ASEA Ingestion, Safety Summary from Human Studies
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INTRODUCTION

Several randomized, placebo-controlled, double blind studies with human subjects have been conducted at the North Carolina Research Campus (NCRC). No adverse symptoms have been reported by subjects ingesting 4 fl. oz. ASEA per day for two to 12 weeks.

This report will focus on data collected on 106 overweight women who ingested 4 fl. oz. ASEA per day for 12 weeks. No adverse symptoms or negative changes in diagnostic chemistries were measured. Four stool samples were also collected from 24 athletes, and the data indicate no effect of 2-weeks ASEA ingestion on microbiome diversity.

HUMAN COMMUNITY TRIAL

This randomized, placebo-controlled study showed that ASEA ingestion by overweight women (4 fl. oz./day) for 12 weeks was safe and not associated with adverse symptoms. All study procedures were conducted under the auspices of the Appalachian State University Institutional Review Board (IRB) for Human Studies. The placebo beverage contained the pure saline solution as found in ASEA, but without catalytic processing.

A total of 106 overweight women (ages 20 to 73 years) ingested 4 fl. oz. of ASEA or placebo (randomized groups) each day for 12 weeks under double blind conditions. Subject characteristics are summarized in Table 1. The data indicate a high degree of obesity (72%) and disease risk factors.

Table 1 **Subject characteristics for entire group (N=106)**

Variable	Mean	SD	Categories	Percentiles
Age (yrs) (20-73)	50	12.1	<50	40%
			≥50	60%
BMI (kg/m^2)	33.9	6.1	<30	28%
			30-39.9	56%
			≥40	16%
Systolic BP (mm Hg)	125	16.2	<120	34%
			120-139	54%
			≥140	12%
Cholesterol (mg/dL)	201	39.8	<200	52%
			200-239	33%
			≥240	15%
C-Reactive Protein (mg/L)	6.42	7.08	<1.0	12%
			1-2.9	29%
			≥3.0	59%
Glucose (mg/dL)	96.4	19.3	<100	74%
			100-125	22%
			≥126	4%

Subjects ingested two 2 fl. oz. doses each day, with one dose in the morning and the other in the evening. Subjects completed 4-week retrospective symptom logs pre-study, and then at 4, 8, and 12 weeks. (See the symptom log in Appendix A). Table 2 indicates that no significant group differences over time were measured.

Table 2 Symptom Log Data	ASEA				PLACEBO				Interaction
	Pre	1-mo	1-mo	3-mo	Pre	1-mo	1-mo	3-mo	P-value
Constipation	1.8	1.5	1.5	1.4	1.7	1.8	1.9	1.9	0.276
Heartburn	2.3	1.6	1.5	1.6	1.6	1.4	1.5	1.9	0.076
Bloating	2.1	1.7	1.5	1.6	1.8	1.5	1.8	1.9	0.247
Diarrhea	1.4	1.5	1.3	1.3	1.4	1.3	1.5	1.3	0.575
Nausea	1.1	1.2	1.1	1.2	1.3	1.2	1.3	1.1	0.261
Hunger, Morning	4.4	3.2	3.6	3.9	5.0	3.4	4.1	3.8	0.465
Hunger, Afternoon	5.1	3.6	4.2	4.2	5.1	3.5	4.6	4.2	0.654
Hunger, Evening	5.4	3.7	4.4	4.5	5.8	4.0	5.2	4.5	0.534
Energy, Morning	6.2	5.3	5.5	5.8	6.1	5.2	5.8	5.7	0.714
Energy, Afternoon	6.1	5.4	5.4	5.7	6.1	5.3	5.9	5.6	0.394
Energy, Evening	5.5	5.2	5.0	5.3	5.4	5.0	5.4	5.3	0.579
Fever	1.1	1.3	1.1	1.0	1.0	1.1	1.2	1.3	0.082
Cough	1.8	1.9	1.6	1.3	1.9	2.2	1.9	1.8	0.738
Throat	1.3	1.7	1.3	1.2	1.1	1.8	1.8	1.5	0.194
Stuffy	2.0	2.1	1.7	1.6	2.1	1.9	2.2	2.0	0.545
Runny	2.1	2.6	2.1	1.8	2.2	2.0	2.3	2.0	0.233
Headache	1.9	1.8	1.7	1.4	2.3	2.2	1.9	2.0	0.649
Joint	2.8	2.4	2.2	3.0	3.1	1.9	2.4	3.0	0.106
Muscle	2.3	2.1	1.8	2.1	2.3	1.4	1.7	2.1	0.069
Back	3.0	2.5	1.9	2.6	2.5	1.5	1.7	1.8	0.101
Allergy	2.5	2.0	2.5	3.2	2.8	2.0	2.9	3.2	0.798
Stress	4.5	3.9	3.9	4.3	4.8	4.1	4.9	4.7	0.223
Focus	7.3	5.8	6.4	6.4	7.6	5.3	7.0	6.6	0.310
Overall	8.6	6.8	7.6	8.0	8.3	7.1	8.0	7.3	0.190

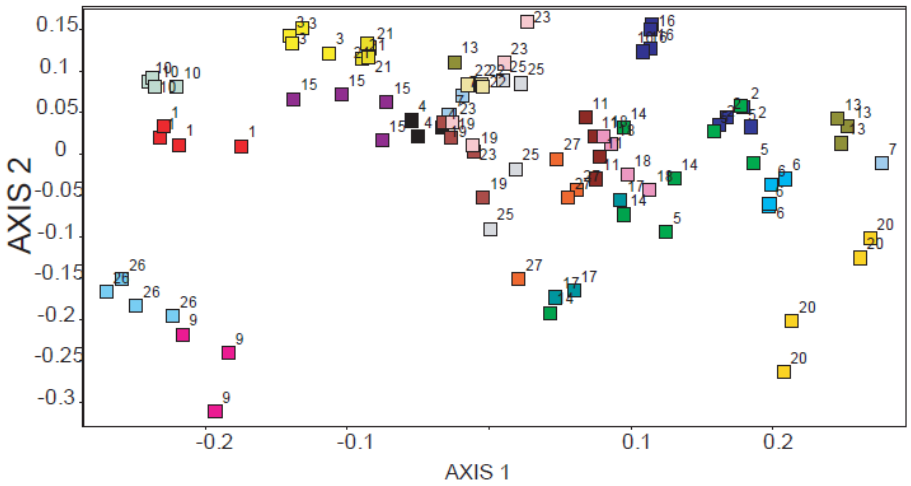
Blood samples were collected pre-study and then monthly during the study, and analyzed for comprehensive diagnostic chemistries at the Carolina Medical Center Clinical Laboratory (Charlotte, North Carolina). ASEA compared to placebo ingestion over 12 weeks was not associated with changes in liver and kidney function in these subjects (Table 3). A slight decrease in bilirubin was measured in the ASEA group, but values were still in the normal range (0.3 to 1.9 mg/dL). Complete blood counts (CBC) were measured pre- and post-study, and showed no group differences over time for hemoglobin, hematocrit, and red blood cell counts.

Table 3 Diagnostic Chemistries and CBC	ASEA				Placebo				Interaction
SERUM	Pre-Study	1-mo	2-mo	3-mo	Pre-Study	1-mo	2-mo	3-mo	P-Value
Sodium (mEq/L)	139	139	139	138	139	139	139	139	0.79
Blood Urea Nitrogen (mg/dL)	11.00	11.30	11.10	11.20	11.40	12.00	11.60	11.70	0.911
Creatinine (mg/dL)	0.80	0.78	0.79	0.77	0.77	0.78	0.79	0.76	0.418
Albumin (g/dL)	4.00	3.87	3.85	3.86	4.01	3.92	3.89	3.92	0.613
Bilirubin (mg/dL)	0.62	0.58	0.46	0.52	0.57	0.56	0.51	0.58	0.043
Alkaline Phosphatase (IU/L)	72.30	71.60	73.70	70.50	68.00	66.80	67.00	66.70	0.276
Aspartate Aminotransferase (U/L) (SGOT)	20.90	21.00	22.20	22.10	21.60	21.40	22.00	23.80	0.328
Alanine Aminotransferase (U/L) (SGPT)	20.70	22.10	22.50	20.30	20.50	20.60	21.60	20.90	0.16
Calcium (mg/dL)	9.38	9.27	9.22	9.16	9.34	9.32	9.27	9.21	0.212
Hemoglobin (g/L)	14.00			13.90	13.90			13.60	0.591
Hematocrit (%)	42.00			41.90	41.60			41.10	0.497
Red Blood Cells (10^6/L)	4.79			4.81	4.67			4.62	0.389

STOOL MICROBIAL COMMUNITY COMPOSITION

Twenty-four endurance runners consumed 4 fl. oz./day ASEA or placebo (randomized, crossover) for two weeks prior to running on treadmills to exhaustion (with intensity clamped at 70% VO_{2max}). Runners provided four stool samples (before and after 2-weeks supplementation with ASEA or placebo). Fecal sample analysis was coordinated under subcontract with Dr. Anthony Fodor of UNC-Charlotte. Microbial DNA was isolated, PCR was performed targeting the 16S rRNA gene, and the microbial community was analyzed with high-throughput Illumina sequencing. Statistical analysis revealed no effect of ASEA ingestion on the microbial community composition (see Figure 1).

Figure 1 Stool sample analysis for microbial community composition. *Data indicate a tight cluster of the 4 stool samples for each subject (2 stool samples taken pre- and post-ASEA and placebo ingestion).*



SUMMARY: Monthly symptom log and blood diagnostic chemistries data obtained from a randomized, placebo-controlled 12-week study conducted under double blind methods with 106 overweight/obese adult females indicated that ASEA ingestion (4 fl. oz. per day, split dose) was safe and no different from placebo. Stool data collected from 24 endurance athletes participating in a randomized, placebo-controlled, crossover trial, showed no effect of 2-weeks ASEA ingestion (4 fl. oz. per day) on microbial diversity or the microbial community composition. These data combined with three other human trials conducted at the ASU-NCRC Human Performance Laboratory indicate that ASEA ingestion is safe with no negative outcomes relative to placebo.

APPENDIX A 4-week retrospective symptom log.

ID Number _____ DATE _____

WEEK 0 4 8 12 (circle week of study)

ASEA Symptom LOG

Please place an "X" in the box that best fits the symptoms or feelings you experienced (from 1 to 12 for each) DURING THE PAST 4 WEEKS. One is none at all, 3=low, 6=moderate, 9=high, and 12=very high levels of the symptom or feeling.

INTENSITY OF SYMPTOMS/FEELINGS

		None		Low		Moderate			High				Very High
CATEGORY	**SYMPTOM**	1	2	3	4	5	6	7	8	9	10	11	12
A. Digestive health	Constipation												
	Heartburn												
	Bloating												
	Diarrhea												
	Nausea												
B. Hunger	Morning												
	Afternoon												
	Evening												
C. Energy Level	Morning												
	Afternoon												
	Evening												
D. Symptoms	Fever												
	Cough												
	Sore throat												
	Stuffy nose												
	Runny nose												
	Headache												
E. Pain	Joint pain												
	Muscle pain												
	Back pain												
F. Allergies													
G. Stress level													
H. Focus/Concentration													
I. Overall well-being													

ANSWER AT WEEKS 4, 8, and 12:

1. Did you consume all of the beverage given you during the past 4 weeks (or if you missed one or two days, then caught up by consuming double the amount?

___Yes; ___No If "no", please explain: _____

ANSWER AT END OF STUDY:

What type of supplement do you think you consumed during the past 12 weeks:

_____ Placebo; _____ ASEA; _____ Do not know