

Purina® Outlast™ Gastric Supplement Optimized pH in a Simulated Gastric Environment More Effectively than Other Similar Products

A SUMMARY OF RESEARCH CONDUCTED AT THE PURINA ANIMAL NUTRITION
CENTER EVALUATING THE EFFICACY OF PURINA OUTLAST™ GASTRIC SUPPLEMENT.¹

< INTRODUCTION >

It has been estimated that over 90% of horses suffer from gastric discomfort related to the stomach lining.² While a variety of risk factors exist for horses to develop gastric imbalance, a commonality amongst all horses is the constant secretion of acid into the gastric lumen. Historically, alfalfa hay and other alfalfa derived products have been identified for their ability to successfully buffer the equine stomach, and recently a number of products have been marketed with similar effects. Previous research conducted by Purina Animal Nutrition has found that horses consuming Purina® Outlast™ Gastric Supplement experienced changes in gastric pH and gastric comfort.³.⁴ The objective of this study was to evaluate the efficacy of Purina® Outlast™ gastric supplement in a simulated gastric environment by measuring the buffering ability, capacity and speed in comparison to a number of currently marketed gastric buffer supplements.

< MATERIALS AND METHODS >

All experiments were conducted in a reaction vessel that was designed to mimic the equine gastric environment. Nine separate gastric buffers were evaluated including Purina® Outlast™ gastric supplement and alfalfa pellets. For all supplements except one (which was tested at a low and high concentration), a ratio was determined based on the manufacturers recommended dosages and the size of the equine stomach in relation to the reaction vessel. All supplements were ground to simulate chewing and placed in a continually stirring (mimicking gastric motility) and heated (37°C; internal body temperature) beaker containing 1 L of a pH=2.0 hydrochloric acid solution. During experiment 1, pH readings were obtained prior to and every 30 min following placing the supplements into the beaker for 6 hours for a total of 13 measurements. This data was utilized to calculate a buffering capacity with the following equation: (Max pH-Min pH)/Time to reach max pH. For experiment 2, Purina® Outlast™ gastric supplement and the top four performers from experiment 1 were subjected to an identical experimental protocol except that pH readings were obtained prior to starting and then again every 2 minutes for 60 minutes resulting in a total of 30 measurements. For all timepoints, pH was measured via a digital pH analyzer (Hach, Loveland CO), and an average of two pH measurements was utilized.

¹ Jacobs, RD, Gordon, ME. HR 246. 2016. Purina Outlast pH comparison.

²Murray, M.J., Schusser, G.F., Pipers, F.S., et al., 1996. Factors associated with gastric lesions in Thoroughbred race horses. *Equine Vet J* 28, 368-374.

³Gordon, ME, Andrews, F. HR 198. 2015. LSU gastric pH study.

⁴Gordon, ME, Jerina, ML, Young, JK, Andrews, F. HR 229, 2016. Draft Horse Field Trial. (Published in abstract form in the 2017 Equine Science Society Proceedings as: The effect of a natural-source mineral supplement on gastric ulceration in horses.)

Results from experiment 1 are presented in Figures 1-2 below. Results from experiment 2 are presented in Figure 3. In experiment 1, Purina® Outlast™ gastric supplement buffered the simulated gastric environment to a higher level than all other measured supplements. Additionally, the buffering capacity of Purina® Outlast™ gastric supplement was higher than that of the other supplements. In experiment 2, Purina® Outlast™ gastric supplement buffered the simulated gastric environment more quickly than the other supplements tested reaching 78.4% of its maximum buffering capacity at 2 minutes.

< IMPLICATIONS >

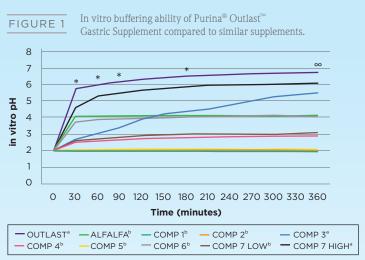
The continual presence of gastric acid in the equine stomach, even during times when the horse is not eating can be considered a major factor in the development of gastric ulcers. Data from this study indicate that Purina® Outlast™ gastric supplement buffered the simulated gastric environment to a higher level than other products tested, and does so quicker and to a greater extent than the other supplements tested, including alfalfa. For horses experiencing stressors such as trailering, exercising, confinement, weaning and others Purina® Outlast™ gastric supplement may have the ability to buffer the gastric acid in the stomach, producing a more favorable gastric environment and reducing the potential for gastric ulceration.

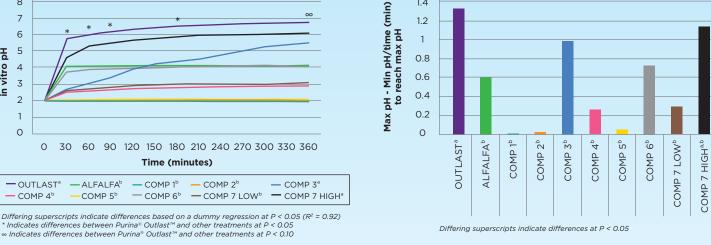
FIGURE 2

1.4

1.2

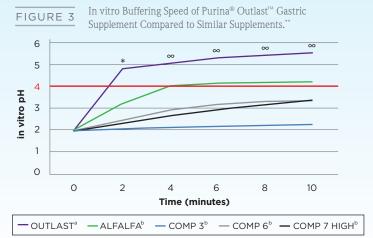
0.8





In vitro buffering ability of Purina® Outlast™ Gastric

Supplement over time compared to similar supplements.



**Comp 3, 6, 7 (high) were chosen for comparison as they were the top performers in HR 246. To review the full data set, see the research review titled "Purina® Outlast™ Gastric Supplement Optimized pH in a Simulated Gastric Environment More Effectively than Other Similar Products Differing superscripts indicate differences based on a dummy regression at P < 0.05 ($R^2 = 0.92$) * Indicates differences between Purina® Outlast™ and other treatments at P < 0.05
∞ Indicates differences between Purina® Outlast™ and other treatments at P < 0.10

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REVIEW >

The Effects of Purina® Outlast™ Gastric Supplement on Equine Gastric Health Parameters

A SUMMARY OF RESEARCH CONDUCTED BY PURINA ANIMAL NUTRITION EVALUATING THE EFFECTS OF A PROPRIETARY MINERAL COMPLEX ON GASTRIC HEALTH IN TRAVELING DRAFT HORSES.¹

< INTRODUCTION >

Horses exercising and traveling for long periods of time can be at greater risk for gastric discomfort. Confinement, meal feeding, new environments and the overall stress of travel can all contribute to this challenge. In addition, research has shown that gastric ulceration is related to low pH levels in the stomach. Nutritional interventions that support optimal gastric pH may help to reduce this risk. Previous research has shown that Purina Outlast Gastric Supplement supports an optimal gastric pH in multiple trials. Herefore, the objective of this study was to determine if feeding Purina Outlast Gastric Supplement would alter gastric imbalance in horses during a 30-day course of exercise and travel.

< MATERIALS AND METHODS >

Fourteen mature, draft geldings (884 ± 14 kg) were initially screened for gastric ulcers via endoscopy. Horses were then assigned to CON (n=7; no added supplement) or OL (n=7; supplement), top-dressed at 227 g/head/d of active ingredient. Next, 9 horses (4 CON, 5 OL) traveled approximately 1610 km via tractor trailer over 3 days, were hitched 4 times over 6 days then returned home over another 3-day trip, covering an additional 1610 km. Housing for the trial varied between permanent and tie stalls. Horses were fed concentrate feeds (Purina® Omolene #100® at 0.9-1.8 kg/d, Enrich Plus® at 0.5 - 0.9 kg/d, Amplify® Supplement at 0.9 kg/d, n=12; Ultium® at 1.8 kg/d, n=1; Enrich Plus® at 0.9 kg/d, n=1) along with 15 kg grass hay/d to meet or exceed NRC requirements. On day 30, horses underwent a second endoscopy. All feed and forage were withheld for 16-18 hours prior to scopings. Findings were noted as: overall equine gastric ulcer score (EGUS), glandular ulcer number (GN), glandular ulcer severity (GS), non-glandular ulcer number (NGN), non-glandular ulcer severity (NGS), desquamation, hyperkeratosis and hyperemia. The veterinarian performing the procedures was blinded to treatment. Body weight was measured via electronic scale. Analysis of variance was done with mixed models using GLIMMIX procedure in SAS, and least squares means were compared using Fisher's least significant difference (P<0.05).

Gordon, ME, Jerina, ML, Young, JK, Andrews, F. HR 229, 2016. Draft Horse Field Trial. (Published in abstract form in the 2017 Equine Science Society Proceedings as: The effect of a natural-source mineral supplement on gastric ulceration in horses.)

² Murray, M.J., Schusser, G.F., Pipers, F.S., et al., 1996. Factors associated with gastric lesions in Thoroughbred race horses. *Equine Vet J* 28, 368-374.

³ Gordon, ME, Vineyard, KR, Andrews, F. HR 198, 2015. LSU gastric pH study

⁴ Gordon, ME, Vineyard, KR, Andrews, F. HR 212, 2016. LSU titration study

< RESULTS

The control group showed an increase in NGN from day 0 to day 30 (P=0.0375), while no change occurred in the Outlast^M supplemented group (P>0.05, Figure 1.) There was also a trend for GN to increase in the control group by day 30 (P=0.067), but no change in the Outlast^M supplement group (P>0.05, Figure 2). There were no differences in EGUS, GS, NGS, desquamation, hyperkeratosis or hyperemia for treatment or time (P>0.05) for all horses overall. When data were analyzed for traveling horses only, an increase in EGUS became evident in the control group (P=0.0474) but not the Outlast^M supplemented group (Figure 3).

< IMPLICATIONS >

In conclusion, control horses with no added $Purina^{\circ}$ Outlast partial gastric supplement had an increase in non-glandular ulcer number and a trend for more glandular ulcers than horses receiving the supplement. Similarly, traveling horses in the control group had an increase in overall EGUS, while the treatment group did not. Providing at-risk horses this mineral complex supports gastric health during times of travel.

FIGURE 1

An increase in non-glandular ulcer number in control (P=0.0375) vs. no increase in $Outlast^{\mbox{\tiny M}}$ horses.

NON-GLANDULAR NUMBER 4 а Non-glandular number ab 3.5 ah 3 2.5 2 b 1.5 1 0.5 0 OUTLAST DAY 30 CONTROL CONTROL OUTLAST DAY 0 DAY 30 DAY 0

FIGURE 2

A trend for an increase in glandular ulcer number in control (P=0.0666) vs. no increase in Outlast^M horses.

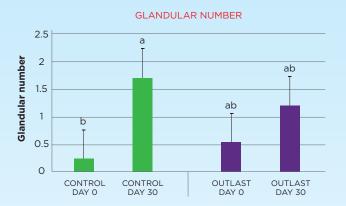
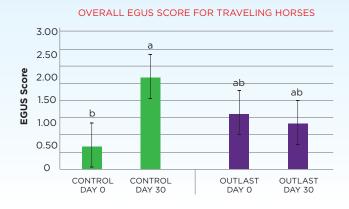


FIGURE 3

An increase in overall EGUS score for traveling horses in control group (P=0.0474) vs. no increase for traveling horses in Outlast^M group.





Alterations in Equine Gastric pH by Purina® Outlast™ Gastric Supplement

A SUMMARY OF RESEARCH CONDUCTED BY PURINA ANIMAL NUTRITION IN CONJUNCTION WITH LOUISIANA STATE UNIVERSITY, EVALUATING THE EFFECTS OF FEEDING A PROPRIETARY MINERAL COMPLEX ON GASTRIC pH IN THOROUGHBRED HORSES.^{1,2}

< INTRODUCTION >

Horses evolved as grazing animals and as such, are meant to devote much of their day to trickle feeding or chewing feedstuffs in small amounts over long periods of time. To that end, horses constantly secrete stomach acid to assist with the digestion of their feed and forage, which is naturally buffered by bicarbonates secreted in saliva as horses chew. In modern management practices, however, horses spend more time confined and eating distinct meals of feed, which can lead to less chewing and higher levels of acid in the stomach. In addition, exercising horses during periods of high stomach acidity has been attributed to increased gastric discomfort. Therefore, a series of research trials were conducted at Louisiana State University, in which horses were fed meals with and without Purina[®] Outlast™ Gastric Supplement to test the hypothesis that this proprietary mineral complex would support optimal stomach acid more than a control feed. Successfully buffering stomach acid for horses could be an important factor in managing horses' gastric comfort.

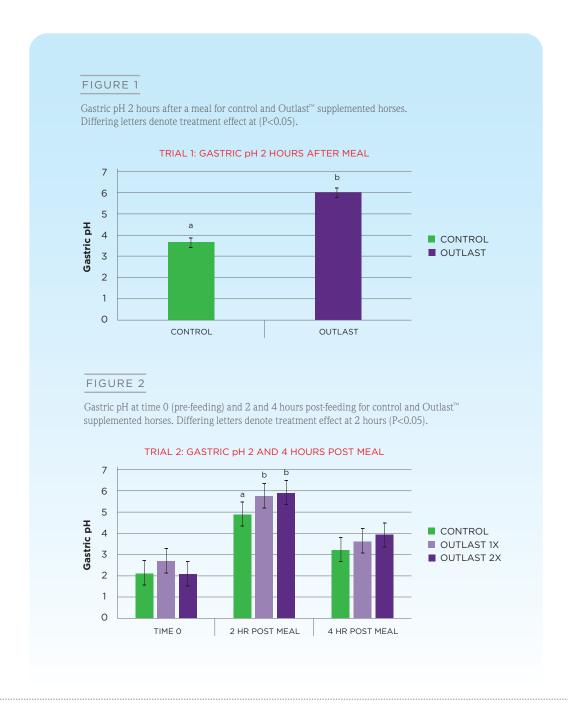
< MATERIALS AND METHODS >

In trial 1, twenty mature, healthy TB geldings were housed in stalls and on testing day were fasted for 16 hours prior to gastric endoscopy. Gastroscopy was performed using a 3-meter endoscope for collection of gastric juice for pH measurement. After collection, horses immediately received either 0.5 lb/500 kg BW of a control pellet or 0.5 lb/500 kg BW of a treatment pellet containing Outlast™ gastric supplement. Two hours later, horses received a second endoscopy for collection of gastric juice and pH measurement. In a second trial, nine TB geldings were housed in stalls and on testing days 0, 7 and 14, the horses received 1 of 3 dietary treatments (CON, Outlast1x, Outlast2x) in a randomized, crossover design at a rate of 45.4 g/100 kg BW along with 0.55 kg Purina® Omolene 100® horse feed. CON contained no added minerals, Outlast1x contained minerals at a lower concentration, and Outlast2x contained minerals at 2x the concentration of Outlast1x. The final on-market formulation of Outlast1™ gastric supplement is formulated at the 2x concentration. All horses underwent gastroscopy prior to feeding the treatments, and at 2 and 4 hours post-feeding. For both trials, gastric juice (60 ml) was aspirated from the biopsy channel and pH measured in duplicate using a benchtop pH meter. Analysis of variance was done with mixed models using GLIMMIX procedure in SAS, and least squares means were compared using Fisher's least significant difference (P<0.05).

For trial 1, at 2 hours post-feeding, gastric pH was higher in the treatment group receiving OutlastTM supplement than the control group (Figure 1). For trial 2, there was a significant time effect (P<0.0001) with an increase in gastric juice pH from time 0 (2.31 \pm 0.48) to 2 hours (5.52 \pm 0.48) and 4 hours (3.59 \pm 0.48). Gastric juice pH at 2 hours was higher (P=0.0122) in Outlast1x (5.78 \pm 0.58) and Outlast2x (5.92 \pm 0.57) than CON (4.88 \pm 0.58, Figure 2).

< IMPLICATIONS >

In conclusion, providing horses at risk for gastric ulcers a feed or supplement containing Purina Outlast Gastric Supplement may help to achieve optimal gastric pH. The increase in pH lasted for at least two hours under these described feeding and management conditions. For horses at risk for gastric ulcers or gastric discomfort, Purina Outlast Gastric Supplement may help support proper gastric pH.





A Comparison of Purina® Outlast™ Gastric Supplement and Calcium Carbonate on TCO₂ Levels in Horses

A SUMMARY OF RESEARCH CONDUCTED AT THE PURINA ANIMAL NUTRITION CENTER EVALUATING MULTIPLE LEVELS OF A PROPRIETARY MINERAL COMPLEX AND CALCIUM CARBONATE ON TCO₂ CONCENTRATIONS IN HORSES.¹

< INTRODUCTION >

When equine athletes exercise, they accumulate lactic acid and hydrogen ions in their tissues as a metabolic product of energy expenditure. The body naturally buffers this acid over time, but a buildup can lead to fatigue and muscle soreness. To combat this acid accumulation, the illegal practice of "milk-shaking" in horse-racing has been described. Briefly, the procedure involves administering large quantities of alkalizing agents via a nasogastric tube to buffer the hydrogen ions and provide a potential ergogenic effect to exercising horses. The TCO_2 blood test allows regulatory agencies to detect this practice, as indicated by concentrations of CO_2 , CO_3 , H_2CO_3 and HCO_3 above 37 mmol/L plasma. Suggestions have been made that feeding smaller amounts of buffers or minerals to horses can increase TCO_2 and result in positive tests in the absence of "milk-shaking." The objective of this study was to test the hypothesis that feeding a mineral complex (Purina® Outlast[™], OL) or isocalcemic amount of calcium carbonate (CC) would not increase TCO_2 due to relatively low feeding rates.

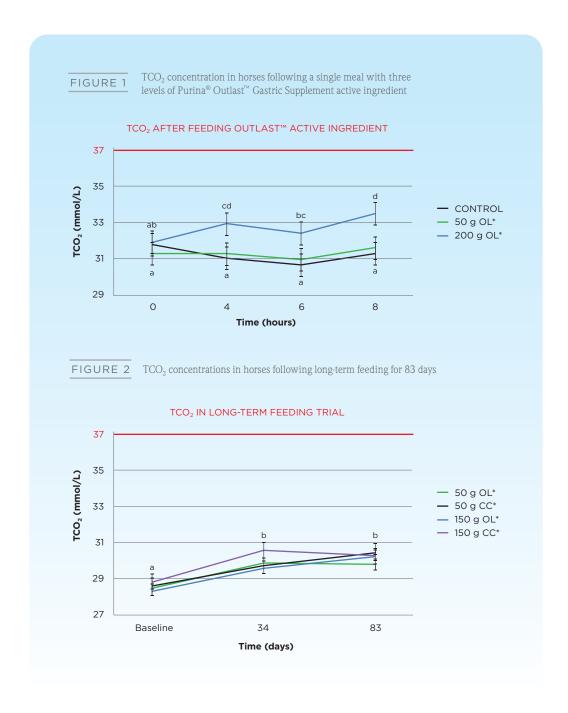
< MATERIALS AND METHODS >

In trial 1, 9 mature, unfit OHs were fed the active ingredient of Purina® Outlast™ Gastric Supplement in a cross-over design, at a rate of 0, 50 and 200 g in a one-time meal mixed with molasses and beet pulp. Blood samples were taken pre-feeding and 4, 6 and 8 hours post-feeding. In a second trial, 47 unfit, mature OHs and TBs were fed either 50 g/head/day of the active ingredient in OL or an isocalcemic amount of CC in 2 meals, or 150 g/head/day OL or isocalcemic amount of CC in 4 meals for 85 days. Blood samples were taken prior to and after 34 and 83 days on diets, with all samples obtained 4-5 hours after 0700 feeding. All blood was taken via jugular venipuncture into 10 mL heparinized tubes, immediately cooled and shipped to the NY Drug Testing and Research Program for analysis via Nova 4 Electrolyte Analyzer. ANOVA was done with mixed models using GLIMMIX procedure in SAS, and least squares means were compared using Fisher's LSD (P<0.05).

All horses remained well below 37 mmol/L of TCO_2 which is the legal limit established for milk-shaking. Horses on the 200 g feeding rate of OL in the first trial did have a small increase in TCO_2 (P = 0.0347, Figure 1). Horses on OL and CC in the second trial also had small increases (P<0.0001) in TCO_2 over time, but there were no treatment (P = 0.4468) or treatment*time differences (P=0.8276, Figure 2). Mean baseline samples were reported as $28.48 \pm 0.19 \text{ mmol/L}$, 34 day samples were $29.90 \pm 0.19 \text{ mmol/L}$, and 83 day samples were $30.14 \pm 0.19 \text{ mmol/L}$.

< IMPLICATIONS >

These results indicate that feeding Purina® Outlast $^{\text{TM}}$ Gastric Supplement or CC as directed does not result in positive TCO $_2$ results under these scientific conditions. This was expected considering the low feeding rates of the minerals that are congruent with typical diets for horses.





Purina® Outlast™ Gastric Supplement Buffers a Simulated Gastric Environment More Effectively than Similar Products when Challenged by an Acid Addition Test

A SUMMARY OF RESEARCH CONDUCTED AT THE PURINA ANIMAL NUTRITION
CENTER EVALUATING THE EFFICACY OF PURINA OUTLAST™ GASTRIC SUPPLEMENT.¹

< INTRODUCTION >

As grazing animals, horses are designed to consume a forage-based diet continually throughout the course of a day. To allow for this, certain physiological mechanisms are in place including the near constant secretion of gastric acid into the lumen of the stomach. However, modern management practices typically restrict horses from maintaining gastric fill, allowing stomach acidity to increase and the pH to drop to levels that may be a causative factor for gastric ulceration. Previous research conducted by Purina Animal Nutrition has found that feeding horses Purina $^{\circ}$ Outlast $^{\circ}$ Gastric Supplement supported a more optimal gastric pH and altered gastric health parameters. 2,3 The objective of the current study was to evaluate the buffering ability of Purina $^{\circ}$ Outlast $^{\circ}$ Gastric Supplement when challenged by an acid test in a simulated equine stomach.

< MATERIALS AND METHODS >

The experiment was conducted in a reaction vessel that was designed to mimic the equine gastric environment. Four separate gastric buffers were evaluated including Purina® Outlast™ Gastric Supplement and alfalfa pellets.¹ For all supplements, a ratio was determined based on the manufacturers recommended dosages and the size of the equine stomach in relation to the reaction vessel. All supplements were ground to simulate chewing and placed in a continually stirring (mimicking gastric motility) and heated (37°C; internal body temperature) beaker containing 1 L of a pH=2.0 hydrochloric acid solution. The supplements were left in the reaction vessel for 6 hours to allow them to reach their maximum buffering capability as determined in a previous experiment.¹ At 6 hours, 5 mL of a pH=1.0 hydrochloric acid solution was added every minute and pH measurements recorded until the pH in the vessel no longer remained above 4.0. For all time points, pH was measured via a digital pH analyzer (Hach, Loveland CO), and an average of two pH measurements was utilized.

¹ Jacobs, RD, Gordon, ME. HR 246. 2016. Purina Outlast pH comparison.

² Gordon, ME, Andrews, F. HR 198. 2015. LSU gastric pH study.

³ Gordon, ME, Jerina, ML, Young, JK, Andrews, F. HR 229, 2016. Draft Horse Field Trial. (Published in abstract form in the 2017 Equine Science Society Proceedings as: The effect of a natural-source mineral supplement on gastric ulceration in horses.)

Results are depicted in Figure 1 below. In this in vitro trial, all other supplements tested against Purina® Outlast $^{\text{\tiny{M}}}$ Gastric Supplement required less acid addition to reach a pH of 4.0. In addition, all other supplements reached a pH of 4.0 more quickly than Outlast $^{\text{\tiny{M}}}$ Gastric Supplement showing the ability of Outlast $^{\text{\tiny{M}}}$ Gastric Supplement to continue buffering for longer periods of time in this simulated environment.

< IMPLICATIONS >

The constant acid secretion by the equine stomach has been identified as a causative factor in gastric ulceration and Equine Gastric Ulcer Syndrome (EGUS). Horses suffering from EGUS may show signs of reduced performance, decreased feed intake, discomfort and other signs. Data from this study demonstrates that Purina® Outlast $^{\text{M}}$ Gastric Supplement can more effectively overcome an acid challenge as compared to similar supplements in a simulated environment. More acid was required to drop the pH below 4.0 and the simulated gastric environment remained above a pH of 4.0 for a longer period of time when challenged by continual acid secretion. These data indicate that Purina® Outlast $^{\text{M}}$ Gastric Supplement may be effective in buffering the acid secreted by the equine stomach, creating a more favorable gastric environment.

TABLE 1 AMOUNT OF ACID (mL) AND TIME NEEDED TO DROP THE pH OF
A SIMULATED IN VITRO GASTRIC ENVIRONMENT BELOW 4.0

	TOTAL ACID ADDED (mL)	TOTAL TIME TO pH < 4.0 (MIN)
OUTLAST ^a	140	38
ALFALFA ^b	10	2
COMP 3 ^b	10	2
COMP 6ª	30	6
COMP 7 HIGH ^b	20	4

