Summary of Commercial Animal Production Studies

Grazix Animal Health, Inc.

A subsidiary of LiveLeaf Inc.



This report summarizes studies performed on commercial and university farms to determine the capability of Grazix solution to promote intestinal health, which may result in increased growth rates, improved feed efficiency and reduction in mortality.

I. Grazix evaluation in milk replacer for piglet stress immediately after post weaning transport

Site: Chen-Leu Yuan Quality Breed Pig Farm, Taiwan **Investigator:** Gin Wu, DVM
Animal: Landrace hybrid pigs
Age: 21 days (at beginning of weaning)
Period: 10/13/09 to 10/21/09

Objective: Pilot study to assess whether use of Grazix solution at weaning can affect weight gain in healthy pigs.

Method: Piglets were segregated into two groups, with one receiving Grazix solution once per day and those in the other group (controls) did not receive any additional supplement. Piglets in both groups were fed with milk replacement only.

Result: At the beginning of the study, the mean weight of piglets that had received Grazix solution was 3% less than the mean weight of those in the control group; however, by the end of the study the mean weight of pigs fed Grazix solution had increased such that there was only a 0.5% difference between the two groups. As a result, the net weight gain for those receiving Grazix was 18% more than the net weight gain of control pigs.

	Test Group (Received Grazix)	Control Group (No Grazix Provided)
No. of Piglets	53	46
Serving size	7.5 µg (1X 15 cc.)	0 µg
Frequency	Once per day	None
Testing period	8 days	8 days
Mean wt. at beginning	7.00 kg	7.22 kg
Mean wt. at the end	8.81 kg	8.76 kg
Mean weight gain	1.81 kg	1.54kg

Conclusion: This study indicated that consumption of Grazix in milk replacer could improve weight gain during high stress periods.

II. Weaning Pig Mortality Reduction Test, Malaysia

Investigator: Kooi Cheng Teo, DVM, PhD PeterLabs, SDN
Site: Local Farm, IPOH Malaysia
Animal: Landrace/local cross pigs
Age: newborn until weaning
Period: November 2009 (weaning at 25 to 28 days after birth)

Objective: to contrast mortality rates of piglets consuming Grazix solution with those not provided with the solution from birth until weaning

Method: Newborn piglets from seven sows (a total of 68 piglets) received 1 mL of Grazix via oral pumps on day 1 of their life and then at the first incidence of diarrhea received 3 mL of Grazix for 5 days; piglets from three sows (25 piglets) received the current treatment regime for this farm, injections of enrofloxacin (a fluoroquinolone carboxylic acid derivative) and lincospectin (lincomycin HCl), when they presented with diarrhea, to serve as controls. Measurement of diarrhea was on a general scale of mild, moderate and severe.

	T1	C2	T3	T4	C5	T6	T7	T8	C9	T10
Sow ID	LY325	LY421	LY386	LY521	LY410	LY350	LD418	LY385	LD430	LY460
Farrow date	2/11	3/11	3/11	3/11	4/11	4/11	4/11	5/11	6/11	6/11
Parity	6	3	4	2	3	5	6	4	3	2
Born alive	10	8	10	7	10	9	12	11	7	9
1st-7th day GI *	++	+++	++	+	++	+	+	+	++	+
8th-14 day GI	+	+	+	_	+	_	+	+	+	+
Mortality	0	1	1	0	2	1	0	1	0	0

This study was performed with an early Grazix formulation and the estimate of dosage was based on dry weight equivalent of the ingredients. The Grazix solution was diluted in farm water (1 part Grazix, 7 parts water) and placed in the hand-held pump. Each activation of the pump delivered 1mL of the mixture (comprising of ~ 150 µg of Grazix active ingredients); 3 pumps delivered 3 mL of the mixture (comprising ~ 450 µg of active ingredients).

Results: The mortality of the piglets fed Grazix was 6%, while 12% of piglets in the control group died before they were weaned—a 50% reduction in the mortality rate for these suckling pigs. In the first week of the study, 8% to 10% of the piglets died; in contrast, in that same time period only 3% of the piglets receiving Grazix died. In addition, piglets fed Grazix were ranked as having only " very mild" diarrhea while those in the control group were ranked as having "severe" diarrhea.

Conclusion: This study indicates Grazix can reduce scours and lower mortality of neonatal piglets

III. GRAZIX Effect on Growth Performance of Post-Wean Pigs, Hungary

Investigator: Bela Denes, DVM, PhD
Szint Istvan University
Budapest, Hungary
Site: Csanyi Farm, Bugyi, Hungary
Animal: Landrace hybrid pigs
Age: ≈ 28 days at the start of the monitoring period
Period: 12/6/09 – 1/14/10
Duration: 35 days

Objective: to measure growth performance in weaned piglets provided with daily consumption of Grazix

Method: Thirty (30) pigs were segregated into two groups, 22 pigs received Grazix solution and 8 pigs did not receive Grazix, in a block design to avoid cross-contamination. All pigs were provided with the standard diet designated for the European Union, but those in the one group were given Grazix (8.8 mL of the solution diluted in 6.6 L of water) provided in the water supply 30 minutes before and 90 minutes after feeding for 35 days. Each was weighed individually daily.

Results: Piglets receiving Grazix solution grew larger than controls—the Average Daily Gain over the 35 day period was 0.230 kg/day (std dev= 0.062) for those in the Grazix group versus 0.183 kg/day (std dev=0.037) for controls (p=0.05).At the end of the 35 days, pigs that had consumed Grazix solution had a an average daily gain per kg baseline weight of 0.0309kg vs 0.0282kg for control.

Conclusion: When a standard diet is supplemented with Grazix solution, a significant increase of 9.6% in average daily weight gain/kg baseline weight was recorded over the test period was recorded over 35 days post weaning.

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Hungary Field Study 12-14-09 to 1-18-10

Weight in kilograms

	Piglet #	Baseline	Week 1	ΔWk1-B	wk1ADG	Week 2	ΔWk2-B	wk2ADG	Week 3	ΔWk3-B	wk3ADG	Week 4	ΔWk4-B	wk4ADG	Week 5	Δ Wk5-B	wk5ADG	%Δ/B	35day ADG
CONTROL	40921	6.00	7.02	1.02	0.15	8.28	2.28	0.18	9.32	3.32	0.15	11.25	5.25	0.28	13.12	7.12	0.27	219%	0.203
	40922	6.86	7.44	0.58	0.08	9.26	2.40	0.26	10.07	3.21	0.12	12.05	5.19	0.28	14.15	7.29	0.30	206%	0.208
	40924	7.00	8.06	1.06	0.15	8.94	1.94	0.13	10.1	3.10	0.17	11.75	4.75	0.24	13.26	6.26	0.22	189%	0.179
	40925	6.16	6.80	0.64	0.09	8.02	1.86	0.17	9.75	3.59	0.25	10.36	4.20	0.09	11.45	5.29	0.16	186%	0.151
	40926	7.00	8.80	1.80	0.26	9.90	2.90	0.16	11.06	4.06	0.17	13.25	6.25	0.31	15.35	8.35	0.30	219%	0.239
	40927	6.52	7.08	0.56	0.08	9.04	2.52	0.28	10.08	3.56	0.15	11.75	5.23	0.24	13.6	7.08	0.26	209%	0.202
	40929	6.84	8.12	1.28	0.18	9.04	2.20	0.13	10.17	3.33	0.16	11.1	4.26	0.13	12.15	5.31	0.15	178%	0.152
	40930	5.20	6.084	0.88	0.13	6.76	1.56	0.10	7.65	2.45	0.13	8.1	2.90	0.06	9.75	4.55	0.24	188%	0.130
	Mean	6.448	7.426	0.978	0.140	8.655	2.208	0.176	9.775	3.328	0.160	11.201	4.754	0.204	12.854	6.406	0.236	199%	0.183
	Std Dev	0.630	0.866	0.419	0.060	0.958	0.419	0.064	0.987	0.462	0.040	1.505	0.992	0.095	1.724	1.278	0.059	0.1602	0.037
	n	8	8	8		8	8		8	8		8	8		8	8		8	
RECEIVED GRAZIX	40931	6.24	7.22	0.98	0.14	8.24	2.00	0.15	9.07	2.83	0.12	11.35	5.11	0.33	13.15	6.91	0.26	211%	0.197
	40933	8.22	10.24	2.02	0.29	12.24	4.02	0.29	13.16	4.94	0.13	16.4	8.18	0.46	18.35	10.13	0.28	223%	0.289
	40934	6.34	6.44	0.10	0.01	8.14	1.80	0.24	9.18	2.84	0.15	11.2	4.86	0.29	13.4	7.06	0.31	211%	0.202
	40935	8.48	10.32	1.84	0.26	12	3.52	0.24	13.76	5.28	0.25	15.2	6.72	0.21	17.1	8.62	0.27	202%	0.246
	40936	7.24	8.22	0.98	0.14	12.64	5.40	0.63	13.85	6.61	0.17	16.95	9.71	0.44	19.05	11.81	0.30	263%	0.337
	40937	7.22	9.10	1.88	0.27	9.30	2.08	0.03	10.6	3.38	0.19	13.45	6.23	0.41	15.05	7.83	0.23	208%	0.224
	40939	7.00	8.36	1.36	0.19	7.66	0.66	-0.10	8.7	1.70	0.15	10.1	3.10	0.20	11.35	4.35	0.18	162%	0.124
	40940	5.28	7.12	1.84	0.26	9.70	4.42	0.37	10.27	4.99	0.08	12.25	6.97	0.28	13.05	7.77	0.11	247%	0.222
	40952	8.78	11.12	2.34	0.33	11.18	2.40	0.01	13.02	4.24	0.26	16.1	7.32	0.44	17.05	8.27	0.14	194%	0.236
	40953	7.50	9.44	1.94	0.28	11.38	3.88	0.28	12.24	4.74	0.12	13.05	5.55	0.12	14.1	6.60	0.15	188%	0.189
	40954	9.20	11.34	2.14	0.31	12.78	3.58	0.21	13.92	4.72	0.16	14.35	5.15	0.06	16.25	7.05	0.27	177%	0.201
	40955	9.68	12.00	2.32	0.33	12.98	3.30	0.14	14.42	4.74	0.21	16.85	7.17	0.35	18.3	8.62	0.21	189%	0.246
	40959	6.56	8.24	1.68	0.24	9.18	2.62	0.13	10.18	3.62	0.14	10.85	4.29	0.10	11.75	5.19	0.13	179%	0.148
	40960	6.52	8.08	1.56	0.22	12.98	6.46	0.70	15.44	8.92	0.35	18.15	11.63	0.39	20.05	13.53	0.27	308%	0.387
	40941	5.12	6.10	0.98	0.14	7.36	2.24	0.18	8.36	3.24	0.14	9.85	4.73	0.21	10.9	5.78	0.15	213%	0.165
	40942	9.90	11.40	1.50	0.21	14.04	4.14	0.38	15.54	5.64	0.21	18.2	8.30	0.38	20.1	10.20	0.27	203%	0.291
	40943	7.26	8.34	1.08	0.15	9.50	2.24	0.17	10.44	3.18	0.13	11.75	4.49	0.19	13.05	5.79	0.19	180%	0.165
	40944	7.16	8.48	1.32	0.19	9.26	2.10	0.11	10.52	3.36	0.18	12.1	4.94	0.23	13.95	6.79	0.26	195%	0.194
	40945	7.98	9.12	1.14	0.16	10.22	2.24	0.16	11.76	3.78	0.22	13.9	5.92	0.31	15.8	7.82	0.27	198%	0.223
	40948	6.54	8.24	1.70	0.24	9.28	2.74	0.15	10.1	3.56	0.12	12.25	5.71	0.31	14.1	7.56	0.26	216%	0.216
	40949	6.92	8.22	1.30	0.19	10.02	3.10	0.26	10.76	3.84	0.11	14.4	7.48	0.52	16.25	9.33	0.26	235%	0.267
	40950	8.32	10.38	2.06	0.29	12.02	3.70	0.23	13.88	5.56	0.27	16.5	8.18	0.37	18.4	10.08	0.27	221%	0.288
	Mean	7.430	8.978	1.548	0.221	10.550	3.120	0.225	11.780	4.350	0.176	13.873	6.443	0.299	15.480	8.050	0.230	210%	0.230
	Std Dev	1.282	1.635	0.540	0.077	1.954	1.302	0.181	2.227	1.529	0.065	2.607	1.979	0.125	2.811	2.172	0.061	0.3208	0.062
	n	22	22	22		22	22		22	22		22	22		22	22		22	

IV. *E. coli* Challenge Study in Post Weaning Piglets, University of Milan, Italy

Principal Investigator:	Valentino Bontempo, PhD Professor, Dept. of Veterinary Sciences and Technology University of Milan, Italy
Study Site:	University of Milan Animal Research Farm Lodi, Italy
Study dates:	September, 30 – November 4, 2010
Study duration:	35 days
Sample size:	144
Study design:	Control and test groups Blocked design to avoid cross contamination Standard EU diet and care
Challenge:	*E. coli* 0149:F4(K88)
Amount:	10^9 cfu
Challenge day:	Day 9
GRAZIX serving:	Day 1-7 8µL/kg BW Day 8-15 200µL/kg BW Day 9-35 8µL/kg BW

Objective: to evaluate the effects of Grazix on the performance and health of weaned piglets fed a mixed diet and then challenged with *E. coli*

Method: At weaning, a total of 144 piglets were allocated to two post-weaning rooms; half of the piglets received Grazix in their water, the other half did not (control). On day 9 of the trial, half of the piglets were injected orally with a 4 mL solution containing 10^9 colony-forming units of *E. coli*. The piglets' growth performance and fecal scores were recorded weekly. On days 0, 14, and 35, fecal samples were collected for microbiological analysis, while on days 0, 6, 19, and 35, blood samples were obtained from one pig per pen. At the end of the trial (day 35), 24 animals (12 from the control group and 12 from the Grazix group) were slaughtered and their distal ileum collected and examined in order to assess the ileum micro-anatomical structure, to perform histometry and immunohistochemistry, and to measure intestinal inflammatory parameters.

Results: When the data were analyzed, it was noted that piglets given the Grazix solution had an increased average daily gain during the last week of the study (p=0.007) and reduced feed conversion rate during the second (p=0.009) and last weeks (p=0.04), and over the entire study period (p=0.01) when compared to piglets in the control group. Also a lower fecal score was observed in the piglets given Grazix (p<0.01). On day 35, fecal *E. coli* and *Entrobacteriaceae* concentrations were lower in animals given Grazix when compared to controls (p=0.02 and p=0.009, respectively). Ileum crypts from piglets given Grazix were deeper in *E. coli* challenged animals than in non-challenged ones (p<0.05), while the number of mucosal macrophages was higher in control piglets challenged with *E. coli* (p<0.05). The number of mucosal macrophages present in piglets given Grazix and then challenged with *E. coli* was comparable to the number present in piglets that were not exposed to *E. coli*. Use of Grazix increased glutathione peroxidase plasma concentration at day 6 (p=0.02), lowered malondialdehyde value at day 6 (p=0.07), and increased total antioxygenic capability value at the end of the trial (p=0.07). The use of plant extracts may be beneficial in the prevention of post-weaning diarrhea, with an associated improvement in performance.

	Weight (kg)				Ave. Daily Gain (g/d)		
	Control	**Grazix**	**p**		**Control**	**Grazix**	**p**
Day 0	6.63	6.53	0.83	Day 0-7	74.03	67.7	0.08
Day 7	7.15	7	0.48	Day 7-14	165.6	184.1	0.47
Day 14	8.31	8.29	0.76	Day 14-21	281.3	307.4	0.96
Day 21	10.28	10.44	0.81	Day 21-28	360.3	353.1	0.72
Day 28	12.8	12.91	0.95	Day 28-35	411.4	499.2	**0.007**
Day 35	15.68	16.41	0.42	Day 0-35	258.6	282.3	ns
	Ave Daily Feed Intake				**eed Conversion Rate (FCR)**		
	Control	**Grazix**	**p**		**Control**	**Grazix**	**p**
Day 0-7	491	406	0.19	Day 0-7	2.7	2.13	0.36
Day 7-14*	1005	931.3	0.44	Day 7-14	2.26	1.75	**0.009**
Day 14-21	1427	1416	0.91	Day 14-21	2.41	1.59	0.46
Day 21-28	1790	1840	0.96	Day 21-28	1.73	1.76	0.56
Day 28-35	2062	2330	0.28	Day 28-35	1.69	1.59	**0.04**
Day 0-35	1355	1385	0.87	Day 0-35	1.78	1.65	**0.01**

* E. coli challenge on Day 9 **FCR in test pigs was 8% better than controls for the entire study period.**

Fecal Analysis

Fecal Microbiogical Counts (log10cfu/g)			
	Control	**Grazix**	**P**
Lactobacilli			
Day 0	8.23	7.67	0.25
Day 11*	11.55	11.05	0.27
Day 35	8.57	8.67	0.51
Clostridia			
Day 0	6.64	7.07	0.40
Day 11*	2.52	2.21	0.37
Day 35	3.28	2.19	0.17
Enterobacteriaceae			
Day 0	7.57	7.17	0.58
Day 11*	8.9	8.01	0.72
Day 35	6.21	5.28	**0.009**
E. coli			
Day 0	6.13	6.26	0.21
Day 11*	6.94	5.85	0.89
Day 35	4.32	3.69	**0.02**

Scour (diarrhea) scores*

* Scour scored as: 1= Hard, dry pellet; 3 = Soft, moist; or 5 = Watery
** *E. coli* challenge occurred on Day 9

	Control	**Grazix**	**p-value**
Day 0	2.00	2.00	1.00
Day 7	3.00	2.83	**0.04**
Day 14**	4.00	2.67	**0.02**
Day 21	3.33	2.83	**0.001**
Day 28	3.00	2.17	**0.0001**
Day 35	2.83	2.17	**0.0003**

Histometry

Histometry of Illeal tissue			
	Control	**Grazix**	**p**
Villus height (V; µm)	355.12 ± 11.09	374.53 ± 11.09	0.47
Crypt depth (C; µm)	285.52 ± 7.16	305.96 ± 7.16	**0.05**
V:C	1.26 ± 0.04	1.24 ± 0.04	0.92
Total area (μm^2)	441239 ± 40864	403213 ± 40864	0.34
Cortex area (μm^2)	169363 ± 7262	173025 ± 7241	0.27
Medulla area (μm^2)	41634 ± 7939	140353 ± 7923	0.64
Corona area (μm^2)	105092 ± 6847	102799 ± 6833	0.90
Lymphatic Follicles Number/mm^2 of mucosa	1.52 ± 0.10	1.45 ± 0.10	0.94
Macrophages number/mm^2 of mucosa	174.61± 14.80	128.56± 14.80	**0.02**

Conclusion: Consumption of Grazix solution improved growth performance in the last phase of the study. These results were associated with significant reductions in scour scores and in the count of fecal *Enterobacteriaceae* and *E.coli* in the Grazix group. Grazix administration also resulted in a lower crypt depth, suggesting a possible reparative action of the product on the small intestinal mucosa following the challenge with *E. coli*. In addition, the number of mucosal macrophages in test piglets was similar to controls, thus confirming the possible protective functional role of Grazix after the bacterial challenge. It is postulated that Grazix interacts in the intestine with feed components, microbiota, and the mucosa in a very complex and dynamic way. The effect should be greatest under an infectious pressure, such as occurs at certain ages, under certain husbandry conditions, and in certain regions. Use of Grazix may be useful in the prevention of post-weaning diarrhea with an associated improvement in growth performance.

Note: A manuscript for this study has been submitted to the *Journal of Animal Science*. A copy of this manuscript can be provided upon request.

V. Survival Rate in Pigs Infected with Porcine Reproductive and Respiratory Syndrome (PRRS) Virus

Investigator: Jonathan Holt, PhD
Director of Swine Nutrition
Standard Nutrition Services
Sioux Falls, SD
Study Site: Dear Park Farm, Parkston, SD
Study dates: 10/20/11 – 3/16/12
Study duration: 148 days
Sample size: 2000
Age of pigs: 19-23 days

Objective: Evaluate the effect of Grazix supplementation on mortality in PRRS infected post weaning.

Method: Two thousand (2000) weaned pigs, some from PRRS-infected sows, were segregated into two groups: 1000 receiving Grazix solution daily in the water system (test group) and 1000 not receiving the product (control group). All pigs were given the same standard diet, with those in the control group receiving feed additives (*e.g.*, zinc, copper, and probiotics) as needed. Grazix was provided based upon mean body weights of those in the test group at the following levels: at days 1 to 4, 400 µL/kg BW; at days 5 to 35, 16 µL/kg BW, and then from day 36 until animal reaches market weight, 16 µL/kg BW.

Results: During the first 35 days of life, 39 piglets (4%) in the control group died versus 16 (2%) out of the 1000 piglets in the group consuming the Grazix solution. At the end of the time in nursery (day 35), piglets consuming Grazix solution averaged 5 pounds heavier than pigs in the control group and their weights had less variability than controls. As pigs grew, those in the test group reached a mean market weight of 273 pounds ten days earlier than those in the control group, which by time of market had a mean weight of 260 pounds.

Conclusion: Consumption of Grazix solution was associated with approx 50% lower mortality rates in the pre-weaning stage and a reduced time to reach market weight.

VI. Deer Park Nursery Wean to Finish Trial #2 (PRRS Infected)

Investigator: Jonathan Holt, PhD
Director of Swine Nutrition
Standard Nutrition Services
Sioux Falls, SD

Study dates: 11/30/11 – 4/23/12
Study duration: 145 days
Sample size: 2000
Age of pigs: 19-23 days
Prior history: Weaned pigs some from PRRS infected sows
Study design: Control (1000) and test (1000) groups; in-feed antibiotics
Control group given feed additives (zinc, copper, probiotics

GRAZIX dosing:		
	Nursery Day 1 – 4	400ul/kg BW
	Day 5 – 35	16ul/kg BW
	Grow – Finish	None

Objective: Evaluate effect of Grazix supplementation on pre-weaning mortality in PRRS infected herd.

Results:

Nursery

1. Mortality: Controls = 49; Test = 19
2. At end of nursery period (35 days), no difference in weight between control group and test group was noted

Grow-Finish

1. Test pigs reached average market weight of 268 lbs 10 days earlier than controls that reached market weight of 257 lbs.
2. There were no underweight test pigs.
3. Individual feed costs for test pigs was calculated at $2 less than controls
4.

Conclusion: Consumption of Grazix solution was associated with 61% lower nursery mortalities, lack of underweight finished pigs, shorter time to market with improved FCR.

VII. GRAZIX™ for Resolution of Neonatal Scour (pilot study)

Investigator: Deborah Murray, DVM
Chief Veterinarian,
New Fashion Pork (60,000 sows)
Jackson, MN
Farm: Waldorf Sow Farm,

Study dates: May 1 to 15, 2012
Sample size: ≈2500 neonatal pigs
Age: 0-8 days old

Objective: Evaluate the effect of Grazix supplementation on scouring piglets

Method: In this pilot study, there was no control group; every pig in the litters that presented with diarrhea (scour) in the farrowing crate was provided with a serving of Grazix solution. The serving was created by diluting 1 part Grazix solution in 2 parts sterile water and administering the solution orally at a rate of 1 cc for piglets 1 to 4 days old and 2 cc for piglets 5 to 8 days old.

Results: 50% of liters treated demonstrated same day resolution of scour; 50% of scours resolved the next morning. No second administration was required in order for the condition to resolve.

VIII. GRAZIX for Resolution of Neonatal Scour (extended study)

Investigator: Deborah Murray, DVM
Company: New Fashion Pork (60,000 sows)
Jackson, MN
60,000 sows
Farm: Company farm operations in IA, IN, and SD
Study date: July 2012
Sample size: 499 litters (>6,000 neonatal pigs)
Age: 0-15 days old

Objective: Evaluate the effect of Grazix supplementation on scouring piglets

Method: In this follow-on study also there was no control group; every pig in the litters that presented with diarrhea (scour) in the farrowing crate was provided with a serving of Grazix solution. The serving was created by diluting 1 part Grazix solution in 2 parts sterile water and administering the solution orally at a rate of 1 cc for piglets 1 to 4 days old, 2 cc for piglets 5 to 8 days old, and 2 to 5 cc of undiluted Grazix solution, with heavier piglets receiving the higher serving sizes. Servings were repeated if there was no improvement in the condition on the following day. A third serving was not provided.

Results:

GRAZIX in neonatal pigs with scour							
0 - 8 day old pigs							
# litters treated	**recovered same day**	**recovered next day**	**given 2nd dose**	**recovered same day**	**recovered next day**	**did not recover**	**recovered with 1 serving**
437	49	336	78	24	39	15	307
	11%	77%	18%	31%*	50%*	3%	70%
Overall success rate: 97%							
Success rate with a single serving: 83%							
9 - 16 day old pigs							
# litters treated	**recovered same day**	**recovered next day**	**given 2nd dose**	**recovered same day**	**recovered next day**	**did not recover**	**recovered with 1 serving**
62	9	36	17	0	5	8	45
	15%	58%	27%	0%	30%*	13%	73%
Overall success rate: 87%							
Success rate with a single serving: 73%							
* % of litters given 2nd serving							

Conclusion: Oral administration of Grazix solution resolved scour in neonatal pigs more effectively and faster than the current standard of care at these farms (as compared to historical data).

IX. High Health Herd Evaluation of GRAZIX, Immediate Post Weaning Phase

Principal investigator:	Deborah Murray, DVM; Gin Wu, DVM, PhD. LiveLeaf Bioscience
Study Site:	New Fashion Pork Research Nursery Buffalo Center, IA
Study dates:	7/15/11 – 8/8/11
Study Duration:	24 days
Sample size:	1008
Age of pigs:	19 – 23 days
Study design:	Control (N=504) and Test (N=504) group Control group provided Antibiotics in feed plus water additive with essential oils, & probiotics. Test group provided Grazix only through water medicator
Grazix dose:	Day 1 – 4 400ul/kg Day 5 - 10 16ul/kg BW Day 11- 12 200ul/kg Day 12 – 24 16ul/kg BW

Objective: Evaluate the effect of Grazix supplementation on high health herds.

Results:

Treatments	Grazix	None
Pens	18	18
No. of Pigs	504	504
Beginning wt., lb.	11.5	11.7
Period 1 (0 to 3d)		
ADG	0.14	0.14
ADFI	0.16	0.15
FG	1.21	1.26
End wt., lb.	11.91	12.12
Gain	0.42	0.42
Individual treatments	0	0
Deads & Culls	0d,0c	0d,0c
Period 2 (3 to 10d)		
ADG	0.51	0.52
ADFI	0.61	0.61
FG	1.20	1.17
End wt., lb.	15.47	15.77
Gain	3.56	3.65

Individual treatments	1	2
Deads & Culls	0d,0c	0d,0c
Period 3 (10 to 17d)		
ADG	0.91	0.95
ADFI	1.11	1.14
FG	1.23	1.21
End wt., lb.	21.85	22.40
Gain	6.38	6.64
Individual treatments	3	3
Deads & Culls	0d,1c	2d,5c
Period 4 (17 to 24d)		
ADG	1.23	1.26
ADFI	1.62	1.67
FG	1.33	1.32
End wt., lb.	30.44	31.26
Gain	8.59	8.80
Individual treatments	0	0
Deads & Culls	0d,2c	0d,0c
Ovll. (0 to 24d)		
ADG	0.79	0.81
ADFI	0.99	1.01
FG	1.26	1.24
Gain	18.95	19.56
Individual treatments	4	5
Deads & Culls	0d,3c	2d,5c

Scour Scores						
% Scours	**0 = no Scours**	**1 = 10%**	**2 = 25%**	**3 = 50%**	**4 = 75%**	**5 = 100% Severe**
Day 3 to 10: Grazix	51.85%	28.70%	12.04%	6.48%	0.93%	0%
Day 3 to 10: Control	39.81%	31.48%	14.81%	8.33%	0%	0%
Day 10 to 17: Grazix	86.51%	13.49%	0%	0%	0%	0%
Day 10 to 17: Control	84.92%	15.08%	0%	0%	0%	0%
Day 17 to 24: Grazix	81.75%	18.25%	0%	0%	0%	0%
Day 17 to 24: Control	75.40%	24.60%	0%	0%	0%	0%

Between day 3 and day 10, 9 pigs with severe scours were isolated in a treatment pen. 5 were given a single treatment (400ul/kg BW) dose of GRAZIX. Scour resolved within 24 hrs. 4 were given SULFATRIM IM daily for 2 days before scour resolved on day 3.

Conclusions:

1. No difference in growth/feed performance between groups were noted.
2. Scour in pigs given a single oral rescue dose of GRAZIX resolved faster than that in pigs given antibiotics
3. During the first phase of the trial (day 0-10) test pigs were observed to have lesser incidence and lower severity of scour than controls.

In high health research nursery, Grazix effectively reduced digestive distress in the period immediately following transport from weaning facility.

VIII. Neonatal Pig trials in 20 Dutch Sow Farms: Comparison of Mortality Between Antibiotics and GRAZIX

Principal Investigator: Sam DeSnoeck, DVM
Lintjeshof Veterinary Practice
Nederweert, The Netherlands
Study Sites: 20 Sow Farms in The Netherlands
Study dates: January 2 to June 30, 2012
Study duration: 6 weeks ~ 3 months
Sample size: 43,745 neonatal piglets
Age of pigs: followed from birth to 28 days

Objective: To evaluate Grazix viability as an antibiotic alternative in European commercial farms

Method: In The Netherlands, 20 farms (units ranging from 400 to 1000 sows) were monitored for use of antibiotics for scour over 6 weeks to 3 months and the incidence of scour and mortality noted for piglets prior to weaning. After that time period, these same farms administered the Grazix solution to individual piglets upon first observation of scouring in the farrowing crate and monitored for an identical 6 weeks to 3 months. Piglets received a volume of the Grazix solution mixed in water (1 part Grazix solution in 4 parts demineralized water) when scour was noted based upon their age: 2 cc of the diluted solution was provided for piglets 1 to 8 days of age; 4 cc of the solution if the piglets were 9 to 14 days old; and 6 cc of the mixture for piglets 15 to 21 days old. If required, a repeat application of the solution was provided 6 to 8 hours after the initial administration. The Grazix solution was only administered on one day and standard EU diet and husbandry practices were followed for the remaining of the time monitored.

Results: Use of **Grazix solution was associated with a reduction in mortality from 21% to 11% (*p*<0.0001)** in these farms during the time periods monitored. Mortality of piglets was mainly due to incidence of unresolved scour. It was determined that by reducing scour duration with consumption of the Grazix solution, these farms also experienced a **reduction in the incidence of subsequent enteric infections by 75% and nonenteric infections by 50%.**

Mortality in neonatal pigs treated with antibiotics and with GRAZIX

Farm	# Sows	Study Duration per tx	Primary Infecting Pathogen	Antibiotics* (abx)	Treatment** Groups (n)		Mortality	
					abx only	GRAZIX only	abx	GRAZIX***
HOOH7	380	6 wk	C. perfringens Rotavirus haem E. coli	Amocolint	1183	1183	22%	12%
SANM7	850	2 mo	C. perfringens haem E. coli	Amocolint Colistine	1516	1455	20%	11%
PLOP7	1500	6 wk	haem E. coli	Amocolint Marbocyl	1558	1401	18%	11%
LEMB10	285	2 mo	C. perfringens haem E. coli	Marbocyl	133	177	19%	12%
BOMK3	700	3 mo	C. perfringens Rotavirus	Amocolint Marbocyl	3230	3200	22%	10%
VERZ1A	500	3 mo	C. perfringens Rotavirus	Amocolint Marbocyl	2200	2210	25%	11%
GORH1	800	6 wk	C. perfringens	Neopen	496	621	22%	11%

JASK3	500	3 mo	C. perfringens	Trimsulint	555	670	23%	12%
CILH24	400	3 mo	C. perfringens Rotavirus haem E. coli	Amocolint Electrolytes	1998	1889	22%	10%
BALB2	550	3 mo	C. perfringens	Trimsulint	481	603	23%	11%
COEG65	150	6 wk	C. perfringens	Amocolint	488	467	19%	9%
SCHB36	450	2 mo	C. perfringens haem E. coli	Amocolint	577	665	22%	9%
JURK1	800	6 wk	Rotavirus	Marbocyl Electolytes	2500	2333	26%	12%
KRUK3	720	6 wk	C. perfringens	Marbocyl	689	809	22%	11%
JONP4	900	3 mo	C. perfringens haem E. coli	Marbocyl	679	688	23%	12%
KURS16	350	6 wk	haem E. coli	Amocolint	300	344	19%	8%
KOPO11	330	3 mo	C. perfringens	Pen30	409	388	22%	12%
MILL6	830	3 mo	haem E. coli	Amocolint	311	345	19%	8%
VERG4	450	6 wk	C. perfringens	Neopen	1499	1525	22%	11%
LOVP7	630	2 mo	haem E. coli	Amocolint	915	1055	17%	11%
*** Antibiotics**					**Total**		**Mean**	
Amocolint = Amoxycilline + Colistine					21,717	22,028	**21%**	**11%**
Marbocyl = Marbofloxacine (quinolone)					**43,745**		**p<0.0001**	
Neopen = Neomycine + Penicilline								
Pen30 = Procaine Penicilline								
Trimsulint = Trim Sulfa product								

IX. Grazix Solution to Address Scour in Neonatal Pigs

Investigator: Tara Donovan, DVM (President of AASV)
Company: Hanor Company of Wisconsin, LLC
Spring Green, WI
Study site: Shellbank Sow Farm, Brattleboro, NC

Objective: Compare effect of consumption of Grazix to address scour versus use of an off-label antibiotic agent

Method: At a single commercial production farm, 80 litters (with over 1000 neonatal piglets) were divided into two groups. Farrowing crates on the left side of the farrowing room were designated as controls and the antibiotic agents bacitracin methylene disalicylate (BMD) or Marquis™ were provided at the first presentation of scour. Typically BMD is used as a 3 day treatment but may be limited to 2 days. Marquis is an equine anti-coccidial antibiotic agent that is often used in production pig farms. Crates on the right side of the farrowing room were designated as test groups and Grazix solution was administered orally once in piglets less than 7 days old at a serving of 1 cc of a mixture of 1 part Grazix in 2 parts sterile water.

Feces were ranked as either 0 (typical feces), 1 (soft, pudding-like feces with the animal's hindquarters remaining clean), 3 (fluid feces with hindquarters dirty but the animal remaining active), or 3 (fluid feces with dirty hindquarters and animals emaciated).

Results:

:

Treatment	# litters treated	Mean Scour Score	Recovered 6-8hrs		Recovered 24hrs		Overall Efficacy
			#	%	#	%	
GRAZIX*	70	1.81	38	54%	14	20%	74%
BMD**	25	1.96	6	24%	7	28%	52%
Marquis***	5	1.4	2	40%	1	20%	60%

* administered only once
** standard treatment is daily for 3 days
*** equine antibiotic used for cocciciosis

Conclusion: Grazix provided a level of scour relief that was faster and more effective than the current antibiotic standards of care used in this farm.

X. Organic Poultry Farm Test, Taiwan

Location : Central Taiwan
Owner: Privately owned commercial poultry farm
Type of farm: Pilot organic production, free range in a open fenced field.
Breed: French naked neck chicken (originated from Hungary and cross bred in France)
Dosage: Low dose 0.045 µg/kg,
Mid dose 0.45 µg/kg,
High dose 4.5 µg/kg (based on total polyphenol concentration 0.9 mg/ml, dry weight 3.0mg/ml)
Dosed once every three days in bell water medicator.
Test started: 4/23/2010

Objective: Pilot feasibility study on the use of Grazix on poultry in the absence of antibiotics

	Group I (0.1x Target dose)				**Group II (Target dose)**				**Group III (10 X target dose)**			
Starting No.	100				100				100			
Dosage	0.045 µg/kg				0.45 µg/kg				4.5 µg/kg			
	Survival	**Weight**	**Diarrhea**	**Mortality**	**Survival**	**Weight**	**Diarrhea**	**Mortality**	**Survival**	**Weight**	**Diarrhea**	**Mortality**
2nd Week	95	—	—	5	97	—	—	3	98	—	—	2
4th Week	85	**0.61 kg**	1	10	93	**0.675kg**	0	4	92	**0.665 kg**	0	6
6th Week	85				93				92			
	Minor blood in feces of a few chickens in Group II (coccidiosis?) on 6th week, treated with 2 X Target dose recovered in 2 days											
10th Week		**1.5 kg**				**1.75 kg**				**1.55 kg**		
	One dead was trampled during feeding. Some healthy ones died due to fighting (mixed population of male & female)											
12th Week		**2.04 kg**				**2.105 kg**				**2.06 kg**		
FCR	**2.45**				**2.30**				**2.36**			

Results:

- Reducing dose frequency of group II & III from every 3 days to once during week 8 resulted in soft stool. Firmed on
- return to original timing week 9
- Reported historic average survival with antibiotics was 94%. Historic average survival for the first 3 week in untreated chickens was ~ 80%
- All test groups were given Grazix without antibiotics.
- Control group I was intentional sub-effective low dose for control. Birds always showed soft stool, notable listlessness and wider growth variation.

Conclusions: **Group II and III showed approx. 50% reduction in mortality in the first 4 weeks over Group I Controls** and reported historic untreated averages. Optimal feed conversion ratio(FCR) was obtained in Group II, with Group III showing possible signs of anti-nutritive effects at high dose, however, lack of fecal blood in this group indicated probable improved intestinal protection as indicated by fecal blood resolution with temporary doubling of dose to Group II. **Grazix Group II provided comparable survival rate to historic levels using antibiotics.**

XI. Grazix-B Pilot Study on Scour and Mortality From Multi-Factor Cause in Newborn Calves

Investigator: Brian Dorsey, DVM
Company: Veterinary Medical Center
Worthington, MN
Study Date: 11/2014
Study site: Client Farm (name withheld)
Animals: 50 Newborn Jersey calves

Objective: Evaluation of performance in a production operation experiencing severe clinical diarrhea and mortality in multi-sourced calves where antibiotic therapy and colostrum supplements were both tried with very limited success.

Method: Animals – Newborn Jersey Calves were used in this study. The calves were born over a three day period and placed in hutches. 70% of the animals were colostrum deficient. Diagnostic results indicated the animals were positive for Bovine Rota Virus, Corona Virus, and Cryptosporidium Parvium.

The animals, born over a three day period,randomly divided into two groups of 25 calves each ,. All calves received colostrum replacement and egg antibody. Group 1 were assigned as controls and Group 2 were given Grazix B in milk replacer according to label directions. Clinical signs were observed while diarrhea scores were given. Antibiotic treatment as a result of clinical symptoms and mortality were recorded.

Results: Antibiotic treatment was administered a total of 26 times to Group 1 versus 19 times in Group 2. Greater than 80% of the antibiotic treatments for both groups were given in the first 12 days. 6/25 (24%) of the calves in Group 1 died versus 2/25 (8%) of the calves in Group 2.

Conclusions: Animals in both groups were infected with Rota and Corona virus as well as Cryptosporidum upon arrival. Bacteriology was not performed, but pathogenic Ecoli was also thought to be involved. Calves in both groups were also colostrum deprived, making them more susceptible to infection. The above factors also seem consistent with the administration of the antibiotic within the first two weeks. Calves in the Group 2 needed less antibiotic treatments and had 16% less mortality than Group 1 animals. **Grazix B had a dramatic effect in reducing the clinical disease present in these animals. The Grazix B group were more robust and healthy overall. Factoring in the cost of the deads , antibiotic treatment and costs of Grazix B there was a greater than 10:1 return by using the product**.