ASSOCIATION AMONG FLUID, GRAIN INTAKE AND WEIGHT GAIN IN HOLSTEIN BULL CALVES

A Thesis

by

MARCELO GONZALEZ FERREIRA

Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Dairy Science

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ABSTRACT

Association Among Fluid, Grain Intake and Weight Gain in Holstein Bull Calves. (May 2009)

Marcelo González Ferreira, D.V.M., National University of Asunción, Paraguay Chair of Advisory Committee: Dr. Michael Tomaszewski

This study was conducted to determine water intake. Forty-four Holstein bull calves were evaluated to investigate the effects of starter intake, body weight, temperature and time to predict water intake. A model was developed using PROC GLM in SAS. Least square means separation were used to identify significant effects.

Starter intake was a significant variable (P < 0.05) in predicting the water intake of a calf, especially after day 21 when starter intake and water intake were both increasing. Water intake was increased by calves with fecal scores of 1 and 2. However, water intake was significantly different for calves with fecal scores of 3 or 4 with a (P < 0.05) which had decreased water intake. The interaction between scours and fecal score were not significant. Water intakes significantly differ in calves that had scour and in calves not experimented scours.

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1. INTRODUCTION

Studies determining the water intake of calves less than 4 wk of age are limited. Thickett et al. (1981) found a significant correlation of both live-weight gain and calf starter intake with water intake prior to weaning at 5 weeks. Jenny et al. (1978) measured daily ad libitum water intake of calves from 3 through 23 days of age. Water intake increased with increasing dry matter concentration of the milk replacer; however, calf starter was not provided. Atkeson et al. (1934) found that milk was the primary source of water during the first few weeks of the calf's life, but represented a decreasing percentage of the total water intake as the calf became older.

Preweaned calves often are not provided water except for that consumed with milk replacer (Kertz et al., 1984). These authors also reported that some dairy producers believe that supplemental water causes calves to scour. Jenny et al. (1978) noted that daily water intake increased 25 to 50% when calves scoured. However, they concluded that it is uncertain whether calves drank more water because of scouring, or if they scoured due to increased water intake. Kertz et al. (1984) observed that calves from either restricted water or ad libitum water treatments did not differ in the number of calves experiencing scours or in the extent of scouring.

Calves offered supplemental water gained more (P<.05) body weight and consumed more (P<.05) calf starter compared to calves without access to free water (Kertz et al., 1984). In that study, water intake remained low and variable until d

This thesis follows the style of the Journal of Dairy Science.

18 of age. Water intake progressively increased after d 21. Beneficial effects of supplemental water for growing calves were attributed to maintaining water equilibrium and encouraging early calf starter intake.

Water intake of Holstein bull calves through 56 day of age was measured in 6 different trials over two years (Quigley, 2000). In these studies water intake was very closely correlated with starter intake. As calves consumed more starter they drank more water. These studies also found that as environmental temperature increased the calves drank more water. Additionally, they found that the amount of water consumed increases in an exponential, not linear function. Thirdly, they found that the amount of liquid in milk replacer affects the amount of additional water consumed. They concluded this relationship is due to the fact that increased water in milk replacer will reduce the need for additional water to be fed as liquid or "free" water.

Other factors need to be considered when allowing free access to water by calves. Water intake has been shown to be restricted due to taste and/or water quality. Beede (2005) concluded that oftentimes the quality and provision of free drinking water does not receive the attention necessary to ensure optimal nutrition. Thomas et al. (2007) reported that the addition of orange or vanilla flavor to the water did not affect (P> 0.05) the consumption of water compared with the control. However, it was found that intake of the dry starter increased in calves offered the orange flavored water treatment compared with the control or the vanilla flavored treatment. The increased dry feed intake agreed with the significant increase in weight gain measured in calves on the 3 orange flavor treatment. The orange flavor in water increased (P < 0.05) calf starter

feed intake by 249 and 217 g/d compared with the control and vanilla flavor, respectively.

The hypothesis of this study is that ad libitum water intake of Holstein bull calves can be affected by the addition of Betaine 96% to milk replacer at 2 g/d and the addition of Protimax® at label recommended amounts to milk replacer during the summer months when heat stress is experienced.

Furthermore, it is believed that consuming more ad libitum water will not increase the incidence of scours nor have any effect on fecal scores of calves during these conditions. However, it is reaffirmed that intake of calf starter will increase the amount of ad libitum water consumed by calves and that as calves become older they will consume more ad libitum water at an increasing amount in a heat stress environment.

The objective of this study was to determine water intake in Holstein bull calves through day 54 as a control and as compared to treatments with the addition of Betaine 96% and Protimax® at label recommended amounts to milk replacer.

In addition, the secondary objective of this study is to determine if calves that scour have an increased consumption of ad libitum water or if water intake is linearly associated with fecal scores.

Another objective of this study is to determine the association between the intake of calf starter and the intake of ad libitum water.

2. MATERIALS AND METHODS

Fifty-two Holstein-marked bull calves (1 to 3 d of age) were purchased from dairy herds located in Eastern New Mexico and the Texas Panhandle. All calves were to have received colostrum no later than two hours after birth, and weighed approximately 45 kg. The calves arrived at the Texas A&M University Farm, located 10 miles West of the Texas A&M University campus in College Station, via covered trailer on May 13, 2008, following 12 h of overnight transport.

Upon arrival, calves were weighed and randomly assigned to individual "Calftel" hutches (Hampel Corp., Germantown, WI). Hutches were placed on a grass sod surface that was slightly sloped to facilitate drainage and faced Southeast into the prevailing wind. At least 3 m meters separated each hutch to prevent physical contact among the calves. Hutches were moved daily to a clean area of sod to maintain cleanliness of the calves and to allow fecal scores to be made routinely. Fecal scores were based on guidelines suggested by Larson et al. (1977). The scores ranged from 1 through 4 and were based on the following characteristics: 1 = Normal, firm but not hard. Original form is distorted slightly after drooping to ground and settling; 2 = Soft, Does not hold form, piles but spreads slightly. (i.e., pudding like consistency) as defined by Dr. Glenn Holub (personal communication); 3 = Runny, Spreads readily to about 6 mm depth, (i.e., pancake batter like consistency); and 4 = Watery, Liquid consistency, splatters, (i.e., orange juice). Scores were assessed at 0600h daily for the entire period of the study (56 days). Scouring events were coded on a daily basis after fecal scores were made. If a calf

was scouring, that is, the feces was liquid or score =4, the calf was scored with a yes as to scouring and a treatment was made to support the calf with Re-Sorb® (Pfizer Animal Health, Exton, PA) commercially available electrolytes . All calves with a fecal score of 1 to 3 were coded with a no treatment for that day.

Environmental data, including ambient temperature and percent relative humidity were recorded every 5 minutes on a HOBO® Recorder (Onset Computer Corp., Bourne, MA). Temperature Humidity Index (THI) were calculated based on the temperature and humidity each observation. The formula used to calculate THI =(1.98*(Ambient Temperature Fahrenheit-(0.55-0.0055*(% Relative Humidity))*(% Relative Humidity-58))-56.83).

On the day of arrival, calves began a 2 day adaptation period with feedings of 4 L of milk replacer split to provide 2 L at 0600 and 2 L at 1800 h daily. The amount of milk replacer powder provided per calf included 0.45 Kg. per calf per day for the first 9 days and then 1.1% of BW adjusted weekly for each treatment group's mean body weight. Calves were fed with milk replacer using bottles with nipples. Milk replacer was prepared by adding powder to water at 43°C and mixing thoroughly with an electric mixer. Each calf received 2000 ml of water per feeding, which did not vary throughout the study, however, the percent of dry matter changed slightly as powder was adjusted weekly to group mean weights to equal 1.1% BW of milk replacer powder per day added to 2 L of water at each feeding. This was done to facilitate the study of the water intake and to have the water input via milk replacer constant. Calves were allowed 30 minutes

to consume the milk replacer or until interest in drinking was no longer evident. Refused feedings were recorded and calves that appeared dehydrated were offered via bottle with nipple 2 liters of a commercially available electrolyte solution Re-Sorb® (Pfizer Animal Health, Exton, PA). Calves that refused both the milk replacer and the electrolyte solution were fed via esophageal feeder to prevent further dehydration. On d one, a blood sample was collected via jugular veinepuncture from each calf for evaluation of (serum or whole blood or plasma) IgG (Immunoglobulins G) and total serum protein (TSP) as an index of health status. All calves received a 7-way clostridial vaccine (Agri-labs, Schering-Plough Animal Health Corp., Union, NJ), an inactivated bacterin used for the prevention of Clostridium chauvoei, septicum, novyi, sordellii, perffringens Types C & D infections. This vaccine was administered at subcutaneously on day 10 to prevent *Clostridial* species diseases.

Following the 2 day adaptation period, calves were stratified by body weight and health status using the IGg blood level and TSP blood level. Within strata, calves were randomly assigned to one of four treatments. Treatments consisted of two animal feed supplements for dairy calves, Protimax® and Betaine 96% added to the milk replacer at the time of mixing. Treatment PRO included the addition of 4 grams per day of Protimax® added to the milk replacer for 7 days and then reduced to 2 grams daily for the next 14 days; Treatment BET consisted of 2 grams daily of Betaine added to the milk replacer from day 3 thru 56; Treatment P-B included 4 grams daily of Protimax for first 7 days and 2 grams daily for the following 14 days and 2 grams daily of Betaine day 3 to 56; Treatment CON was the control, milk replacer (Land O. Lakes Maxi Care 25%

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crude protein, 20% crude fat). Calves received ad libitum water and ad libitum commercially available 18% protein calf starter feed (Calf Niblets, Gore Bros. Inc., Comanche, TX) beginning on the third day after calves were placed on treatments. Calf starter feed refusal was measured daily at 1800 h.

Body weight, wither height and heart girth was measured every 3.5 d.

Water intake was recorded daily at 0600h. Water buckets were initially filled with 6 L. of water. Daily at 1800 h measured amounts of water were added so that ad libitum water remained available. The following day at 0600 h, the remaining water was measured and subtracted from the total amount made available to the calf for 24 hours. Daily changes in water due to evaporation or rainfall was recorded via the use of a control bucket placed near the research site. That amount of rainwater collected or water lost via evaporation was recorded and adjustments were made for each calf's intake based on amount lost or gained for the day because the buckets were exposed to the open environment. Occasionally, buckets were contaminated with fecal material by the calves. When this happened, the buckets were removed, washed, rinsed with a chlorine solution, and refilled. Intake was not recorded for that calf for that day.

The study ended with forty-four calves remaining on study. Five calves died and 3 were deemed morbid during the trial. The main reason was severe cases of diarrhea during the first two weeks due to Salmonella species. Calves were weaned on Day 54 of the study.

Means, standard deviations and scatter plots were used to determine if there were

any data outliers and/or missing values. A model was developed using General Linear Models procedures of SAS (SAS Institute, Cary, NC, USA). Least square means separation were used to identify significant effects.

The mathematical model used to describe an individual observation was:

 $Yijklmpo = \mu + T_i + D_j + C(T)_{ki} + F_l + S_m + T_p + W_o + ijklmpo$

Where: μ is the overall population mean of water intake, T is the effect of treatments (i = 1,2,3,4), D is the effect of day as a continuous variable (j = 1,... 16), C is the effect of the kth calf (k=1, ,44) within ith treatments, F is the effect of fecal score (i = 1,2,3,4), S is the class variable of scours treatment on any single day (m = 0,1), T is the continuous effect of starter intake, W is the continuous effect of body weight, and is the random residual error term.

Analysis of Variance and Least Square Means were computed using PROC GLM of SAS. Three models were applied to the dataset. The first model included day 1 through day 54. The second model was associated with calves receiving four treatments for the first 21 days of the trial. After day 21, calves no longer received Protimax®. Thus, Treatment PRO or P-B calves no longer received Protimax. Effectively, Treatment PRO became the same as Treatment CON and Treatment P-B tested only Betaine exactly the same as treatment BET for the remainder of the trial. Therefore, from day 21, calves in treatment PRO and CON and Treatment BET and P-B were treated alike. The interaction between both, fecal scores and scours with treatment were left out of the model because they were not significant.

3. RESULTS AND DISCUSSION

Figure 1, total fluid intake by day, includes the intake of 4L of water received with the milk replacer in addition to voluntary water intake by the calf. Total fluid intake generally increases across time for all treatments. On d 21 and 46 rainwater was collected into water buckets. The amount of rainwater collected in the control bucket was subtracted from the amount of water in the calves' buckets. On d 21 this amounted to a reduction of 340 ml. and on d 46 a reduction of 500 ml. On d 21, water consumption decreased for each treatment, except Treatment PRO. However, on d 46 all calves reduced their water intake because the ambient temperature was cooler than previous days and that the calves spent much of their time in the hutches and did not drink as much water as previous days.



Figure 1. Average Total Fluid Intake by Treatments by Days

Figure 2 depicts average starter feed intake by day. In general, starter consumption increased with the calves' age. Just as there was a decrease in fluid intake on d 21, there was also a decrease in the amount of starter consumed. On d 46, starter intake was not measured and was deleted because rainwater covered the feed in the buckets and calves spent much of their time in the hutches during the rain.



Figure 2. Average Starter Intake by Treatment by Days

Figure 3 illustrates average body weights by treatment. Average body weight tended to decrease through day 9 and started to increase after that. Calves did not recover their initial body weight until d 19. This agrees with the work of Kertz et al. (1984) who found that water intake remains low and is variable until d 18 and reported that providing supplemental water is beneficial to the growing calf maintaining water equilibrium and encouraging calf starter intake.



Figure 3. Average Body Weight by Treatments by Days

3.1 Analysis of Data

Table 1 illustrates the ANOVA associated with water intake as the dependent variable from day 1 to 54.

Table 1. ANOVA of Water Intake from Day 0 to 54.

ANOVA Table				
Source	DF	Mean Square	Pr > F	
Treatment	3	12743807	< 0.0001	
Day	16	10264197	< 0.0001	
Calf (Treatment)	40	13756469	< 0.0001	
Fecal	3	3373195	0.0097	
Scours	1	6775077	0.0057	
Starter	1	19812350	< 0.0001	
Body Weight	1	10698550	0.0005	
Error	602	879346		

The adjusted average daily water intake was 3548 ml per day with an R₂ of .765. No interactions were found to be significant and were not included in the analysis. When fecal scores and scours variables were added separately to the model, they were not significant and only slightly reduced the R₂. Since they were measuring two different sources of variation, they were both included in the model.

Source of variation were separated using least square means and the results are presented in the following tables. Table 2 depicts the least squares mean water intake by treatments. Calves fed Trt BET consumed more water than calves fed any other treatment (P < 0.05). Calves fed Trt PRO consumed less water than those fed Trt CON (control; P < 0.05), While those consuming Trt P-B consumed a quantity of water intermediate between Trt PRO and CON.

Least Square Means of Additional Water Intake				
Treatment PRO (Protimax)	3316 ^b			
Treatment BET (Betaine)	3940 ^a			
Treatment CON (Control)	3543 ^⁵			
Treatment P-B (Protimax x Betaine)	3350 ^{ab}			

Table 2. LSM for Treatment of Water Intake from Day 0 to 54.

^{a, b}. Rows with the same superscripts are not significantly different from each other (P < 0.05).

Table 3 provides mean water intake by day. Water intake on d 2 was the lowest observed (P < 0.05). Water intake on d 0 was intermediate to d 1 and most other observations. This was the initial day after arrival and all calves were stressed and not

functioning normally. On d 9, 12, and 44 water intake was greater than on other days. In general, after adjusting water for other effects in the model, water consumption was relatively stable over time once the initial stress of relocation was overcome.

	LSM for water (ml)
Day	
0	2174 ^c
2	1733°
5	3459 ^b
9	4016 ^ª
12	4012 ^a
16	3590 ^b
19	3645 ^b
23	3596 ^b
26	3581 ^b
30	3712 ^{bc}
33	3750 ^b
37	3609 ^b
40	3813 ^b
44	4367ª
47	3503 ^b
51	3660 ^b
54	3808 ^b

Table 3. LMS for Days of Water Intake from Day 0 to 54.

^{a, b}. Rows with the same superscripts are not significantly different from each other (P < 0.05).

Table 4 provides Least Squares Means water intake associated with different levels of fecal scores. Calves with fecal score of 1 through 3 consumed significantly more water than calves with a fecal score of 4. Scores 1 and 2 consumed more water than calves with scores of 3 and 4. Calves with a 2 consumed the most water. Calves with a 3 and 4 also were not different in amount of water consumed.

5			
	Total Water LSMEAN (ml)		
Fecal			
1	3697 ^{abc}		
2	3836 ^{ab}		
3	3533 ^{acd}		
4	3082 ^{cd}		

Table 4. LSM for Fecal Scores of Water Intake from Day 0 to 54.

^{a,b,c,d}. Rows having different superscripts are significantly different form each other (P < 0.05).

Scours in the model indicated whether the calf was treated for scours or dehydration for that day and were binomial and were scored 1 for yes and 0 for no treatment. Fecal Scores were different than Scours. Calves that had scours drank 3811 ml. of water versus calves not having scours 3263 ml. The interaction between scours and fecal score were not significant.

Table 5 presents the Analysis of Variance for day 0 to 21 of the study.

ANOVA Table				
Source	DF	Mean Square	Pr > F	
Treatment	3	2116744	0.0643	
Day	16	15782706	< 0.0001	
Calf (Treatment)	40	5641829	< 0.0001	
Fecal	3	3032702	0.0161	
Scours	1	2546988	0.0872	
Starter	1	2600649	0.084	
Body Weight	1	1561885	0.1798	
Error	197	861965		

Table 5. ANOVA of Water Intake from Day 0 to 21.

The original model for day 1-54 treatment, scours, starter and body weight were significant. However, in the model for day 0 to 21, they were not. However, these variables were left in the model to test if the least square means were different. The R₂ for this model was 0.71. The mean was 2403 ml. of water daily intake for days 0 to 21.

Table 6 contains the Least Square Mean values for treatments in the model for 0 to 19 days. Significant differences were found among the least squares means for treatments. Treatment PRO, CON and P-T were significantly different than BET while BET, CON and P-B were significantly different from PRO.

Tuele e. Estimet de duitient en mane ment aug e te 19.			
Least Square Means of Additional Water Intake			
Treatment PRO	2069 ^a		
Treatment BET	2547 ^b		
Treatment CON	2404 ^{ab}		
Treatment P-B	2352 ^{ab}		

 Table 6. LSM for treatment of Water Intake from day 0 to 19.

^{a, b}. Data having different superscripts are significantly different form each other (P < 0.05).

Table 7 illustrates Least Squares Means of days for water intake. Day 2 is significantly different from all days except from day 0. This was anticipated due to the stress experienced by calves at this part of the experiment.

2	
	LSM for Water (ml)
Day	
0	1328 ^b
2	971 ^b
5	2672 ^a
9	3014 ^a
12	3020 ^a
16	2514 ^a
19	2880 ^a

Table 7. LSM for Days of Water Intake from Day 0 to 19.

^a. Rows with the same superscripts are not significantly different from each other (P < 0.05).

Table 8 contains the LSM for Fecal Score. Calves with more "normal" fecal

scores of 1 or 2 drank more water than calves that had "loose" fecal scores of 3 or 4.

 Water Intake LSMEAN (ml)

 Fecal

 1
 2665^a

 2
 2625^a

 3
 2206^b

 4
 1875^b

Table 8. LSM for Fecal Scores of Total Water from Day 0 to 21.

^a. Data having different superscripts are significantly different form each other (P < 0.05).

Table 9 contains the ANOVA associated with total water intake from day

21 to 54 of the study.

ANOVA Table				
Source	DF	Mean Square	Pr > F	
Treatment	3	8125646	< 0.0001	
Day	16	1997258	0.0069	
Calf (Treatment)	40	9468991	< 0.0001	
Fecal	3	1837743	0.0701	
Scours	1	24716	0.8583	
Starter	1	13402637	< 0.0001	
Body Weight	1	11391401	0.0001	
Error	356	275758223		

Table 9. ANOVA of Water Intake from Day 21 to 54.

This model had an R² of 0.757, which is very close to the R² obtained in the overall ANOVA of the original model including all days. However, in this model, scours and fecal score were not significant due to the low number of calves experiencing scours after day 21. The mean daily water intake is 4246 ml.

Table 10 contains the LSM by treatment for days 21 to 54. Treatment PRO, CON and P-B were significantly different from BET. Calves that were on Protimax®, consumed less water than all other treatments. Treatment P-B should be increased after day 21, because only Betaine remains in this treatment. However, treatment P-B was not significantly different from the control.

Least Square Means of	of Additional Water Intake
Treatment PRO	3803 ^b
Treatment BET	4356 ^ª
Treatment CON	3885 ^b
Treatment P-B	3686 ^b

Table 10. LSM for Treatment of Water Intake from Day 21 to
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^a. Data having different superscripts are significantly different form each other (P < 0.05).

Table 11 illustrates that no differences were found in water intake among the differing fecal scores after day 21.

	Total Water LSMEAN (ml)
Fecal	
1	8113.03
2	8305.29
3	8316.32
4	6995.58

Table 11. LSM for Fecal Scours of Total Water from Day 21 to 54.

4. CONCLUSION

Water appears to have a relationship with starter intake as starter intake is a significant variable in predicting the water intake of a calf, especially after day 21 when starter intake and water intake are both increasing. Prior to 21 days, the relationship between water intake and starter intake is not significant but does trend to be related.

Water intake is increased by calves with fecal scores of 1 and 2. These fecal scores are acceptable for calf raisers and indicate normal stools. However, calves with fecal scores of 3 or 4 have decreased water intake which may contradict Jenny et al, 1978. This finding is especially significant during the first 21 days of a calf's life when water intake is more variable and the stress of scours is more likely to occur.

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APPENDIX A

	J.)		AI	9443	11595	15569	17276	17259	16507	16314	16410	16298	16204	15903	15557	15670	16330	14571	14825	14250			15293
	g. (ml./Kg		P-B	9316	11256	15575	17269	16244	16108	15809	15925	15180	15735	15771	15209	15260	15416	13739	13815	12525			14715
	er 100 K(reatment	CON	9412	11510	15257	17575	17808	16657	16145	16823	16491	16256	15312	15194	16262	16132	15181	14736	15048			15400
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call pel	Tot		PRO	9427	11726	14809	16125	17064	15933	15081	16075	15789	15828	15651	15368	15094	16836	13339	14652	13891			14864
g. per			AII	0,00	0,02	0,03	0,04	0,07	0,18	0,18	0,37	0,41	0,47	0,50	0,58	0,64	0,74	0,82	0,90	0,88	1,03	1,36	0,43
	Kg.		P-B	0,00	0,01	0,03	0,04	0,05	0,14	0,18	0,34	0,37	0,40	0,45	0,50	0,55	0,73	0,80	0,92	0,86	1,03	1,30	0,40
e per	ntake in	atment	CON	0,00	0,02	0,03	0,05	0,08	0,19	0,18	0,43	0,45	0,60	0,60	0,74	0,69	0,82	0,99	0,94	0,98	1,20	1,44	0,48
1 In tak	tarter Ir	Tre	BET (00'c	0,01	0,05	0,05	0,10	0,22	0,20	0,43	0,50	0,50),58),62	0,73	0,74),82	0,89	06'0	1,06	1,14	0,46
al Tiluic	S		RO	,00	,04	,02	,03	,06 (,15 (,15 (,30	,31	,38	,38	,46 (,58 (,69	,65 (,87 (,78 (,84	,54 、	,38 (
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i able A. Average dally iluid intake and	Total Fluid in ml. Body Weight in Kg.	Treatment	BET CON P-B AII PRO BET CON P-B	4000 4000 4000 4000 42 42 43 43	4769 4723 4660 4749 41 40 41 41	6583 6219 6443 6327 41 39 41 41	6966 6932 6878 6767 39 38 39 40	7039 7075 6581 6876 40 39 40 41	7004 6745 6611 6689 40 40 40 41	7740 6818 6791 6945 43 42 42 43	7413 7303 7124 7215 44 44 43 45	8191 7571 7147 7517 45 46 46 47	8083 7675 7537 7672 47 48 47 48	8380 7586 7760 7833 49 50 50 49	8455 7779 7812 7933 50 51 51 51	8695 8675 8075 8360 53 54 53 53	9548 9233 8695 9178 55 56 57 56	9490 9065 8327 8639 58 59 60 61	9711 9025 8503 8994 60 60 61 62 (9891 9881 8350 9223 63 63 66 67 6	· · · · · ·		8061 7595 7236 7348 48 48 48 49
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