AN ABSTRACT OF THE THESIS OF

Mark S. Aseltine for the degree of Doctor of Philosophy in Animal Science presented on January 31, 1979

Title: NUTRIENT EVALUATION OF SECONDARY CLARIFIER MICROBIAL PAPER SLUDGE FOR RUMINANTS

Abstract approved: Redacted for privacy

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The objectives of this study were to nutritionally evaluate the feeding of secondary clarifier single cell protein (SCP) to ruminants.

Seven digestion trials were conducted to determine nutrient digestibility and nitrogen retention by mature crossbred wether sheep. Single cell protein samples were obtained from secondary clarifiers of five different paper mills in the Pacific Northwest. Samples were dried by sonic dehydration (SD), triethylamine extraction (TEA) or the Carver-Greenfield method (CG). Samples were labeled by lab number, drying method and percent of protein replaced. The single cell protein was added to the basal ration replacing 10, 30, 50 or 100% of the protein supplied by cottonseed meal (CSM). Crude protein (CP) for the SCP ranged from 32.44 to 44.40%. Results of the trials indicate that drying the SCP by sonic dehydration, triethylamine extraction or the Carver-Greenfield method apparently had no appreciable adverse

effects on digestibility of the feed. In some cases there was a tendency for reduced digestibilities of crude protein and dry matter and on nitrogen retention when the SCP replaced more than 30% of the nitrogen supplied by cottonseed meal. Inclusion of urea in the diets apparently tended to increase digestibility of the ration.

A feedlot trial also was conducted with steers approximately 13 months of age and weighing 285 kg at the start of the experiment. Animals were randomly allotted to four treatments of 5 animals/treatment. The treatment diets contained 30% roughage and were formulated to have 13% crude protein with either cottonseed meal or three different SCP sources supplying the supplemental protein. The SCP ranged from 32.4 to 44.4% in crude protein. Animals were slaughtered and carcass data were collected. There were no differences ($P>.05$) between the CSM control and any SCP treatment for average daily gain (ADG). Carcass characteristics of steers fed either the CSM control or SCP diets showed no differences ($P>.05$).

Samples of longissimus muscles were used for sensory evaluation. Steaks were evaluated by a 14-member trained sensory panel. The 212 CZ-TEA treatment resulted in increased steak tenderness ($P<.05$), but there were no other significant differences for flavor, aroma, juiciness or overall desirability.

A study was carried out to determine the effects of ruminal pH, NH_4 and VFA concentrations when either 10, 30, 50 or 100% of the protein from cottonseed meal was replaced by protein from secondary clarifier single cell protein. Four crossbred wethers were prepared with rumen fistulas and a feeding trial was conducted. Single cell protein samples were obtained from pulp and paper mills in the Pacific Northwest. The samples were either dried by sonic dehydration or triethylamine extraction. When 206 Sonic and 1439 Sonic diets were fed, there was a treatment x hour interaction for ammonia. Ammonia values decreased from hours 4 to 7 for the 206-Sonic 50 and 100% diets. Ammonia values for 1439-Sonic 30% were consistently high during the entire sampling period. All other parameters tested were similar when compared with the cottonseed meal control diet. In all trials sheep consumed the diets readily and no unusual digestive or metabolic disorders were observed.

Nutrient Evaluation of Secondary Clarifier Microbial Paper Sludge For Ruminants

by

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Apparent *In Vivo* Nutrient Digestibility of Secondary Clarifier Single Cell Protein with Sheep[1,2]

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Key Words: Single Cell Protein, Digestibility, Sheep

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SUMMARY

Seven digestion trials were conducted to determine the effects of feeding secondary clarifier single cell protein on nutrient digestibility by mature cross-bred wether sheep. Single cell protein samples were obtained from secondary clarifiers of five different paper mills in the Pacific Northwest. Samples were dried by sonic dehydration, triethylamine extraction or the Carver-Greenfield method. The single cell protein was added to the basal ration replacing either 10, 30, 50 or 100% of the protein supplied by cottonseed meal. Crude protein for the SCP ranged from 32.44 to 44.40%. Results of the trials indicate that drying the SCP by sonic dehydration, triethylamine extraction or the Carver-Greenfield method apparently had no appreciable adverse effects on digestibility of the feed. In some cases there was a tendency for reduced digestibilities of crude protein and dry matter and on nitrogen retention when the SCP replaced more than 30% of the nitrogen supplied by cottonseed meal. Inclusion of urea in the diets apparently tended to increase digestibility of the ration. In all trials sheep consumed the diets readily and no unusual digestive or metabolic disorders were observed.

INTRODUCTION

One of the ecological problems facing the pulp and paper industry today is wastewater treatment. The pulp and paper industry provides primary treatment for the removal of sedimented solids from their wastewater effluents. Handling and disposing of excess solids is a major problem and expense to the industry. However, this wastewater from the pulping process contains considerable amounts of soluble nutrients that can support large quantities of microbial or single cell protein growth. This process utilizes one pond for aerobic digestion of paper mill effluents, and secondary clarifiers for the recovery of the SCP product (figure 1). The resulting product, when dried, contains approximately 40% crude protein (dry basis).

The purpose of this study was to determine the apparent nutrient digestibility of secondary clarifier single cell protein when fed to sheep.

MATERIALS AND METHODS

Five digestion trials were conducted using 20 crossbred wethers with 5 animals per treatment. Trials consisted of a cottonseed meal control diet and 3 test diets in which the SCP replaced either 10, 30 or 50% of the CSM

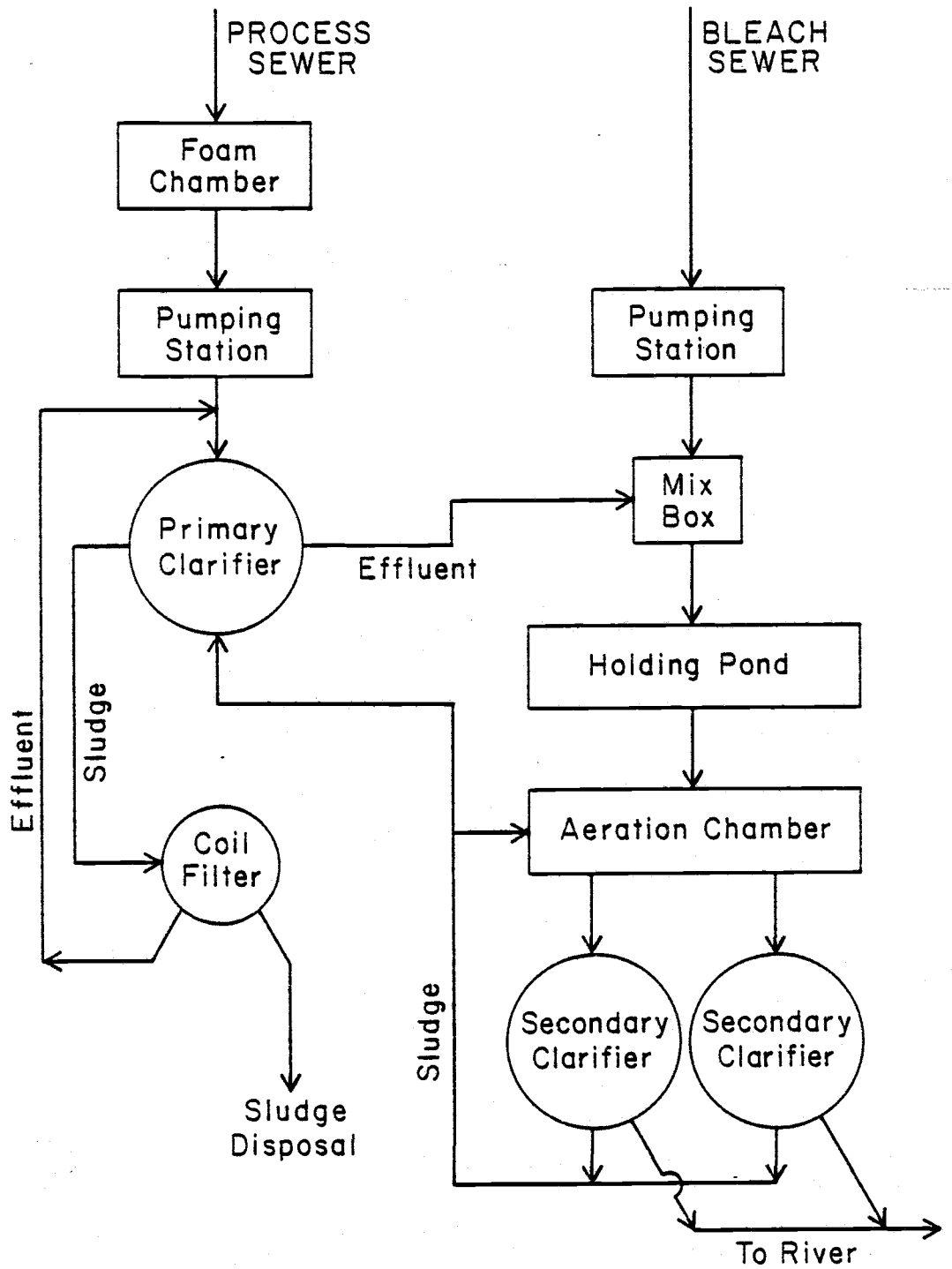

Figure 1. Effluent flow of a large pulp mill utilizing secondary clarifiers for single-cell protein recovery.

protein (table 1). Two additional trials were completed using 15 wethers with 5 animals per treatment (trial III, VI); in these trials the CSM protein was replaced 100% by the SCP. Trial VII was conducted to determine the effects of feeding SCP with three levels of urea. On the basis of nitrogen content, the sludge:urea ratios used were 50/50, 40/60 and 30/70. All diets were formulated to contain 15% CP (dry basis).

Five different SCP samples were obtained from pulp mills in the Pacific Northwest. Two samples were dried using triethylamine extraction. In this process SCP is introduced and mixed with cold recycled solvent at a ratio of 6 parts of solvent to one part SCP. The mixture is then subjected to solid-bowl cetnrifugation during which 95% or more of the solids are captured and discharged as a solvent/water/solids cake. The solids-wet cake enters a closed cycle dryer where the solvent and water are evaporated to produce "dry" solids. Two other samples were dried using a sonic dehydrator located at Roy, Washington. This drying method is based on the principles of the ramjet engine. The wet sample is introduced into a compartment where the pulsing ramjet engines dry the sample. The sonic dehydrator heats the sample to about 65 C and the TEA-dried SCP is heated slightly higher. The last sample was dried using

the Carver-Greenfield method. This method of drying involves mixing the wet sample (4% solids) with oil and then vaporizing this mixture to remove the water. The oil is then extracted from the sample and recycled. The final product is a pelletized deoiled solid with an average moisture content of 5%.

The animals were housed in metabolism cages designed to allow separation of urine and feces. Animals were fed 500 g of their respective diets twice daily with trace mineral salt available at all times. Each trial consisted of a 10-day pre-adjustment period, a 10-day adjustment period and an 8-day collection period. During the pre-adjustment period, all lambs received the control ration. Records were kept of feed offered and feed refused during the entire test period. Feed samples were collected daily, weighed, and a 10% sample was removed and stored in a cooler at 4 C. Urine was collected daily and a 10% sample was composited for analysis. To minimize urinary nitrogen losses, 5 ml of phosphoric acid were added to the collection buckets before each collection. Urine samples were also stored in a cooler at 4 C until analyzed.

Data obtained from the chemical analyses and energy determinations of the feeds and feces were used to calculate digestibilities for energy, crude protein and dry matter. Total nitrogen, dry matter, ash and fat in the

feed and feces, and total urinary nitrogen were determined by methods described in AOAC (1971). Acid detergent fiber of the feed was determined using the method of Van Soest (1963) as described in the modified micro-procedure of Waldern (1971). Gross energy in the feed and feces was determined in a Parr oxygen bomb calorimeter. Amino acid analyses were performed on the samples by the Dept. of Biochemistry and Biophysics, Oregon State University. The data in each trial were analyzed statistically by use of a one-way analysis of variance procedure as outlined by Steel and Torrie (1960). Means were compared using the LSD as described by Cochran and Cox (1957).

RESULTS AND DISCUSSION

Results for Trials I through VII are shown in table 2. In Trial I where the TEA SCP protein replaced either 10, 30 or 50% of the protein from CSM, there were no significant differences ($P>.05$) for energy (DE), dry matter (DM), crude protein (CP), acid detergent fiber (ADF) or nitrogen retention (NR). However, there was a nonsignificant tendency for lowered NR as the SCP was increased in the diet (1.9% vs 5.81 and 9.91%).

In Trial II the SCP was from a different pulp mill and was dried in a different manner (sonic dehydration). Results indicate that there were no ($P>.05$) differences for

DM, DE, ADF or NR. There was, however, a reduction in the CP digestibility for the Sonic-30% and Sonic-50% diets, but not for the Sonic-10% diet. Again, there was a tendency for the NR to be lowered as the amount of SCP increased. The NR for the Sonic-10% was 40.14 g whereas the NR for the Sonic-30 and 50% was 24.14 and 32.11 g, respectively. The Sonic-10, 30 and 50% diets contained 1.7, 5.15 and 8.66% SCP. Single cell protein in the diet was slightly lower than in Trial I due to the increased CP content.

In Trial III the TEA and Sonic SCP replaced all of the protein supplied by the cottonseed meal in the control ration. This was equivalent to 17.65% of the diet for the sonic and 21% of the diet for TEA. Results of Trial III show that there were no significant differences ($P>.05$) for DM, DE, CP, ADF digestibilities or NR. Nitrogen retention for the Sonic-100% was not significantly higher than the control while the TEA-100% was somewhat lower.

In Trial IV SCP was dried using the Carver-Greenfield method. The CG 10, 30 and 50% diets contained 1.88, 5.75 and 9.80 SCP expressed as a percent of the diet. Results indicate that there were no significant differences ($P>.05$) for DE or ADF digestibility. There were significant differences for DM and CP digestibilities and NR. Dry matter digestibility was lower for CG-50% compared with the control diet. There was also a difference in digestibility between

CG-10 and CG-30 and 50%, but not between CG-30 and CG-50%. There was a decrease in crude protein digestibility ($P<.05$) when the control diet was compared with CG-50%. Decreases in CP digestibility were present between CG-10% and those diets containing CG-30 and 50%, but not between 30 and 50% when the CP from CSM was replaced by 30 or 50% SCP. There was a decrease ($P<.05$) in NR when the 30 or 50% diets were fed, but there was no significant difference ($P>.05$) between the control and CG-10% diet.

In Trial V, SCP was from the same pulp mill as that in Trial IV, but was dried by sonic dehydration. There were no significant differences for energy, but all other digestibility coefficients showed some degree of difference. The 1439-Sonic 10, 30 and 50% diets contained 2.34, 7.21 and 12.34% SCP.

Dry matter and CP digestibility decreased significantly when the 1439-Sonic 50% was fed. This level was significantly different ($P<.05$) from both the control and the 1439 Sonic-10 and 30% diets. Digestibility for ADF increased ($P<.05$) from the control when either 1439 Sonic 10, 30 or 50% was fed, but there was no difference ($P>.05$) between the 10, 30 and 50% treatments. Nitrogen retention showed a decrease ($P<.05$) when the CP was replaced with either 30 or 50% SCP. There was no difference between the Sonic 10, 30 or 50% diets.

In Trial VI the SCP replaced all of the protein supplied by the CSM in the control ration. There were differences ($P<.05$) in digestibilities for DM, CP, DE, ADF and NR. Dry matter digestibility was lower than the control ($P<.05$) when either the CG-100 or 1439-Sonic 100% was fed. There also was a decrease between the two different types of SCP used. The 1439-Sonic diet was lower ($P<.05$) than the CG-100% diet (61.97 vs 58.32). Crude protein, DE and ADF were lower ($P<.05$) for both the CG-100 and 1439 Sonic 100 SCP. Nitrogen retention was lower ($P<.05$) when the 1439-Sonic 100 was fed, but not for the CG-100% diet.

Trial VII was conducted to determine the effects of feeding 212-TEA SCP with varying levels of urea. The SCP/urea nitrogen ratios used were: 50/50, 40/60 and 30/70. This was equivalent to 7.41/1.01, 5.78/1.18 and 4.23/1.34 SCP/urea expressed as a percent of the diet.

Results of the trial indicate that, as the amount of urea is increased and SCP is decreased, digestibilities for DM, CP and ADF increase. Dry matter digestibility increased ($P<.05$) when 40/60 and 30/70 levels were compared with the control and 50/50 level. Crude protein increased ($P<.05$) at the 40/60 and 30/70 level compared with the control. There were no differences between the

50/50, 40/60 and 30/70 levels. Acid detergent fiber increased at the 30/70 level when compared with the control and the other levels. There was no difference ($P>.05$) for DE or NR.

Amino acid analyses and chemical composition and pulp mill process of the different SCP are shown in tables 3 and 4. The differences in amino acid composition of the samples indicate the variations in composition that were found in all samples obtained from different pulp mills using different pulping procedures. The amounts of CP, ash and ADF were also quite variable. The high ash content of the 1439-Sonic could be one reason why it did not perform as well as some of the other SCP samples.

Chemical compositions of the rations for Trials I through VII are shown in table 5. Here again there was some variation in the chemical make up of the rations.

Comparison of our results with published data is difficult due to the limited number of publications on this particular type of SCP. However, in Finland a group called the SITU has carried out research in order to find a competitive method for the production of protein from carbohydrate-containing solutions. This research has led to the development of the PEKILO process, where microfungi are cultured in the spent liquor of a sulfite pulping operation. About one-third of the dissolved organic material

of the spent sulfite liquor is made up of monosaccharides, polysaccharides, carbohydrate derivatives and acetic acid, most of them utilizable in the PEKILO process (Romantschuk and Ab, 1975). The PEKILO protein has proven to be a suitable protein replacement for pigs and poultry and, when used with whey, it is also suitable for feeding calves.

Another dried SCP product that could be used as a possible alternative to skim milk protein in calf milk replacers is produced by the Imperial Chemical Industries Ltd. This product is called "Pruteen" and is produced from a culture of Methylomonas (pseuodomonas) methylotropha. Digestibility studies with growing pigs (Whittemore and Moffat, 1976) indicate the nitrogen in "Pruteen" is highly digestible (digestibility coefficient = .91). Pruteen also has a well-balanced amino acid composition.

Roth and Kirchgessner (1976) have also reported results from feeding "Pruteen" in a comparative growth trial with veal calves, in which approximately 20% of the milk protein in the diet was replaced by "Pruteen". No negative effects on performance were reported.

The effect of either drying the SCP by sonic dehydration, triethylamine extraction or the Carver-Greenfield method apparently had no appreciable adverse effects on the digestibility of the feed. In some cases there was a tendency for reduced digestibilities when the SCP replaced

more than 30% of the nitrogen supplied by CSM. However, the inclusion of urea into the diets tended to increase the digestibility of the ration. In all trials the sheep consumed the diets readily and no unusual digestive or metabolic disorders were observed.

In order to insure the safety of the animals and handlers, plate counts for pathogenic organisms were performed on the SCP samples by the Department of Microbiology, Oregon State University. Their report indicated that there were no pathogenic organisms associated with any of the samples.

Utilization of secondary clarifier single cell protein by ruminants would be beneficial to the pulp and paper industry as well as to the animal industry. There are more than 1.7 million tons of pulping residue produced annually. The present problem is producing a product of uniform composition and nutritive value.

TABLE 1. COMPOSITION OF RATIONS FOR TRIALS I THROUGH VII[a]

Treatment	% of Ration			
	Cottonseed meal (5-01-621)	Ground barley (4-07-939)	Single cell protein	Urea
Trial I				
Control	16.00	26.00	---	
205-TEA 10	14.70	25.40	1.90	
205-TEA 30	11.69	24.50	5.81	
205-TEA 50	8.54	23.55	9.91	
Trial II				
Control	16.00	26.00	---	
206-Sonic 10	14.64	25.66	1.70	
206-Sonic 30	11.49	25.36	5.15	
206-Sonic 50	8.29	25.05	8.66	
Trial III				
Control	16.00	26.00	---	
206-Sonic 100	---	24.35	17.65	
205-TEA 100	---	21.00	21.00	
Trial IV				
Control	16.00	26.00	---	
1444-CG 10	14.69	25.43	1.88	
1444-CG 30	11.66	24.59	5.75	
1444-CG 50	8.49	23.71	9.80	
Trial V				
Control	16.00	26.00	---	
1439-Sonic 10	14.74	24.92	2.34	
1439-Sonic 30	11.79	23.00	7.21	
1439-Sonic 50	8.64	21.02	12.34	
Trial VI				
Control	16.00	26.00	---	
1444-CG 100	---	21.37	20.63	
1439-Sonic 100	---	15.46	26.54	
Trial VII				
Control	16.00	26.00	---	---
212-TEA 50/50	---	33.58	7.41	1.01
212-TEA 40/60	---	35.04	5.78	1.18
212-TEA 30/70	---	36.43	4.23	1.34

[a]All diets contained 30% ryegrass straw (1-04-059), 10% chopped alfalfa (1-00-063), 10% beet pulp (4-00-672), 7% cane molasses (4-04-696), and 1% limestone.

TABLE 2. APPARENT DIGESTIBILITY COEFFICIENTS AND NITROGEN RETENTION FOR TRIAL I THROUGH VII

Lab number and treatment	Dry matter, %	Crude protein, %	Energy, %	Acid detergent fiber, %	Nitrogen retention, g
Trial I					
Control	64.71	58.77	66.81	50.48	43.82
205-TEA 10	66.63	62.75	69.76	57.72	41.48
205-TEA 30	63.48	57.23	64.63	56.46	25.33
205-TEA 50	63.49	58.85	64.82	54.46	32.86
Trial II					
Control	56.00	59.66[a]	55.10	30.93	30.15
206-Sonic 10	54.30	56.64[a]	54.79	23.98	40.14
206-Sonic 30	54.90	48.98[b]	54.35	33.21	24.15
206-Sonic 50	52.92	51.35[b]	51.53	28.23	32.11
Trial III					
Control	67.57	66.96	68.40	47.51	29.69
206-Sonic 100	66.50	65.54	65.37	48.21	43.25
205-TEA 100	66.58	64.06	66.98	58.07	24.89
Trial IV					
Control	69.46[a,b]	65.09[a,b]	67.44	54.74	47.06[a]
1444-CG 10	69.79[a]	68.23[a]	67.97	55.81	49.68[a]
1444-CG 30	67.35[b,c]	61.57[b,c]	68.35	54.82	24.37[b]
1444-CG 50	66.79[c]	58.15[c]	67.24	52.63	27.74[b]
Trial V					
Control	74.28[a]	69.19[a]	73.74	52.79[a]	32.61[a]
1439-Sonic 10	73.37[a]	68.92[a]	73.27	60.15[b]	24.72[a,b]
1439-Sonic 30	73.07[a]	69.63[a]	73.41	63.12[b]	15.29[b]
1439-Sonic 50	69.54[b]	60.93[b]	69.53	62.51[b]	1.57[b]
Trial VI					
Control	70.39[a]	69.34[a]	70.13[a]	48.92[a]	46.07[a]
1444-CG 100	61.97[b]	50.23[b]	61.33[b]	42.76[b]	36.25[a]
1439-Sonic 100	58.32[c]	51.74[b]	60.56[b]	40.81[b]	19.17[b]
Trial VII					
Control	70.24[a]	66.69[a]	70.81	50.62[a]	63.65
212-TEA 50/50	70.09[a]	68.77[a,b]	70.57	52.07[a]	55.84
212-TEA 40/60	72.23[b]	69.94[b]	71.55	51.81[a]	62.96
212-TEA 30/70	73.14[b]	70.86[b]	71.91	56.40[b]	69.63

[a,b,c]Significantly different within each trial ($P<.05$).

TABLE 3. AMINO ACID ANALYSIS OF SECONDARY CLARIFIER SINGLE CELL PROTEIN, SOYBEAN MEAL AND COTTONSEED MEAL, % OF TOTAL PROTEIN

	SBM	CSM	206-Sonic	205-TEA	1444 CG	1439 Sonic	212-TEA
CP % of dry matter	49.00	44.80	41.30	36.80	37.30	32.44	44.40
Lysine	6.81	4.85	5.35	2.90	3.63	6.48	4.68
Histidine	3.14	3.45	2.47	2.16	2.39	2.59	2.21
Arginine	8.01	12.88	6.81	5.75	6.47	5.74	6.31
Aspartic acid	12.22	10.61	10.96	13.16	10.65	11.12	11.83
Threonine	4.09	3.53	6.31	6.08	5.35	4.89	5.44
Serine	5.15	4.65	4.03	3.78	4.03	4.88	4.24
Glutamic acid	19.06	21.74	13.04	14.23	15.28	15.20	13.34
Proline	4.89	3.75	4.33	5.00	4.04	3.79	4.47
Glycine	3.87	4.02	5.85	6.90	5.66	5.53	6.17
Alanine	4.10	3.91	8.16	9.22	8.36	7.76	8.40
Valine	4.83	5.32	6.26	7.40	6.41	5.91	6.52
Methionine	1.01	.83	2.08	.78	3.23	2.26	1.73
Isoleucine	4.81	3.58	4.57	5.13	4.54	4.77	4.83
Leucine	7.67	6.10	8.00	5.37	8.00	7.77	8.26
Tyrosine	4.15	3.60	4.29	4.06	5.94	5.10	5.26
Phenylalanine	5.36	6.14	6.94	7.92	5.64	5.76	5.94
Cys acid	--	.31	.59	.14	--	.50	.37

TABLE 4. CHEMICAL COMPOSITION AND PULP MILL PROCESS OF SCP USED IN DIGESTIBILITY TRIALS

	205-TEA	206-Sonic	1444-CG	1439-Sonic	212-TEA
DM, %	93.79	95.00	95.30	88.39	85.95
Crude protein, % of DM	36.80	41.27	37.30	32.44	44.40
ASH, % of DM	20.62	14.71	17.61	22.55	16.04
ADF, % of DM	8.76	25.76	35.44	16.74	18.10
Lignin, % of DM	2.44	4.04	11.34	6.83	9.41
EE, % of DM	.31	1.06	.80	.77	.45
Pulp mill process	Sulphite, Mg base	Kraft	Sulphite, Na base	Sulphite, Na base	Kraft

TABLE 5. CHEMICAL COMPOSITION OF RATIONS FOR TRIALS I THROUGH VII

Lab number and treatment	DM, %	CP, % of DM	ASH, % of DM	ADF, % of DM	EE, % of DM	GE, kcal/g
Trial I						
Control	86.69	11.71	6.67	27.26	1.23	4.42
205-TEA 10	87.23	13.89	6.28	29.89	1.51	4.60
205-TEA 30	86.77	13.62	6.26	29.42	1.17	4.42
205-TEA 50	87.02	14.20	6.96	26.66	1.09	4.46
Trial II						
Control	90.97	14.72	5.69	24.16	1.21	4.29
206-Sonic 10	90.95	15.69	6.59	23.08	1.52	4.36
206-Sonic 30	91.70	14.02	6.62	25.18	1.76	4.30
206-Sonic 50	92.59	15.14	6.47	24.31	1.84	4.36
Trial III						
Control	86.79	14.44	6.49	24.03	1.39	4.56
206-Sonic 100	88.75	16.28	7.38	24.58	1.80	4.41
205-TEA 100	87.42	14.46	9.46	25.58	1.33	4.36
Trial IV						
Control	88.68	16.25	6.23	26.50	1.80	4.31
1444-CG 10	88.57	16.61	6.15	24.61	1.68	4.36
1444-CG 30	87.48	14.67	6.51	27.82	1.65	4.48
1444-CG 50	89.09	15.36	7.10	26.49	1.72	4.39
Trial V						
Control	91.79	13.05	5.12	23.28	1.38	4.48
1439-Sonic 10	91.88	13.65	6.11	27.95	1.03	4.45
1439-Sonic 30	91.98	13.83	6.15	28.95	.98	4.39
1439-Sonic 50	92.84	12.28	6.50	31.88	.79	4.42
Trial VI						
Control	90.00	15.48	6.12	24.83	1.25	4.43
1444-CG 100	91.00	15.80	5.95	29.12	1.04	4.15
1439-Sonic 100	91.00	14.21	6.40	27.16	1.01	4.35
Trial VII						
Control	92.32	14.36	6.76	25.52	1.10	4.37
212-TEA 50/50	92.06	15.16	6.26	24.79	1.30	4.28
212-TEA 40/60	92.17	14.85	6.62	22.01	1.74	4.27
212-TEA 30/70	92.50	15.17	6.74	22.71	1.58	4.15

LITERATURE CITED

AOAC. 1971. Official Methods of Analysis. (11th ed.). Association of Official Agricultural Chemists. Washington, D. C.

Cochran, W. G. and G. M. Cox. 1957. Experimental Designs. 2nd ed. John Wiley and Son, Inc., New York.

Romantschuk, H. and T. O. Ab. 1975. In: S. R. Tannenbaum and D. I. C. Wang (eds.). Single Cell Protein II. M.I.T. Press. Cambridge, MA.

Roth, F. X. and M. Kirchgessner. 1976. Methanol-fermentation protein in veal calf nutrition. Anim. Feed Sci. Technol. 1:33.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Official Agr. Chem. 46:829.

Waldern, D. C. 1971. A rapid micro-digesting procedure for neutral-acid detergent fiber. Can. J. Anim. Sci. 51:67.

Running Head: Ruminal effects of feeding a single cell protein to sheep.

The Effect of Feeding Secondary Clarifier Single Cell Protein on Rumen Parameters[1,2]

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Key Words: Single Cell Protein, Rumen pH, VFA, Ammonia

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SUMMARY

Four crossbred wethers were prepared with rumen fistulas and a feeding trial was conducted to determine the effects on ruminal pH, NH_4 and VFA concentrations when either 10, 30, 50 or 100% of the protein from cottonseed meal was replaced by protein from secondary clarifier single cell protein. Single cell protein samples were obtained from pulp and paper mills in the Pacific Northwest. The samples were either dried by sonic dehydration or triethylamine extraction

When 206 Sonic and 1439 Sonic diets were fed, there was a treatment x hour interaction for ammonia. Ammonia values decreased from hours 4 to 7 for the 206-Sonic 50 and 100% diets. Ammonia values for 1439-Sonic 30% were consistently high during the entire sampling period. All other parameters tested were similar when compared with the cottonseed meal control diet. All animals consumed the diets readily and no digestive or metabolic disorders were observed.

INTRODUCTION

Copious amounts of soluble or non-filterable nutrients end up in the wash water effluent of pulp and paper mills. This effluent provides an excellent source of nutrients to support microbial or single cell protein growth. Recovery of the solids utilizes one pond for aerobic digestion, and secondary clarifiers for the recovery of the SCP product. The resulting product when dried contains approximately 40% crude protein (dry basis) depending on the amount of fibrous material it contains. The production of the SCP product is dependent upon the type of mill, type and quantity of the fiber used, and other operation variables.

Projected protein needs for human consumption are high. Conventional sources of protein will not likely meet these needs. Single cell protein from the fermentation of paper mill effluents could serve as sources of additional protein for ruminant animals. The following trial was conducted to determine the effects on ruminal pH, NH_4 and VFA concentrations when either 10, 30, 50 or 100% of the protein from cottonseed meal was replaced by SCP.

MATERIALS AND METHODS

Animals and Feeding

Four crossbred wethers weighing 48 to 55 kg were prepared with rumen fistulas following the procedures outlined

by McCann et al. (1973). Animals were given a minimum of 60 days to recover before testing began. The sheep were maintained on grass hay and alfalfa prior to the studies. Sheep were placed in separate metabolism crates designed to allow easy access to the animal. All animals had access to water and trace mineral salt ad libitum. The SCP diets tested were 205-TEA 10, 30 and 50%; 206-Sonic 10, 30, 50 and 100%; and 1439-Sonic 10, 30, 50 and 100%. These SCP were previously described in a digestibility experiment with sheep (Aseltine, 1979). Experimental diets are shown in table 1. Each trial consisted of a 10-day adjustment period and a 1 day, 8-hr collection period. Animals were fed 750 g of their respective diets twice daily. The first feeding was always 1 hr before the first sampling.

Sampling

Samples of about 200 ml of rumen fluid were taken from the middle of the rumen by gentle suction through a perforated tube every hour for 8 hr post-feeding. After pH measurements were made, 25% meta-phosphoric acid was added and the samples were chilled. Samples were then centrifuged at 12,500 rpm for 30 min and the supernatant was stored (-20 C) until analyzed.

Analysis of SCP and Feed

Total nitrogen, dry matter, ash and ether extract in the feed and SCP were determined by methods described in

AOAC (1971). Acid detergent fiber of the SCP was determined using the method of Van Soest (1963). The modified micro-procedure for ADF (Waldern, 1971) was used for the feed. Gross energy in the feed was determined in an adiabatic Parr oxygen bomb calorimeter.

Ammonia Nitrogen Determination

Ammonia-nitrogen (NH_3-N) was determined using a method described by Hawk et al. (1954). Duplicate 5 ml samples of rumen fluid obtained from the hourly collections were placed in test tubes in an aeration train.

Hydrogen Ion Activity

The pH of the sample was taken within one min of removal using a digital pH meter.

VFA Analysis

Volatile fatty acids (VFA) in the rumen fluid, including acetic, propionic, butyric, isobutyric, valeric and isovaleric were determined by gas chromatography, as described previously (Aseltine, 1979).

Statistics

Data were analyzed statistically by use of analysis of variance. Differences between means were tested for significance by the Newman-Keul's sequential variation procedure as outlined by Steel and Torrie (1961).

SCP Samples

Three different SCP samples were obtained from secondary clarifiers of pulp and paper mills in the Pacific Northwest. Samples were dried either by sonic dehydration or triethylamine extraction (Aseltine, 1979).

RESULTS AND DISCUSSION

The effect of replacing either 10, 30, 50 or 100% of the nitrogen supplied by cottonseed meal with secondary clarifier single cell protein on rumen pH, NH_4, total VFA, acetic propionic ratio (A/P) and VFA molar ratio are summarized in table 2. The results are expressed as daily means averaged over an 8-hr collection period.

205-TEA SCP

This sample was obtained from a pulp mill utilizing the sulphite, Mg base pulp mill process. The sample was dried using triethylamine extraction. The TEA-100% level was omitted due to lack of sufficient SCP sample.

Fermentation of the TEA-10 diet resulted in a lower ($P<.05$) pH value compared with the CSM control diet. No differences ($P>.05$) in pH were observed for the TEA 30 or 50% diets compared with the CSM control. The minimum pH recorded was by the TEA-10% diet. The average values were 6.26, 6.11, 6.25 and 6.21 for the control, TEA 10, 30 and

50% diets, respectively. The pH tended to increase from hour 1 to hour 7 or 8 for all treatments. The highest pH value was 6.62 for the control diet at hour 8. Ruminal samples were collected through a rumen canula at a location near the top of the rumen. Wheaton et al. (1970) collected samples at 4 and 8 hr after a morning feeding of hay and reported, in most cases, higher pH values near the top of the rumen fluid.

The daily mean concentrations of ammonia were highest for the TEA-10% diet and lowest for the TEA-50% diet. All treatments had the highest ammonia levels 1-3 hr post feeding. These data indicate that the solubility of the rations were similar.

There were no significant differences for daily mean concentrations of total VFA for any treatments. The highest VFA productions were observed at 2-3 hr post-feeding. The A/P ratio was significantly higher for the TEA-50% diet compared with the control diet (3.44 vs 2.98). Molar percents of acetic acid for all treatments were similar compared with the control diet with the exception of the TEA-30% treatment. A decrease in molar percent of propionic acid was observed for diets 10 and 50%. Diet TEA-10% was significantly higher than the control diet.

Whitelaw et al. (1972) stated that it is difficult to predict the proportions of propionic acid from cereal diets

because of the high variability influenced by the level of feeding and the composition of the rumen microorganisms. Molar percentages of butyric acid were highest ($P<.05$) for the TEA 10 and 50% diets. No differences were observed between the control and TEA-30% diets.

Results for the control diet are consistent with other workers. Balch and Rowland (1957) found molar compositions of the VFA mixture to be 68.9% C_2, 17.3% C_3 and 9.9% C_4. Sutton and Johnson (1969) reported values of 68.3% C_2, 17.8% C_3 and 12.1% C_4 for animals receiving mixed diets. Results from the present study for the CSM control diet were 63.06% C_2, 21.44% C_3 and 13.07% C_4.

Molar percents of isovaleric were higher ($P<.05$) for the TEA-30% diet compared with the control diet. No differences were observed for the TEA-10 or 50% diets compared with the control. This indicates a possible increase in protein degradation. Faichney (1968) stated that this branched-chain acid came from the degradation of protein by microbial action.

There was a significant difference for mean molar percent for valeric acid for both treatment and hour. However, there was a treatment x hour interaction due to the inconsistency of hour values. This may have been due to sampling technique or analysis.

206-Sonic SCP

Mean concentrations for pH were lowest for the Sonic-10% diet and highest for the Sonic-100% diet (6.15 vs 6.39). Again, as with the 205-TEA diets, pH values increased with time and there was apparently an inverse relationship with total VFA. There were no differences in ammonia means due to a treatment x hour interaction which was due to irregular values for ammonia concentrations for diets Sonic-50 and 100%. Values for ammonia were consistent with other treatments. In both Sonic-50 and 100% diets ammonia levels dropped at hour 5 and remained low until hour 8.

Mean concentrations for total VFA were highest for the control diet compared with all treatments. Mean concentrations for Sonic-10 and 30% diets were higher ($P<.05$) than both Sonic-50 or Sonic-100% diets.

The A/P ratios were highest for Sonic-30 and Sonic-100% diets. No differences ($P>.05$) were observed between the control ration and Sonic-10 or 50% diets. Molar percents of acetic acid were highest for Sonic-30 and 100% diets. The control diet was lower ($P<.05$) than the Sonic-10% diet. Molar percents of propionic acid were highest for the control diet and Sonic-50%. No differences ($P>.05$) were observed between Sonic 10, 30 and 50%. Molar percent of butyric acid was highest for Sonic-10% compared with the control and all other treatment diets. Significant

differences for isobutyric, isovaleric and valeric acids were omitted due to a treatment x hour interaction.

1439-Sonic SCP

Significant differences between mean concentrations for pH and ammonia were omitted due to a treatment x hour interaction. Although the rumen ammonia levels were somewhat irregular, values recorded for diets 10, 50 and 100% were somewhat similar to the control ration. Rumen ammonia data for the Sonic-30% diet showed high levels throughout the 8-hr collection period, resulting in the interaction.

Mean molar percents for acetic acid were higher for all SCP treatments compared to the control ration. Molar percents for propionic acid was lower for all treatments compared with the control. Butyric acid was higher ($P<.05$) for the control diet compared to diet 100%. However, diet 30% was higher ($P<.05$) compared with the control. Data for isobutyric and isovaleric were omitted due to a treatment interaction.

It is difficult to compare the mean values obtained directly with results reported by other workers, since the results are dependent upon the amount of feed administered, chemical composition of the feed, and the sampling frequency. Bath and Rook (1963), in experiments with feeding cycles, emphasized the necessity of sampling digesta at frequent

intervals throughout the feeding cycle in order to achieve representative mean values of the parameters measured. Church (1975) also reported that different diets support different microbial populations and that the end-products of different organisms differ, resulting in changes in VFA production.

Chemical compositions of the SCP rations are summarized in table 3. Chemical compositions and pulp mill processes of the SCP are summarized in table 4. All three SCP products were obtained from different pulp mills using different pulping processes. Crude protein of the SCP ranged from 32.44% CP to 41.27% CP. The percent of SCP in the ration also varied considerably (table 1). Percent SCP in the diets ranged from 1.7% to as high as 26.54%.

The effect of feeding SCP at various levels of protein replacement of CSM apparently had no adverse effects on pH, A/P ratio or molar percent of acetic, propionic or butyric acids compared with a CSM control diet. Treatment x hour interactions were observed for isovaleric and valeric acid for all treatments except 205-TEA. Ammonia values for Sonic-50 and Sonic-100% were lower at hours 4 to 7, indicating decreased protein degradation or increased ammonia absorption. The amounts of measurable ammonia are dependent upon the rate of hydrolysis of the protein and rate of uptake. The actual concentration of ammonia at any one time are

only a reflection of the combined effects of all the factors involved.

TABLE 1. COMPOSITION OF RATIONS

Treatment	% of ration		
	Cottonseed meal (5-01-621)	Ground barley (4-07-939)	Single cell protein
Control	16.00	26.00	---
205-TEA 10	14.70	25.40	1.90
205-TEA 30	11.69	24.50	5.81
205-TEA 50	8.54	23.55	9.91
206-Sonic 10	14.64	25.66	1.70
206-Sonic 30	11.49	25.36	5.15
206-Sonic 50	8.29	25.05	8.66
206-Sonic 100	---	24.35	17.65
1439-Sonic 10	14.74	24.92	2.34
1439-Sonic 30	11.79	23.00	7.21
1439-Sonic 50	8.64	21.02	12.34
1439-Sonic 100	---	15.46	26.54

[a] All diets contained 30% ryegrass straw (1-04-059), 10% chopped alfalfa (1-00-063), 10% beet pulp (4-00-672), 7% cane molasses (4-04-696), and 1% limestone.

TABLE 2. EFFECT OF SECONDARY CLARIFIER SINGLE CELL PROTEIN ON RUMEN pH, AMMONIA, A/P RATIO, TOTAL VFA CONCENTRATION AND VFA MOLAR RATIO[1]

Treatment and lab no.	Sampling time	pH	NH_4 (mg %)	Total VFA (μm/ml)	A/P	Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric
						Molar %					
Control	1	5.99	31.96	85.53	2.49	60.42	24.45	.74	12.18	.85	1.35
	2	5.97	28.18	97.38	2.55	61.06	24.29	.61	12.24	.58	1.22
	3	5.99	25.91	100.73	2.75	62.36	22.93	.63	12.39	.54	1.17
	4	6.14	21.82	110.68	2.81	62.42	22.06	.93	12.94	.55	1.09
	5	6.39	18.63	96.83	3.05	64.23	20.72	.68	12.87	.54	.97
	6	6.46	16.06	102.78	3.15	64.48	20.32	.85	12.92	.55	.88
	7	6.53	14.39	94.24	3.44	64.34	18.69	.92	14.59	.69	.78
	8	6.62_a	$13.78_{a,b}$	100.15	3.63_b	65.16_a	18.07_a	$.91_c$	14.41_c	$.63_b$	.81
	$\bar{x}$	6.26	$21.34_{a,b}$	98.54	2.98_b	63.06_a	21.44_a	$.78_c$	13.07_c	$.62_b$	1.03
205-TEA 10	1	5.98	29.99	80.85	2.58	59.64	23.34	.99	13.74	1.12	1.15
	2	5.98	30.45	89.05	2.54	58.94	23.15	.99	13.38	.64	2.82
	3	5.96	30.45	93.69	2.71	62.14	22.57	.85	13.56	.74	1.27
	4	6.04	28.33	94.33	2.91	61.39	21.22	.83	14.88	.52	1.18
	5	6.26	24.69	86.61	3.05	63.09	19.87	.66	14.77	.56	1.34
	6	6.19	21.51	79.87	3.29	62.90	18.83	.80	15.71	.59	1.17
	7	6.19	21.05	81.62	3.49	63.78	17.69	.96	15.81	.75	1.01
	8	6.31_b	19.09_c	70.93	3.79_b	$63.50_{a,b}$	16.72_b	1.00	16.82	1.03	.93
	$\bar{x}$	6.11_b	25.69_c	84.62	3.04_b	$61.92_{a,b}$	20.43_b	$.88_{b,c}$	14.83_b	$.74_{a,b}$	1.35
205-TEA 30	1	6.12	28.93	77.72	2.38	58.87	24.85	1.11	12.05	1.43	1.68
	2	6.17	27.57	99.23	2.39	59.01	24.67	1.13	12.79	.92	1.48
	3	6.21	29.24	96.87	2.43	59.71	23.96	1.01	13.03	.81	1.48
	4	6.28	24.54	99.82	3.89	60.36	22.88	1.01	13.69	.69	1.38
	5	6.24	19.69	94.13	2.76	60.59	22.03	1.06	14.39	.67	1.24
	6	6.26	17.73	102.28	2.99	62.35	20.89	1.13	14.09	.66	.88
	7	6.32	15.30	89.65	3.14	62.74	20.04	1.08	14.34	.70	1.09
	8	6.46	13.94	70.61	3.39	63.76	19.22	1.14	14.08	.79	1.02
	$\bar{x}$	6.25_a	22.12_a	91.28	2.92_b	60.92_b	22.32_c	1.08_a	13.56_c	$.83_a$	1.28
205-TEA 50	1	6.10	29.54	97.64	2.95	59.22	20.43	1.01	15.27	1.09	1.65
	2	6.14	24.03	88.97	3.26	61.17	19.02	1.07	16.89	.67	1.16
	3	6.06	21.07	91.97	3.10	60.77	20.27	.84	16.34	.60	1.19
	4	6.15	19.08	89.52	3.15	61.75	19.61	1.01	15.92	.57	1.14
	5	6.19	17.41	87.56	3.63	62.96	17.24	1.03	17.00	.63	1.15
	6	6.25	14.72	81.19	3.73	64.08	16.86	.87	16.45	.66	1.07
	7	6.32	14.75	74.12	3.64	62.48	17.18	1.15	17.52	.72	.95
	8	6.45	13.96	69.63	4.07	65.32	16.05	1.09	15.79	.90	.84
	$\bar{x}$	$6.21_{a,b}$	19.32_b	85.07	3.44_a	$62.22_{a,b}$	18.33_d	$1.01_{a,b}$	16.40_a	.73	1.14

TABLE 2 CONTINUED. EFFECT OF SECONDARY CLARIFIER SINGLE CELL PROTEIN ON RUMEN pH, AMMONIA, A/P RATIO, TOTAL VFA CONCENTRATION AND VFA MOLAR RATIO[1]

Treatment and lab no.	Sampling time	pH	NH_4 (mg %)	Total VFA (μm/ml)	A/P	Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric
						Molar %					
Control	$\bar{x}$	6.26[b]	21.34	98.54[c]	2.98[b]	63.06[b]	21.44[a]	.78	13.07[a]	.62	1.03
206-Sonic 10	1	5.98	25.75	95.37	3.31	61.60	18.97	.83	16.43	.57	1.61
	2	6.01	22.63	91.33	3.18	60.37	19.41	.70	17.42	.69	1.41
	3	6.19	21.49	90.92	3.12	59.82	19.60	.68	17.91	.58	1.42
	4	6.22	19.96	86.09	3.25	62.09	19.31	.75	15.80	.59	1.47
	5	6.23	18.73	88.13	3.16	60.91	19.46	.68	17.00	.55	1.40
	6	6.19	18.70	84.13	2.89	59.40	20.87	.94	16.92	.52	1.36
	7	6.26	18.01	95.49	2.72	58.72	21.87	1.06	16.36	.62	1.37
	8	6.19	16.38	85.97	3.15	61.44	19.64	1.16	15.78	.65	1.33
	$\bar{x}$	6.15[c]	20.20	89.68[a]	3.09[b]	60.06[c]	19.88[b]	.85	16.70	.59	1.42
206-Sonic 30	1	6.17	33.44	87.85	3.14	62.69	20.03	1.04	13.32	1.01	1.86
	2	6.09	31.65	93.54	3.24	63.72	19.73	.74	13.85	.63	1.33
	3	6.21	30.80	89.07	3.34	64.96	19.51	.61	12.97	.62	1.35
	4	6.17	23.18	89.14	3.45	65.85	19.24	.79	12.41	.55	1.16
	5	6.31	18.25	83.50	3.57	66.20	18.75	.89	12.64	.53	1.01
	6	6.39	15.73	80.47	3.74	66.74	19.55	.69	13.04	.57	.92
	7	6.50	14.14	73.49	3.95	67.59	17.24	.71	13.05	.60	.83
	8	6.59	13.23	73.84	4.11	67.88	16.73	.95	13.24	.59	.60
	$\bar{x}$	6.31[a,b]	22.55	83.86[a]	3.57[a]	65.70[a]	18.85[b]	.80	13.06[a]	.64	1.13
206-Sonic 50	1	6.19	26.68	85.91	2.91	62.43	21.90	.95	11.45	1.11	1.99
	2	6.14	15.20	79.42	2.84	62.33	22.65	.84	12.25	.56	1.37
	3	6.14	11.02	75.55	2.91	63.82	22.90	.65	10.97	.50	1.16
	4	6.12	8.65	78.05	2.89	63.51	23.07	.84	11.09	.51	.97
	5	6.19	6.54	79.31	3.02	64.35	22.51	.67	10.99	.52	.96
	6	6.50	6.92	65.44	3.21	65.46	21.24	.97	10.98	.51	.84
	7	6.58	7.60	63.53	3.35	65.95	20.88	.65	11.67	.61	.84
	8	6.78	9.27	63.92	3.45	66.25	19.41	.77	11.97	.66	.87
	$\bar{x}$	6.33[a,b]	11.49	73.89[b]	3.07[b]	64.26[d]	21.75[a]	.79	11.42[b]	.62	1.13
206-Sonic 100	1	6.34	34.65	79.73	3.18	62.29	19.99	1.30	12.17	1.65	2.60
	2	6.27	18.24	78.30	3.14	63.49	20.51	.87	13.14	.72	1.27
	3	6.26	13.68	72.90	3.25	65.29	20.53	.69	11.82	.59	1.11
	4	6.37	10.75	70.16	3.40	66.14	20.02	.92	11.50	.52	.91
	5	6.30	8.54	73.27	3.45	66.19	79.75	.82	11.88	.50	.86
	6	6.47	9.20	64.84	3.66	67.13	18.96	.73	11.86	.53	.78
	7	6.45	8.97	62.76	3.95	67.97	17.79	.86	12.10	.52	.75
	8	6.61	10.87	57.50	4.09	68.22	17.19	.84	12.39	.64	.73
	$\bar{x}$	6.39[a]	14.36	69.93[b]	3.51[a]	65.84[a]	19.34[b]	.88	12.11[a,b]	.71	1.22

TABLE 2 CONTINUED. EFFECT OF SECONDARY CLARIFIER SINGLE CELL PROTEIN ON RUMEN pH, AMMONIA, A/P RATIO, TOTAL VFA CONCENTRATION AND VFA MOLAR RATIO[1]

Treatment and lab no.	Sampling time	pH	NH_4 (mg %)	Total VFA (μm/ml)	A/P	Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric
						Molar %					
Control	$\bar{x}$	6.26	21.34	98.54[a]	2.98[d]	63.06[a]	21.44[a]	.78[a]	13.07[b,c]	.62	1.03
1439-Sonic 10	1	6.11	40.97	88.61	3.49	62.03	18.06	1.28	13.80	1.71	3.14
	2	6.26	28.88	81.78	3.17	61.62	19.92	1.03	14.78	.92	1.73
	3	6.16	27.59	82.03	3.40	63.56	18.98	1.20	13.93	.86	1.48
	4	6.18	24.32	77.62	3.53	64.44	18.57	1.45	13.67	.77	1.40
	5	6.23	19.76	71.27	3.68	64.90	17.92	1.44	13.78	.74	1.23
	6	6.28	17.79	68.24	3.95	66.18	16.99	1.22	13.84	.75	1.10
	7	6.33	17.10	64.11	4.12	67.12	16.41	1.04	13.56	.85	1.04
	8	6.37	15.86	63.48	4.24	66.68	15.87	1.35	14.32	.83	.97
	$\bar{x}$	6.23	24.04	74.64[c]	3.69[c]	64.56[b]	17.84[b]	1.20[b]	13.96[a,b]	.93	1.51
1439-Sonic 30	1	6.20	36.51	82.05	3.86	64.92	16.98	1.11	13.49	1.46	2.05
	2	6.14	34.74	86.57	3.69	64.36	17.54	1.08	14.78	.87	1.37
	3	6.10	37.40	89.81	3.80	65.49	17.33	.92	13.96	.82	1.47
	4	6.14	35.57	92.53	3.98	65.83	16.65	1.08	14.31	.77	1.36
	5	6.30	34.85	85.21	4.19	66.24	16.01	1.12	14.57	.83	1.24
	6	6.32	32.91	85.63	4.34	66.72	15.44	1.20	14.66	.83	1.17
	7	6.39	30.19	80.44	4.49	67.03	14.96	1.29	14.73	.84	1.13
	8	6.44	33.05	80.70	4.50	66.70	14.83	1.38	15.02	1.00	1.12
	$\bar{x}$	6.25	34.40	85.37[b]	4.10[b]	65.91[c]	16.21[c]	1.15[b]	14.44[a]	.93	1.36
1439-Sonic 50	1	6.17	37.43	82.61	3.64	63.28	17.45	1.33	14.10	1.46	2.38
	2	6.20	30.14	80.20	3.76	64.48	17.18	1.14	14.70	.90	1.60
	3	6.23	25.15	80.28	4.12	67.34	16.41	1.00	13.33	.68	1.25
	4	6.27	21.36	74.72	4.21	67.09	16.00	1.05	14.13	.63	1.11
	5	6.33	18.85	74.88	4.32	67.75	15.74	1.09	13.83	.61	.98
	6	6.32	15.43	66.88	4.46	68.21	15.39	1.02	13.87	.60	.89
	7	6.44	14.75	67.32	4.63	69.07	14.97	1.02	13.46	.64	.80
	8	6.28	13.45	52.35	4.75	69.70	14.70	.84	13.35	.65	.75
	$\bar{x}$	6.28	22.07	72.40[c]	4.23[b]	67.12[d]	15.98[c]	1.06[b]	13.84[a,b]	.77	1.22
1439-Sonic 100	1	6.15	35.03	82.73	3.73	64.89	17.52	1.30	12.29	1.54	2.47
	2	6.18	21.05	79.17	4.01	67.65	16.93	1.60	11.81	.71	1.30
	3	6.24	18.24	72.82	4.27	68.84	16.21	.94	12.12	.64	1.25
	4	6.29	14.36	66.90	4.45	69.23	15.65	.93	12.52	.57	1.10
	5	6.27	12.47	63.11	4.61	69.85	15.25	.82	12.45	.58	1.04
	6	6.29	11.28	66.40	4.75	70.20	14.94	.80	12.59	.57	.90
	7	6.33	10.49	65.51	4.83	70.06	14.59	.88	12.98	.63	.86
	8	6.41	9.88	62.86	4.88	70.75	14.56	.93	12.45	.60	.72
	$\bar{x}$	6.27	16.60	69.94[c]	4.44[a]	68.93[c]	15.71[c]	1.02[b]	12.40c	.73	1.21

[1]Four animals per observation. Column means with the same letter are not different (P<.05).

TABLE 3. CHEMICAL COMPOSITION OF RATIONS[a]

Lab number and treatment	DM, %	CP, % of DM	ASH, % of DM	ADF, % of DM	EE, % of DM	GE, kcal/g
Control	86.69	11.71	6.67	27.26	1.23	4.42
205-TEA	87.01	13.90	6.50	28.66	1.26	4.49
206-Sonic	91.00	15.28	6.77	24.29	1.73	4.36
1493-Sonic	91.93	13.49	6.29	28.99	1.00	4.40

[a]Mean of all diets.

TABLE 4. CHEMICAL COMPOSITION AND PULP MILL PROCESS OF SCP USED IN RATIONS

	205-TEA	206-Sonic	1439-Sonic
DM, %	93.79	95.00	88.39
Crude protein, % of DM	36.80	41.27	32.44
ASH, % of DM	20.62	14.71	22.55
ADF, % of DM	8.76	25.76	16.74
Lignin, % of DM	2.44	4.04	6.83
EE, % of DM	.31	1.06	.77
Pulp mill process	sulphite, mg base	Kraft	sulphite, N base

LITERATURE CITED

AOAC. 1971. Official Methods of Analysis. (11th ed.). Association of Official Agricultural Chemists. Washington, D. C.

Aseltine, M. S. 1979. Nutrient evaluation of secondary clarifier microbial paper sludge for ruminants. Ph.D. Thesis. Oregon State University, Corvallis, OR.

Balch, D. A. and S. J. Rowland. 1957. Volatile fatty acids and lactic acid in the rumen of dairy cows receiving a variety of diets. Brit. J. Nutr. 11:288.

Bath, I. H. and J. A. F. Rook. 1963. The evaluation of cattle foods and diets in terms of the ruminal concentration of volatile fatty acids. I. The effects of level of intake, frequency of feeding, the ratio of hay to concentrates in the diet, and of supplementary feeds. J. Agr. Sci. 61:341.

Church, D. C. 1975. Digestive Physiology and Nutrition of Ruminants, Vol. 1, Digestive Physiology. (2nd ed.). O & B Books, Corvallis, Oregon.

Faichney, G. J. 1968. The production and adsorption of volatile fatty acids from the rumen of the sheep. Aust. J. Agr. Res. 19:791.

Hawk, P. H., B. L. Oser and W. H. Summerson. 1954. Practical Physiological Chemistry. (13th ed.). The Blakiston Co., Philadelphia, PA.

McCann, C. P., R. J. McLaren, R. L. Delay and J. K. Matsushima. 1973. Digestive tract cannulas for ruminants. Proc. West. Sec. Amer. Soc. Anim. Sci. 24:377.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

Sutton, J. D. and V. W. Johnson. 1969. Fermentation in the rumen of cows given rations containing hay and flaked maize or rolled barley in widely different proportions. J. Agr. Sci. 73:459.

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Official Agr. Chem. 46:829.

Waldren, D. C. 1971. A rapid micro-digesting procedure for neutral-acid detergent fiber. Can. J. Anim. Sci. 5:67.

Wheaton, N. H., N. W. Bradley, G. E. Mitchell, Jr., C. O. Little and J. A. Boling. 1970. Distribution of volatile fatty acids in rumen ingesta of steers fed concentrate and roughage diets. J. Anim. Sci. 30:601.

Whitelaw, F. G., J. Margaret Eadie, S. O. Mann and R. S. Reid. 1972. Some effects on rumen ciliate protozoa in cattle given restricted amounts of barley diet. Brit. J. Nutr. 27:425.

Running Head: Feedlot performance of steers fed single cell protein.

Feedlot Performance and Sensory Evaluation of Meat from Steers Fed a Single Cell Protein From Secondary Clarifiers of Pulp Mills[1,2]

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Key Words: Feedlot, Microbial Protein, Palatability, Single Cell Protein

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SUMMARY

Studies were conducted to evaluate three secondary clarifier single cell protein samples as sources of protein for ruminants. A feedlot trial was conducted with steers approximately 13 months of age and weighing 285 kg at the start of the experiment. Animals were randomly allotted to four treatments of 5 animals/treatment. The treatment diets contained 30% roughage and were formulated to have 13% crude protein with either cottonseed meal or three different SCP sources supplying the supplemental protein. The SCP ranged from 32.4 to 44.4% in crude protein. Animals were slaughtered and carcass data were collected. There were no differences ($P>.05$) between the CSM control and any SCP treatment for average daily gain. Carcass characteristics of steers fed either the CSM control or SCP diets showed no differences ($P>.05$).

Samples of longissimus muscles were used for sensory evaluation. Steaks were evaluated by a 14-member trained sensory panel. The 212 CZ-TEA treatment resulted in increased steak tenderness ($P<.05$), but there were no other significant differences for flavor, aroma, juiciness or overall desirability.

INTRODUCTION

Secondary clarifier single cell protein is a by-product of the pulp and paper industry. This SCP contains proteinaceous material formed during the digestion process involving microbial decomposition of the cellulosic material in the waste water from large pulp and paper mills. The use of single cell protein as a ruminant feed supplement would reduce a waste management problem for the paper industry as well as create a new protein source that should be less expensive than conventional sources.

The objective of the present study was to determine the effects of feeding secondary clarifier single cell protein on feedlot performance with steers and to determine if there were any adverse effects on the palatability of the meat.

MATERIALS AND METHODS

Feedlot Trial

A feedlot trial was conducted with 20 crossbred steers approximately 13 months of age which weighed 285 kg at the beginning of the experiment. Steers were randomly allotted to four treatments of 5 animals per treatment. The treatment diets contained 30% roughage and they were formulated to have 13% crude protein on a dry basis with either CSM or three different SCP (211 CZ-Sonic, 213 ITT-Sonic or

212 CZ-TEA) supplying the supplemental protein. All SCP samples were obtained from pulp mills in the Pacific Northwest. Two samples (211 CZ-Sonic and 212 CZ-TEA) were from the same pulp mill, but were dried differently (SD: sonic dehydration or TEA; triethylamine extraction; Aseltine, 1979).

Ration composition and nutrient analysis are shown in table 1. The steers were fed ad libitum with access to trace mineral salt and water at all times. Animals were implanted initially with 36 mg zeranol and again at approximately 370 kg body weight. Animals were weighed at weekly intervals and the experiment was terminated for each steer upon reaching a minimum of 8 mm backfat measured by an ultrasonic device. Final live weights were calculated by dividing cold carcass weight by .58. All animals were slaughtered at the Oregon State University Meats Laboratory and data were collected for carcass characteristics.

Total nitrogen, dry matter, ash and ether extract for SCP samples were determined by methods described in AOAC (1971). Acid detergent fiber was determined by use of the method of Van Soest (1963).

Taste Evaluation

Samples of longissimus muscle from the 11 to 13th rib were frozen and stored (-34 C) for subsequent palatability analysis by the Dept. of Food Science and Technology,

Oregon State University. Frozen steaks were cooked in matching gas ovens for 10 min on each side on broiler pans 18 cm from the flame. After 20 min thermocouples were inserted into the center of the steak which was cooked to a final internal temperature of 70 C. Three steaks from each of the four treatments were sampled by a 14-member trained sensory panel. Steaks were evaluated for aroma, tenderness, juiciness, flavor and overall desirability. The steaks were cut in such a way as to insure that each member of the panel always received a portion of steak from the same location on the steak.

Data from the feedlot trial were analyzed by use of one-way analysis of variance. Differences between means were tested for significance by the Newman-Keuls procedure as outlined by Steel and Torrie (1961). Sensory evaluation data were analyzed by use of a 3 factor analysis of variance and treatment means were compared using the LSD as described by Cochran and Cox (1957).

RESULTS AND DISCUSSION

The results of the feedlot trial are summarized in table 2. Animals consuming diet 211 CZ-Sonic (table 1) refused to eat all of the ration fines, therefore the amount of SCP was reduced in the diet from 7.75% to 6.00%. Particle size of the sonic-dried SCP was smaller than the

TEA SCP. However, there was only minimal feed refusal for the 213 ITT-Sonic diet which contained single cell protein dried the same way as the 211 CZ-Sonic.

No significant differences (P>.05) were found between treatments for average daily gain at trial termination. However, there was a slight reduction in average daily gain for all SCP treatments compared with the cottonseed control. Steers on the SCP diets began to reject the ration fines toward the end of the trial and this probably reduced performance somewhat. Feed conversion also was lowest (P>.05) for the cottonseed meal control. Average time on feed to reach a minimum of 8 mm of backfat was lower for the cottonseed meal control, however, this difference was slight compared with the other SCP treatments.

The carcass characteristics of steers fed either the control or SCP diets are shown in table 3. There were no significant differences (P> .05) for any characteristic studied. USDA grade did show some improvement for the 211 CZ-Sonic diet, however, this was not significant. The 211 CZ-Sonic and the cottonseed control treatments both had four choice and one good out of five steers. Ribeye area for 211 CZ-Sonic was slightly less than for the control, however, the marbling score was higher for the 211 CZ-Sonic compared to the control. Fat color score and lean color scores were not different (P >.05) for any treatments.

Sensory evaluation data of longissimus muscle steaks from the four treatment groups are summarized in table 4. When compared to the cottonseed meal control, the 212 CZ-TEA showed increased tenderness ($P<.05$). There were no other significant differences for flavor, aroma, juiciness, or overall desirability. There was a slight improvement for all treatments with regards to overall desirability when compared with the cottonseed meal treatment.

Data on chemical composition of SCP are summarized in table 5. It should be noted that the two SCP samples from the same paper mill (211 CZ-Sonic and 212 CZ-TEA) varied in chemical composition, especially with regards to crude protein and ether extract. The differences in crude protein indicate the variations in composition that were found in all samples obtained from the pulp mills. Single cell protein from the same pulp mill, but collected at a different time showed differences in composition.

Results indicate that the feeding of secondary clarifier single cell protein to beef steers had no apparent adverse effects upon animal performance. Single cell protein treatments also did not affect the aroma, tenderness, juiciness or overall desirability of the red meat.

One animal from the 213 ITT-Sonic treatment became ill at the end of the trial and those data were omitted from the results. However, there was no reason to believe that the illness was related to diet.

TABLE 1. PERCENT DIET COMPOSITION AND NUTRIENT ANALYSIS FOR FEEDLOT TRIAL

	NRC Reference No.	Treatment 211 CZ-Sonic	213 ITT-Sonic	212 CZ-TEA	Cotton-seed meal
Ingredient					
Straw, ryegrass	1-04-059	26.10	25.25	25.70	27.25
Alfalfa, ground	1-00-063	2.50	2.50	2.50	2.50
Molasses	4-04-696	7.50	7.50	7.50	7.50
Wheat, rolled	4-05-210	20.00	20.00	20.00	20.00
Barley, rolled	4-07-939	34.45	33.50	33.30	32.35
Tallow	4-07-880	1.25	1.25	1.25	1.25
Cottonseed meal	5-01-621	---	---	---	7.15
Urea	---	1.20	1.25	1.00	1.00
211 CZ-Sonic	---	6.00	---	---	---
213 ITT-Sonic	---	---	7.75	---	---
212 CZ-TEA	---	---	---	7.75	---
Salt, TM	---	.10	.10	.10	---
Limestone	---	.80	.80	.80	.90
Rumensin[a]	---	.05	.05	.05	.05
Vitamin A premix[b]	---	.05	.05	.05	.05
Analysis					
CP, %		13.02	13.03	13.06	12.99
Estimated TDN[c], %		66.00	65.98	65.99	66.08
Ca, %		.54	.55	.55	.55
P, %		.31	.32	.32	.33
CP from supplement, % of total		17.00	17.10	22.6	22.60
CP from urea, % of total		25.90	27.00	21.5	21.60

[a]Premixed at 66 g/kg.
[b]Premixed at 4,400,000 IU/kg.
[c]Assumes a value of 60% TDN for all sludges.

TABLE 2. FEEDLOT PERFORMANCE OF STEERS FED CONTROL AND SINGLE CELL PROTEIN DIETS

Variable	Treatment			
	211 CZ-Sonic	213 ITT-Sonic	212 CZ-TEA	Cottonseed Meal
Initial wt., kg	284.55	272.73	285.91	285.91
Final wt., kg[a]	460.00	465.91	469.09	463.64
Days on feed[b]				
Mean	129	132	131	121
Range	107-163	114-149	107-163	79-163
Gain, kg				
Total for 5 steers	877.27	965.00	915.45	887.73
Av. daily	1.36	1.45	1.41	1.50
Av. daily, range	1.24-1.49	1.23-1.74	1.05-1.80	1.11-1.70
Feed consumption, kg[c]				
Av. daily	9.29	10.20	9.73	9.81
Feed conversion				
kg feed/kg gain[c]	6.83	6.97	6.96	6.69

[a]Calculated from carcass weight divided by .58.
[b]Cattle slaughtered when back fat measured a minimum of 7 mm with an ultrasonic measuring device.
[c]Dry matter basis.

TABLE 3. CARCASS CHARACTERISTICS OF STEERS FED CONTROL OR SINGLE CELL PROTEIN DIETS

Variable	Treatment			
	211 CZ-Sonic	213 ITT-Sonic	212 CZ-TEA	Cottonseed meal
Maturity[a]	A-	A-	A-	A-
Kidney fat, %	2.60	2.75	2.40	2.50
Marbling score[b]	13.00	12.80	12.00	11.80
Fat color score[c]	5.60	5.25	5.60	5.40
Lean color score[d]	5.40	5.50	6.20	5.80
Fat thickness, cm	.96	.92	.97	.94
Ribeye, sq cm	67.08	70.31	71.98	71.98
USDA grade[e]	11.40	10.80	10.60	10.60

[a]A-; young A; older.
[b]Modest + = 16; small + = 13; trace = 6.
[c]1 = dark yellow; 6 = white
[d]8 = very dark red; 1 = bleached red
[e]Standard = 6; good = 9; choice = 11.

TABLE 4. SENSORY EVALUATION OF CONTROL AND SINGLE CELL PROTEIN-FED STEERS[a]

Variable[b]	Treatment			
	211 CZ-Sonic	213 ITT-Sonic	212 CZ-TEA	Cottonseed Meal
Aroma	5.83	5.96	5.93	5.83
Tenderness	5.91[c]	5.56[c]	6.16[d]	5.53[c]
Juiciness	5.83	5.30	5.57	5.43
Flavor	5.99	5.86	6.06	5.66
Overall desirability	5.80	5.53	5.79	5.37

[a]5 replications/treatment; 14 judgements/replication.
[b]Score range: 8, highest value to 1, lowest value.
[c,d]Means in the same line with different superscripts differ significantly ($P<.05$).

TABLE 5. CHEMICAL COMPOSITION OF SINGLE CELL PROTEIN USED IN FEEDLOT TRIAL

	Single cell protein type		
	211 CZ-Sonic	212 CZ-TEA	213 ITT-Sonic
DM, %	89.07	85.95	88.39
Crude protein, % of DM	41.34	44.40	32.44
Ash, % of DM	20.22	16.04	22.55
ADF, % of DM	19.49	18.10	16.74
Lignin, % of DM	10.60	9.41	6.83
EE, % of DM	1.10	.45	.77

LITERATURE CITED

AOAC. 1971. Official Methods of Analysis (11th ed.). Association of Official Agricultural Chemists. Washington, D.C.

Aseltine, M.S. 1979. Nutrient evaluation of secondary clarifier microbial paper sludge for ruminants. Thesis. Oregon State University, Corvallis, OR.

Cochran, W. G. and G. M. Cox. 1957. Experimental Designs. 2nd ed. John Wiley and Son, Inc., New York.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Official Agr. Chem. 46:829.

CONCLUSION

This study has produced considerable information regarding the use of single-cell protein from secondary clarifiers of pulp and paper mills.

Specific recommendations and conclusions that can be derived from this study include the following:

1. More specific information on the chemical make-up of the SCP product needs to be conduced. This includes analysis for nucleic acid and true protein.

2. Specific information on the solubility of the SCP.

3. Analysis for heavy metals and microtoxins.

4. Isolation and identification of specific microorganisms from the pulp mill SCP.

5. The effects of various drying methods on the solubility of the SCP.

6. It appears from this study that waste biological solids from pulp and paper mills are suitable for cattle and sheep feeding.