taken at 0700 h (just prior the first glucose challenge), and from the second challenge (d 2, 4, 6, or 8) at 1 h before and 2, 4, 6, 8, 28, 52, 124, 196 and 268 h. As the time between fluctuation of energy intake increased, ruminal fluid pH ($P < 0.05$) and ruminal L-lactic acid increased linearly ($P < 0.10$) after the first challenge. However, ruminal pH and L-lactic acid were not related ($P > 0.10$). During the first 6 h following the second glucose challenge ruminal fluid pH decreased. No effects of treatments on ruminal pH were observed ($P > 0.10$) among treatments from 3 days after the second challenge. Ruminal fluid osmotic pressure increased ($P < 0.10$) after dosed glucose with all treatments. Ruminal osmolality increased ($P < 0.10$) as the time between challenges were 2 or 4 days. After dosed glucose, total volatile fatty acids increased, except by treatment 1 after second challenge. Total volatile fatty acid and pH were related positively ($R^2 = 0.69$). As the time increased, a tendency on increment of concentrations of protozoa was observed. Ruminal glucose concentration decreased linearly ($P < 0.10$) 2 h after the second fluctuation of energy intake. We conclude that ruminal alterations are magnified as the time between glucose challenge decreases.

Key words: Cattle; acidosis; glucose; lactate.

RESUMEN

La acidosis ruminal láctica es uno de los problemas metabólicos más importantes que se presentan en la engorda de bovinos de carne. Una adaptación transitoria a dietas de finalización puede reducir el riesgo de acidosis ruminal proporcionando el tiempo suficiente para su adaptación. Este proceso de adaptación de los bovinos a dietas altas en concentrados involucra marcados cambios en el ambiente ruminal, para lo cual se requiere de cierto tiempo con la finalidad de que las condiciones ruminales se mantengan estables. De cualquier manera, solo pocos estudios han evaluado este

proceso de adaptación en bovinos. Se condujo un ensayo de metabolismo para evaluar los efectos de dos desafíos consecutivos con glucosa (500 g) en la función ruminal de novillos alimentados con una dieta de finalización alta en energía. Se usaron cuatro novillos Holstein (320 kg PV) con cánula ruminal, en un diseño de cuadrado latino 4 x 4. Se evaluaron cuatro tratamientos que consistieron en el tiempo transcurrido entre los desafíos con glucosa (2, 4, 6 u 8 d). La dieta basal contenía 72% de trigo hojueleado al vapor, 3% de grasa amarilla y 8% de forraje. Los desafíos de glucosa se introdujeron al rumen vía la cánula ruminal simultáneamente a la oferta de la ración matutina. Se colectaron muestras ruminales a las 0700 h (justo antes del primer desafío con glucosa), y del segundo desafío (d 2, 4, 6, u 8) a 1 h antes y a 2, 4, 6, 8, 28, 52, 124, 196 y 268 h. A medida que el tiempo entre la fluctuación en el consumo de energía se incrementó, el pH del fluido ruminal ($P < 0.05$) y el ácido L-láctico se incrementaron linealmente ($P < 0.10$) después del primer desafío. Sin embargo, no se observó relación ($P > 0.10$) entre el pH y el ácido L-láctico. Durante las primeras 6 h siguientes al segundo desafío con glucosa el pH ruminal disminuyó. No se observó ($P > 0.10$) efecto de los tratamientos en el pH ruminal a partir del tercer día después del segundo desafío. La presión osmótica del fluido ruminal se incrementó ($P < 0.10$) después de la dosis de glucosa con todos los tratamientos. La osmolaridad ruminal se incrementó ($P < 0.10$) a medida que el tiempo entre desafíos fue de 2 a 4 d. Después de dosificar la glucosa, los ácidos grasos volátiles totales se incrementaron, excepto en el tratamiento 1, posterior al segundo desafío. Los ácidos grasos volátiles totales y el pH se relacionaron positivamente ($R^2 = 0.69$). A medida que el lapso entre desafíos se incrementó, se observó tendencia al incremento de la concentración de protozoarios. La concentración ruminal de glucosa disminuyó linealmente ($P < 0.10$) 2 h después de la segunda fluctuación en el consumo de energía. Se concluye que las fluctuaciones en el consumo de alimento pueden afectar la función ruminal, observándose mayores alteraciones a medida que disminuye el tiempo entre desafíos.

Palabras clave: Ganado; acidosis; glucosa; lactato.

INTRODUCTION

When a high amount of free glucose is accumulated in the rumen of animals fed a high-starch ration, ruminal lactic acidosis may be present (Allison, 1976). This nutritional alteration is considered the most important metabolic problem in feedlot (Irwin *et al*., 1979). Vogel and Parrot (1994), observed that death due to digestive disorders comprised 30% of the total death loss in feedlot cattle. Likewise, Slyter (1976), observed that time required for ruminal stabilization after a digestive disturbance may be a week or more. The prophylactic methods used in the feedlot industry to prevent lactic acidosis such as restriction of feed intake or slow incremental adaptations to increasing dietary energy concentration have resulted in reduced gain and feed efficiency (Tremere *et al*., 1968; Muir *et al*., 1980). Daily fluctuations in feed intake affect behavior in feedlot presumably by its influence on ruminal function (Britton and Stock, 1987; Zinn, 1987). Although fluctuations in feed intake have been observed without effect on the parameters of feedlot cattle performance (Stock *et al*., 1995; Soto *et al*., 1998), Fulton *et al*. (1979) and Galyean *et al*. (1992) observed that intake variation decreased animal gain and feed efficiency. Stock *et al*. (1995), reported that the magnitude of intake variance was 5 to 10 times greater for the individual steer within a pen than for the average of the commercial pen lot. The physiological mechanisms affected as cattle adapt to wide variation in intake cattle fed high-energy diets are not clear, but may be related to the interval between respective challenges. Therefore, the objective of this study was to evaluate the influence of two challenges of glucose at different times on rumen function in steers fed a high-concentrate finishing diet.

MATERIALS AND METHODS

Four Holstein steers (320 kg ± 15 LW) with ruminal cannulas were used in a 4 x 4 Latin square design experiment to evaluate the effect of two consecutive glucose challenges on ruminal characteristics. Steers were housed individually on slotted-floor pens (1.42 x 2.74 m) equipped with automatic drinkers. Ambient temperature in the metabolism unit was maintained between 21 and 26ºC. The trial consisted of four 21-d periods, 11 d for treatment application and sampling and 10 d for adaptation. Dry matter intake was restricted to 2.2% of body weight. A steam-flaked wheat-based finishing diet (Table 1) was fed in equal proportions at 0800 and 2000 h daily. Wheat was selected as it may cause greater ruminal acidity than others grains (Tremere *et al*., 1968; Slyter, 1976; Owens, 1998). Two single doses of 500 g of glucose mixed in 2 L of water at 40° C were administered via the ruminal cannula at 0800 h. The first dose was administered on day 0 and the second dose at the following days: d2, d4, d6 or d8 (treatment 1, 2, 3 or 4, respectively). Ruminal fluid samples (250 ml approx.) were collected via the ruminal cannula according to the following schedule: d 0: 0700 h (just prior the first glucose challenge). On day of the second challenge (d 2, 4, 6, or 8) ruminal samples were obtained at 0700,

1000, 1200, 1400, and 1600 h. During days 1, 2, 5, 8 and 11 after the second glucose challenge, ruminal samples were obtained at 1200 h. Ruminal fluid pH was determined on fresh samples. Ruminal fluid sample were strained through 4 layers of cheesecloth. Freshly prepared 25% (w/v) *m*-phosphoric acid (2 mL) was added to 8 mL of strained ruminal fluid. Samples were then centrifuged (17,000 x g for 10 min) and supernatant fluid stored at -20ºC for analysis of L (+) lactic acid (Sigma Technical Bulletin 826-UV, 1990), Glucose (Zinn, 1990) and VFA concentrations (gas chromatography; Zinn, 1991). A separate sample of strained ruminal fluid (100 mL) was used for measuring osmotic pressure (Micro Osmette), and total protozoa (1 mL of strained ruminal fluid was mixed with 8 mL of 0.16 N saline solution plus 1 mL of a 10% formol solution, and protozal counts determined using a Neubauer counter). This trial was analyzed as a 4 x 4 Latin square (Hicks, 1973).

Table 1. Composition of experimental diet (% DM basis)

Item	%
Alfalfa hay	4.00
Sudangrass hay	4.15
Steam-flaked wheat	72.50
Yellow grease	3.00
Cane molasses	8.95
Cottonseeds meal	5.05
Urea	0.50
Limestone	1.40
Trace mineral salt[a]	0.45
Nutrient composition (DM basis)	
NE, Mcal/kg	
Maintenance	2.24
Gain	1.56
Crude protein, %	12.50
Lipid, %	6.40
Calcium, %	0.68
Phosphorus, %	0.34

[a]Trace mineral salt contained: $CoSO_4$, .068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%, ZnO, 1.24; $MnSO_4$, 1.07%; KCI, .052%; and NaCl, 92.96%.

RESULTS AND DISCUSSION

Treatment effects on ruminal glucose concentration are shown in Table 2. Timing of second glucose challenges (days between first and second challenge) caused very little change in ruminal glucose concentration. This is consistent with the results of Zinn (1994), who did not observe effect of 20% of variation in daily feed intake in feedlot, on ruminal starch digestion. As was expected, ruminal glucose increased 2 h after the glucose challenge and returned close prefeeding levels by 4 h after fed. Low levels of glucose (from 1.83 to 12.59 mg/dL) were observed during the rest of sampling times. Counotte *et al*. (1983), reported that concentrations of soluble sugars in the rumen fluid became maximal by 30 min postfeeding, but by 90 min no sugars were detectable in cows adapted to a diet of *ad libitum* hay plus 12 kg of concentrate. Furthermore, our results are in close agreement with others (Hart, 1985) who observed ruminal fluid glucose concentration ranging between 28 to 63 mg/dL. The contrasting results of the studies by Slyter (1976), and the present trial may be associated with differences in ruminal pH. Meissner and Du Preez (1996) compared several protein and energy concentrations in feedlot diets and concluded that several combinations of dietary protein, rumen degradable protein and starch will result in similar quantities of amino acids and glucose in the small intestine. At blood level, Owens *et al.* (2008) did observe an effect of either sucrose or concentrate supplementation on the mean glucose or plasma urea levels. Concentrate supplementation increased ($P < 0.001$) plasma β-hydroxybutyrate levels, while supplementation with sucrose had no effect.

Presence of elevated ruminal glucose concentration 2 h after the glucose challenge indicates that the metabolism of free glucose by ruminal microorganisms may be much slower than has been suggested (91 to 99%, Ghedalia and Salomon, 1987; Ghedalia *et al*., 1989; or 500%/h, NRC, 1996). Effects of fluctuating energy intake on ruminal L-lactic acid level are shown in Table 3. As the laps in time between the first and second glucose challenge increased, ruminal L-lactic acid 1 h before the first and second challenge increased (linear effect, $P < 0.10$). Similar linear relationship was present at 6 and 8 h following second glucose challenge. Consistent with results of previous studies (Mackie and Gilchrist, 1981), ruminal L-lactic acid peaked at 2 h after glucose challenge. The increased levels of L-lactic acid at 2 h after second challenge occurred when time between challenges decreased from 8 to 2 days. L-lactic acid levels returned to prefeeding values 6 h later. Others workers (Telle and Preston, 1971; Kezar and Church, 1979; Nagaraja *et al*., 1981) observed higher levels of ruminal L-lactic acid, but critical drops in rumen pH (pH < 5.00) were present. Though low pH values were observed in rumen at the time that increased L-lactic acid was observed (Tables 3 and 4), the relation of pH and L-lactic acid was not strong ($R^2 = .20$).

Table 2. Influence of treatments on ruminal Glucose (mg/dL).

	Days between glucose challenges				
	1	2	3	4	SEM
1h before 1st challenge	44.00	34.08	33.47	29.82	6.4
1h before 2nd challenge	32.29	45.12	40.35	34.88	9.7
Following 2nd challenge					
2h	81.04	46.98	66.88	60.14	13.4
4h[a]	45.96	22.18	42.70	38.12	9.5
6h[b]	34.33	32.66	39.59	26.32	12.3
8h[a]	45.98	32.46	42.25	38.56	6.8
28h	43.50	46.57	51.36	40.42	3.8
52h[a]	43.44	50.13	41.60	49.43	3.1
124h	44.90	47.65	38.95	45.47	4.4
196h	37.83	46.57	43.71	46.01	8.2
268h[c]	34.34	47.31	45.59	41.72	7.4

[a]Cubic effect, (P <.05)
[b]Cubic effect, (P <.10)
[c]Linear effect, (P <.05)

Table 3. Influence of treatments on ruminal L-lactic acid (mg/dL).

	Days between glucose challenges				
	1	2	3	4	SEM
1h before 1st challenge[a]	9.38	10.64	15.13	16.56	2.47
1h before 2nd challenge[a]	14.15	13.75	15.99	19.13	1.91
Following 2nd challenge					
2h	81.20	88.13	65.66	71.76	8.72
4h	47.76	15.42	25.57	21.50	12.16
6h[b]	13.49	13.38	15.58	20.80	2.48
8h[b]	12.68	14.07	14.88	18.60	2.10
38h	15.09	13.91	16.76	19.25	1.92
52h	17.04	18.67	16.06	23.81	3.55
124h	17.41	18.14	16.96	19.86	2.64
196h	16.10	20.63	16.68	20.39	2.71
268h	14.68	16.17	16.15	19.39	2.11

[a]Linear effect, (P <.10).
[b]Linear effect, (P <.05).

According to Bond *et al*. (1975), a lack of influence of lactic acid on ruminal pH may be due the rapid return of ruminal glucose concentration to prefeeding values (Table 2). Hence, under the conditions of this experiment lactic acid was not produced or accumulated at amounts sufficient to influence ruminal pH values, possibly due to ruminal microbial adaptation to the high concentrate diet. After 8h following the second glucose challenge, no differences (P>0.10) were observed among treatments. Consistent with the present study, Huntington and Britton (1979) observed little variation in rumen fluid lactate of lambs after 4 days after being switched from an all-forage diet to a high-concentrate diet. Likewise, Fulton *et al*. (1976) observed low ruminal lactate concentrations in steers fed a high-wheat finishing diet. The small increase in lactate concentration observed with treatment 4, without significant fall of the ruminal pH, may reflect the

complete return of the rumen to normal conditions. Because a ruminal pH of less than 5.0 increases markedly increases growth of lactic acid producing bacteria (Suda *et al*., 1995), maintenance of ruminal pH above 5.0 should be considered a "critical point" if we wish to maintain the ruminal physiological conditions within a normal range.

Treatment effects on ruminal pH are shown in table 4. After the first challenge, ruminal pH increased (linear effect, P <0.05). Differences in ruminal pH 1 h before the first and second challenge decreased from 13% for treatment 1 to 4% for treatment 4. Ruminal pH after the second glucose challenge was not affected by treatment. Others (Muir *et al*., 1981; Nagaraja *et al*., 1981) have reported markedly lower ruminal pH values following a high-energy challenge. Consistent with Muir *et al*. (1981), ruminal pH dropped during the first 5 to 6 h following the second glucose challenge. Ruminal pH values observed at 1200 h for all treatments are considered within the normal (not indicative of subacute acidosis) and expected range following a feeding bout, notwithstanding a glucose challenge. Ruminal pH tended to stabilized at 8 hours *postprandium*, approximately, and remained lower than prefeeding values the rest of the sampling periods. There were not significant differences (P >0.10) between treatments during the subsequent sampling times. Others (Huntington and Britton, 1979) have observed critical ruminal pH values (4.48), or observed lower ruminal fluid pH values during four days following a high-energy challenge (Kezar and Church, 1979). Effects of fluctuating high-energy feed intake in feedlot cattle are similar to those observed in dairy cows (Malestein *et al*., 1984), or in sheep and goat (Mirgani and Bakhit, 1990). Irwin *et al*. (1979) observed that after 58 h of the administration of glucose (11 g kg^{-1} BW), the rumen was unable to restore pH to that of initial pH level in ewes fed pasture. In the present study, within 8 hours following the second glucose challenge, ruminal pH for all treatments returned to values consistent with that observed 4 h after feeding during the subsequent 10 d of sampling. Similar responses to variable energy intake have been reported by Zinn (1994), who observed no influence of a 20% variation in daily feed intake on ruminal pH in steers fed a high-concentrate diet. Likewise, no relationship was observed (P >0.10) between pH and ruminal L-lactic acid, osmolality, or glucose concentration. Results demonstrate that as the time between about of readily available energy intake fluctuation increases, the adaptive response to the challenge is enhanced.

Table 4. Influence of treatments on ruminal fluid pH

	Days between glucose challenges				
	1	2	3	4	SEM
1h before 1st challenge	6.53	6.46	6.52	6.54	0.07
1h before 2nd challenge[a]	5.76	6.12	6.06	6.26	0.11
Following 2nd challenge					
2h	5.33	5.44	5.38	5.21	0.10
4h	5.45	5.36	5.58	5.57	0.15
6h	5.39	5.55	5.68	5.86	0.16
8h[a]	5.64	5.59	5.85	6.05	0.14
28h[b]	5.56	5.81	5.46	5.71	0.09
52h	5.66	5.53	5.60	5.49	0.06
124h	5.49	5.28	5.55	5.63	0.15
196h	5.57	5.47	5.61	5.63	0.13
268h	5.63	5.52	5.50	5.84	0.10

[a]Linear effect, (P <.05)
[b]Cubic effect, (P <.10).

Treatment effect on ruminal total volatile fatty acids (TVFA) are shown in table 5. Ruminal TVFA concentration tended to decrease (linear effect, P < 0.10) 1 h before the second glucose challenge with increasing days between challenge. Otherwise, there were not treatment effects on ruminal TVFA. Likewise, Zinn (1994), did not observed an effect of a 20% of variation of intake on ruminal VFA. Uhart and Carroll (1967) observed that when steers were suddenly switched for an all forage to a high concentrate diet, ruminal pH dropped below 5.00 and ruminal lactic acid approached 100 mg/dL. In their study, feed intake and ruminal TVFA did not stabilize to a normal level for approximately 6 days. In the present study, ruminal TVFA explained very little (R^2 = 0.20) of the variation in ruminal lactic acid concentration. Wilson *et al*. (1975) attributed this lack of relationship to a decline in fermentation and changes in dilution rates. Gaebel *et al.* (1987) suggested that the lack of relationship between TVFA and lactic acid concentration might be also attributable to increased ruminal fluid osmolality. In contrast, ruminal TVFA was more closely associated (R^2 = 0 .69) with ruminal pH. Indeed, it is expected that ruminal TVFA concentration is the principal factor modulating pH when ruminal pH is 5.5 or greater (Van Campen., 1986; Schoonmaker, et al., 2003).

Treatment effect on ruminal fluid osmolality are shown in table 6. Changes in ruminal osmolality are important as they may impact ruminal liquid dilution rate (Zhao *et al*., 1995) and VFA absorption rate (Lopez *et al*., 1994). Greater ruminal osmolality was observed with treatment 1 following the first challenge. However, the response was not constant. There tended to be a quadratic effect of treatment on osmolality (P <0.10) at two and four hours following the second glucose challenge, with greater osmolality observed with treatment 2 and lower values observed for treatment 4. Ruminal osmolality is expected to increase postprandially as a result of salt intake and VFA production (Garza *et al*., 1989; Kapoor and Puri, 1994). Telle and Preston (1971) observed that ruminal osmotic pressure increased as ruminal lactic acid concentration increased. In contrast, in the present study ruminal lactate concentration explained very little (R^2 = 0.20) of the variation in ruminal osmolality. Likewise, ruminal TVFA explained very little of the variation (R^2 = 0.36) in ruminal osmolality. Kezar and Church (1979), observed that in the event of hypertonicity, ruminal chyme is characteristically yellowish-green in color with very low viscosity. These effects were not apparent in the present study.

Table 5. Influence of treatments on ruminal total volatile fatty acids (mmol/mol).

	Days between glucose challenges				
	1	2	3	4	SEM
1h before 1st challenge	76.39	81.48	81.14	82.12	4.2
1h before 2nd challenge[a]	109.06	99.40	102.93	90.59	4.8
Following 2nd challenge					
2h	107.69	123.49	122.71	113.74	1.1
4h	110.74	116.80	125.16	116.55	.01
6h	110.18	107.32	121.21	105.73	1.2
8h	114.31	110.79	108.39	95.08	.08
28h	113.65	125.59	124.36	116.83	1.4
52h	116.63	134.90	119.52	123.59	.08
124h	114.63	135.05	100.70	117.84	1.6
196h	120.90	130.57	114.31	130.12	1.2
268h	113.97	112.46	129.79	117.92	1.3

[a]Linear effect, (P <.10)

Table 6. Influence of treatments on ruminal osmolality (mOsmol/kg)

	Days between glucose challenges				
	1	2	3	4	SEM
1h before 1st challenge	351	349	358	340	26
1h before 2nd challenge	403	378	371	373	15
Following 2nd challenge					
2h[a]	412	458	411	380	19
4h	390	418	374	394	31
6h	380	373	391	343	31
8h[a]	372	393	372	332	13
38h	382	385	435	387	14
52h	381	424	377	398	39
124h	412	394	340	398	44
196h	386	409	385	442	25
268h	415	362	420	416	19

[a]Quadratic effect, (P <.10)

Treatment effect on ruminal total protozoa are shown in table 7. No effects (P >0.10) of treatments were observed during most of the sampling times. The small effect registered on days 7 and 13 are nevertheless within the normal expected range (Allison, 1976). Although, compared with one hour before the first glucose challenge, ruminal protozoal counts one hour before the second challenge decreased 107 and 68% with treatment 1 and 2, respectively, and increased 33 and 52% with treatments 3 and 4, respectivley. Consistent with (Counotte *et al*., 1983), the date clearly demonstrate that ruminal protozoa population is able to recover within 3 days following an initial challenge, but not following the second challenge. As previously reported (Dehority and Males, 1974; Allison, 1976), maximum numbers occurred just prior to feeding, then decrease rather rapidly and remain quite low for the following hours. Consistent with Nikolov (1966), protozoa counts were positively associated (R^2 = 0.55) with ruminal pH. Numerous studies (Slyter *et al*., 1970; Slyter, 1976; Gabel, 1990; Suda *et al*., 1994) have likewise demonstrate that ruminal protozoal counts are markedly reduced or eliminated during lactic acidosis. Ruminal protozoal counts were not directly associated (P>0.10) with ruminal fluid osmotic pressure, as reported by Dehority and Males (1974).

Table 7. Influence of treatments on ruminal total protozoa (nx10^5/ml)

	Days between glucose challenges				
	1	2	3	4	SEM
1h before 1st challenge	16.8	9.6	12.0	8.4	3.1
1h before 2nd challenge	8.1	5.7	16.0	12.7	4.9
Following 2nd challenge					
2h	4.7	2.6	8.3	9.4	2.8
4h	3.6	1.7	7.6	9.2	2.7
6h	2.9	1.9	7.6	6.2	2.1
8h	4.3	2.0	8.1	6.0	2.0
28h	6.4	2.2	6.2	8.7	2.0
52h	6.5	3.1	7.4	5.2	2.3
124h[a]	5.8	1.6	6.9	2.3	1.9
196h	7.6	4.1	5.6	8.7	2.0
268h[b]	5.2	1.7	6.7	11.3	2.6

[a]Cubic effect, (P <.10)
[b]Quadratic effect, (P <.05)

CONCLUSIONS

Ruminal alterations are magnified as the time between glucose challenge decreases. However, the gradual increase in ruminal pH, L-lactic acid, and total protozoa indicated that within approximately 7 days after fluctuation in feed intake the conditions in the rumen are stabilizing, and are better capable of supporting a subsequent challenge.

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