AN ABSTRACT OF THE THESIS OF

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in Animal Science presented in May 3, 1982

Title: A STUDY OF FEEDING VALUE AND POTENTIAL TOXICITY OF PULP MILL SINGLE CELL PROTEIN IN RUMINANTS.

Redacted for Privacy

Abstract approved: ______________________

D. C. Church

The objectives of this study were to evaluate the feeding value and potential toxicity of single cell protein (SCP) when fed to ruminants.

Three in vivo digestibility trials with lambs, one feedlot trial with steers and one long term feeding trial of high levels of SCP with sheep were conducted to accomplish the objectives of this study.

The in vivo digestibility trial with lambs showed that the SCP was utilized efficiently by the lambs at both 50 and 100% crude protein (CP) replacements of cottonseed meal (CSM). Supplementation of urea in the presence of SCP tended to increase ($P<.05$) the digestibility coefficients of the dietary nutrients.

Performance of the feedlot steers fed increased levels of CP supplementation from SCP were similar ($P>.05$) to CSM supplemented steers. There were increased ($P<.05$) Cd concentrations in the liver and spleen of steers fed with SCP protein supplementations. There were no toxicity problems due to any heavy metals present in the SCP.

Histological examinations further revealed no uncommon pathological signs in liver, kidney or muscle of the steers.

The long term feeding of growing sheep showed a significant increase of Cd concentrations in the liver (.445 and .614 ppm) and kidney (1.286 and 1.236 ppm) of sheep that were fed with Grays Harbor and Port Angeles SCP. Significant amounts of Pb (1.421 ppm) were found in the liver of sheep that were fed with Port Angeles SCP. However, the levels of Cd and Pb concentrated in these tissues were not considered too high to cause any ill effect to the sheep or to humans as a major consumer of animal products. Performance of the sheep throughout the experimental period was normal except for severe diarrhea in the group fed high levels of polyacrylamide which was used to concentrate the SCP during the drying process.

A Study of Feeding Value And Potential Toxicity of Pulp Mill Single Cell Protein In Ruminants

by

Osman Bin Atil

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed May 3, 1982

Commencement June 1982

APPROVED:

Redacted for Privacy

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Date thesis is presented May 3, 1982

Typed by Khadijah Bte Baharum for Osman Bin Atil

ACKNOWLEDGEMENT

I wish to express my sincere appreciation to Dr. D. C. Church for assistance and guidance throughout my graduate study at Oregon State University. Also, to the Public Service Department and Pusat Penyelidekan Atom Tun Ismail (PUSPATI) of Malaysia for their financial support.

I would also like to thank the Department of Animal Science and the Experimental Station for the assistance received.

I am indebted to Drs. J. E. Oldfield, W. H. Kennick, H. S. Nakaue, J. A. Harper and D. N. Moss for serving as members of my graduate committee.

My appreciation to Dean Hanson, Drs. P. D. Whanger, F. W. Adams, D. R. Buhler and others in the Department of Soil Science and Agricultural Chemistry for their technical assistance.

A deep appreciation to my family and deceased father, Encik Atil bin Maon in Malaysia, for their encouragement which made my study at Oregon State University a reality.

A special thanks to my dearest wife Lin, a great helper in typing my manuscripts and also a special acknowledgement for showing patience and love through some difficult times.

I owe this degree especially to ALLAH, my deceased father and to my dearest wife.

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Running Head: Single Cell Protein for Sheep.

Comparative Evaluation of Single Cell Protein from Potato and Pulp Mill Industries as Protein Supplements for Sheep[1]

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Key Words: Single Cell Protein, Pulp Mill, Potato Waste, Digestibility, Sheep

[1]Oregon Agricultural Experiment Station Technical Paper No._________.

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SUMMARY

Three in vivo digestibility trials with wether lambs were conducted: (1) to compare cottonseed meal (CSM) with single cell protein (SCP) from potato and pulp mill industrial waste water, (2) to investigate the effects of urea supplementation on the utilization of SCP, and (3) to evaluate the effects of levels of supplementation on the digestibility of diets containing high levels of SCP. SCP derived from potato industrial waste water tended to have high levels of ash. This SCP had a crude protein (CP) digestibility equivalent to 62% of CSM. Growing wether lambs did not show any signs of rejecting the high levels of SCP fed with the basal diets. However, the overall nutrient digestibility of diets tended to decrease as levels of SCP from potato waste increased. Supplementation of urea in the presence of pulp mill SCP tended to increase CP digestibility ($P<.05$) of the diets. When SCP (100SCP) provided all of the supplementary CP, digestible CP was equal to 50SCP:50CSM and 50SCP:50UREA ($P>.05$) but higher ($P<.05$) than the control group and less than ($P<.05$) 30SCP:70UREA. However, 100SCP lambs retained ($P<.05$) more nitrogen (N) as compared to control and were equally as efficient ($P>.05$) when compared to 50SCP:50CSM groups. There were no differences ($P>.05$) in digestible dry matter (DM), CP, organic matter (OM), ash, ether extract (EE), gross energy (GE) and N retention among the groups fed premixed diets containing CSM and pulp mill SCP dried in a Carver-Greenfield apparatus. Acid detergent fiber (ADF) digestibility of the positive control, 25-SCP and 50-SCP groups were

similar (P>.05), but they were higher (P<.05) when compared to the negative control. Estimated values of supplemental CP digestibility varied according to the method of calculation. Results suggested that calculation by difference will provide better results against negative control using data from groups of animals rather than using individual animals as their own control. The results further suggested that adaptation of lambs in sequence to both negative and positive control diets tends to produce a better overall dietary digestibility.

INTRODUCTION

Pulp mill single cell protein (SCP) or any other SCP produced via waste water treatment has a tremendous potential to increase the supply of such protein sources for feeding animals. The role of this novel product in animal feeding may depend on several factors; most important factors will be animal utilization and the cost of SCP relative to other conventional proteins such as cottonseed meal (CSM) or soybean meal (SBM).

In vitro and in vivo studies have been done in this laboratory (Aseltine, 1979; Kellems et al., 1981) with pulp mill SCP. Since the products tested were from pilot plants, the nutrient compositions of the SCP tend to vary from one batch to another. Estimated digestibility of SCP using sheep indicated that the crude protein of SCP from pulp mills varied from 66.3 to 69.9% of CSM.

Kellems and Church (1979) and Ortega (1981) have shown that the utilization of SCP by cattle tends to be improved when it was incorporated into liquid supplements. Several other workers have showed that urea supplementation improved utilization of SCP (Aseltine, 1979) and feather meal (Daugherty and Church, 1978; Aderibigbe, 1981).

The objectives of this study were : (1) to compare CSM to SCP from potato waste and pulp mills as protein supplements for wether lambs; (2) to further investigate the effects of urea supplementation on the utilization of SCP; and (3) to evaluate the effects of level of supplementation on the digestibility of diets containing high levels of SCP by wether lambs.

MATERIALS AND METHOD

Three in vivo trials were conducted with 20 crossbred wether lambs assigned at random according to initial body weight to respective treatments. There were 4 treatments of 5 lambs/treatment in trial 1: (1) Control (lambs were fed with basal diet only); (2) CSM-50 (CSM supplied 50% of the total N in the diet); (3) SCP-40 (SCP supplied 40% of the total N in the diet); and (4) SCP-50 (SCP supplied 50% of the total N in the diet).

In trial 2 there were 5 treatments of 4 lambs/treatment: (1) Control (lambs were fed with basal diet only); (2) 100SCP (100% of supplemental protein added was from SCP, which supplied 50% of the total N in the diet); (3) 50SCP:50CSM (SCP and CSM of supplemental protein were added at equal amounts based on N content and each supplied 25% of the total N in the diet); (4) 50SCP:50UREA (SCP and urea provided the supplemental N and each supplied 25% of the total N in the diet); and (5) 30SCP:70UREA (SCP and urea were added at a ratio of 3:7 based on the N content and each supplied 15% and 35% of the total N in the diet, respectively).

Trial 3 consisted of 4 treatments, with 2 treatments of 4 lambs/ treatment; (1) negative control, (2) positive control, and the other 2 treatments utilized 6 lambs/treatment; (3) 25-SCP (25% of the CSM protein was replaced with SCP) and (4) 50-SCP (50% of the CSM protein was replaced with SCP).

SCP of trial 1, which was furnished by OreIda Potato Co. (Caldwell, Id), had been prepared from potato waste by centrifugation followed by drum drying. ITT Rayonier Inc. (Port Angeles, WA) furnished the SCP in trials 2 and 3. This product was prepared from SCP grown on pulp mill effluent. It was concentrated using polyacrylamide and a belt press followed by drying in a Carver-Greenfield apparatus. The product (Ray Pro™) used in trial 2 was produced in a pilot plant. That used in trial 3 was produced in an on-line full scale plant. The chemical compositions of these SCP are listed in tables 3, 5 and 7 for trials 1, 2 and 3, respectively. The ingredients and chemical compositions of the experimental diets are listed in tables 1, 2 and 3, 5 and 7, respectively.

In trials 1 and 2, the lambs were preadjusted to the diets for 14 days in their respective pens. Following preadjustment, they were housed individually for 17 days in metabolism crates designed to allow individual feeding and separation of urine and feces with a 7-day adjustment period and a 10-day collection period. In trial 3, the 12 lambs which were allotted to receive 25-SCP and 50-SCP diets were placed on the negative control and followed by positive control diets. They were preadjusted for a period of 7 days followed by a 7-day adjustment period and a 5-day collection period in the metabolism crates. These 12 lambs were later randomly assigned to (3) 25-SCP and (4) 50-SCP diets with 6 lambs/ treatment. Four lambs/treatment were assigned to (1) negative control and (2) positive control diets. They were preadjusted for 7 days in pens, and housed in metabolism crates with an adjustment period of 7 days and a collection period of 5 days. The experimental plan was designed to determine if variability in estimating digestibility could be reduced by feeding each lamb in sequence the negative control, positive control, 25-SCP and 50-SCP as compared to the more typical method of having different animals on different diets at the same time or the more complicated Latin square method.

In trials 1 and 2, the basal diet and supplemental proteins (for supplemental diets) were weighed separately and thoroughly mixed together before feeding. Daily feed was divided into two equal parts and fed at 0800 h and 1600 h. The levels of CP in the negative control diet in trial 3 was similar to that in trial 1. The levels of CP in the experimental diets were also very similar to the experimental diets used in trial 1. However, the dietary CP in this trial were higher than that of trial 2. Protein supplements in trial 3 were premixed, and in trial 1 and 2, the CP supplements were added on top of the basal diet and thoroughly mixed before being fed to the lambs.

Daily records of feed consumption, feed refused and notations on the overall health of the lambs were recorded. Water and trace mineral salt blocks were made available to the lambs ad libitum throughout the experimental period. Total fecal and urinary excretions were collected daily from each lamb during the collection periods. Fecal samples were weighed and urinary excretions were measured using a graduated cylinder.

Ten percent aliquots of fecal and urinary excretions were removed, stored in individual containers at 5 C for later analyses. Five ml of phosphoric acid (85%) were added to the urinary collection buckets to prevent loss of N as ammonia.

Methods of analyses as described by AOAC (1975) were used to analyze total dry matter (DM), crude protein (CP), ash, organic matter (OM), ether extract (EE) and urinary N. Acid detergent fiber (ADF) was determined by using the modified micro-procedure of Van Soest (1963) described by Waldern (1971). Gross Energy (GE) was determined with a Parr adiabatic oxygen bomb calorimeter. Apparent digestibility coefficients for diet components and estimated apparent digestibility of supplemental proteins (by difference) were computed by the methods described by Schneider and Flatt (1975). In trial 3, an estimated apparent digestibility coefficient for components of supplemental protein were also computed using formulae in appendix table 1.

The data from each trial were analyzed statistically by use of a one-way analysis of variance as outlined by Steel and Torrie (1980). Means were compared using the LSD procedure as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Trial 1

Chemical composition of the basal diet and protein supplements are shown in table 3. CP contents of the SCP were lower than for CSM (38.51 vs 45.93%), but the levels of ash in the SCP were approximately 4 times (31.60 vs 7.16%) higher than CSM. Higher levels of ash in the

SCP were presumably due to soil contamination or chemicals used for removing peelings. The higher ash observed in the SCP contributed to the lower OM and GE values.

Mean apparent digestibility coefficients of the diets are presented in table 4. The percents DM, CP, ash, GE digestibilities and N retention (g/kg $BW^{.75}$) were different ($P<.01$) among the treatment groups. Increased levels of SCP decreased ($P<.05$) the DM, CP, ash and GE digestibilities of the diets. Even though the basal diet was supplemented with CP from SCP, the dietary DCP observed was lowest ($P<.05$) among the SCP treatments. The results indicate that the CP from SCP is less digestible than that from CSM. Calculations were made to estimate the apparent digestibility of supplemental protein used (table 4). The estimated DCP of SCP were 51.3 and 51.2% from SCP-40 and SCP-50, respectively. The estimated values of DCP of SCP relative to DCP from CSM was about 62%, a value not markedly different from other reports (Kellems et al., 1981). Fiber digestibility was somewhat lower than for CSM, and ash was markedly lower. The latter might be expected if soil made a significant contribution to the ash content. However, no good explanation is available for the reduced fiber or energy digestibility of the SCP. Perhaps the microorganisms in this product are particularly resistant to digestion.

N retentions expressed as grams per kilogram of metabolic weight (g/kg $W^{.75}$), were different ($P<.01$) among the groups. The CSM-50 diet was highest ($P<.05$) among the groups but there were differences ($P<.05$) among the control (6.2), SCP-40 (2.2) and SCP-50 (3.0) diets. When N retention was expressed as a percentage of N intake, the values obtained were no different (P>.05) among CSM-50, Control and SCP-40 diets, however the mean was highest for CSM and lowest for SCP-50. These data provide additional information indicating that CSM protein was utilized more efficiently than SCP.

Trial 2

Chemical composition of the basal diet and protein supplements in trial 2 are shown in table 5. CP of SCP and CSM in this study were 35.10 and 44.49%, respectively. Concentration of EE in the SCP was very high (42.44%) because the product was not put through a screw press as is done in a full scale Carver-Greenfield plant. Thus, the high EE increased the GE of the SCP (6.82 Kcal/g) considerably.

Apparent digestibility coefficients of the diets, protein supplement and N retention results were shown in table 6. CP, EE, Fecal N, urinary N excreted, N retention expressed per kg of metabolic body weight (g/Kg $BW^{.75}$) and as percent of N intake were different (P<.01) among the dietary treatments.

The order of the digestible CP values obtained were: 30SCP:70UREA > 50SCP:50UREA > 100SCP > 50SCP:50CSM > Control. These results were in agreement with previous findings by other workers (Daugherty and Church, 1978; Aseltine, 1979; Ortega, 1981; Aderibigbe, 1981; Shquier, 1981; Atil, unpublished data) indicating that urea supplementation tended to improve digestibility of dietary CP. There were smaller amounts of fecal N at higher levels (P<.05) of SCP in the diets. Higher amounts (P<.05) of urinary N were observed as urea increased in the diets. Data on both digestible CP and N retention further showed that either the SCP or SCP:CSM mixture were utilized efficiently by lambs. However, the SCP:UREA mixtures, although resulting in high DCP, also resulted in reduced N retention, a finding relatively common with diets high in urea (Hungate, 1966; McDonald and Warner, 1975; NRC, 1976; Church, 1979). Estimated digestibilities of CP of protein supplements were in the order of: 30SCP:70UREA > 50SCP:50UREA > 100SCP > 50SCP:50CSM, however the trend in values of N retention was the other way, except that 100SCP diet resulted in the highest N retention.

Digestibility of dietary EE was high in the SCP diets ($P<.05$), and estimated digestibility coefficients of protein supplements were all in the upper 95% range, no doubt a reflection of the large amounts of tallow in the SCP product.

There were no differences ($P>.05$) in digestibility coefficients of DM, ADF, OM, Ash and DGE. Computation of digestibility coefficients of protein supplements is shown in table 6. The digestibility of ADF from 30SCP:70UREA was highest. In spite of relatively similar intakes of ADF (table 5), the results observed may suggest that urea supplementation tended to increase the ADF digestibility. Dietary ash digestibility coefficients were relatively similar across the treatments. However, supplementation of SCP:CSM showed higher digestibility coefficients of ash. This result further indicates that the mineral in CSM was better utilized by the lambs.

Trial 3

Chemical composition of the supplemental protein and diets used in trial 3 were presented in table 7. CP levels of negative control (NC), positive control (PC), 25-SCP and 50-SCP were 14.6, 17.2, 18.3 and 19.4% of dry weight, respectively. SCP in 25-SCP and 50-SCP contributed 6 and 12% (table 2) of the total diets ingredients, respectively.

Apparent digestibility coefficients and N retention for trial 3 were shown in table 8. There were differences (P<.01) among treatments for digestibility coefficients of ADF. Higher (P<.05) values of ADF digestibility coefficients were observed in 25-SCP and PC followed by 50-SCP, but they were not different (P>.05) from the PC. The NC was lowest (P<.05) ADF digestibility coefficient in NC[a] was slightly higher than NC[d] (20.7 vs 18.6); however, the digestibility coefficient of ADF for PC[b] was slightly lower than the PC[d] (24.2 vs 27.3). The differences in this observation may be explained because the NC[a] and PC[b] were average values of 12 lambs and average values of 4 lambs were used in NC[d] and PC[d]. Thus, the former should be a better estimate of diet digestibility. However, the trends in digestibility coefficients of ADF observed were very consistent due to the fact that higher CP contents of diets generally increased the nutrient digestibility (Church, 1979): PC[b] (24.2) > NC[b] (20.7) and PC[d] (27.3) > NC[d] (18.6).

There were no differences (P>.05) in other nutrients or N retention among the treatment diets. The digestibility coefficients of DM among NC[a], PC[b] and dietary treatments[c] (DT[c]) and within the DT[c] were not much different. Within DT[c] a slight increase in the digestibility coefficients of CP were observed in these groups as supplemental SCP increased in the diets. This may be due to a slightly higher total CP present in the total diet of these groups (table 7). The 50-SCP diet allowed higher (P>.05) N retention (3.6 g) per unit of metabolic body weight than other treatments group. The N retention of 50-SCP was comparable to PC[d] and PC[b], the 50-SCP tended to be similar to PC[b] (3.6 g) and slightly higher than PC[d] (3.4 g). These results suggested that the SCP of this nature was comparable to CSM in its CP value when fed to growing lambs at 12% of the total diets. Data on fecal and urinary N indicated that, as the CP intake increased, higher amounts of N will be excreted in the feces and urine. This suggests that excess N in the diets was not utilized efficiently for synthesis of proteins. A higher percentage of N retention was observed in NC[a] and PC[b] when compared to DT[c]. Since the lambs in NC[a] and PC[b] were younger than when they were used later in DT[c], therefore a higher percentage of N retention was expected in the earlier groups. However, NC[d] and PC[d] were high in the percentage of N retention compared to 25-SCP or 50-SCP. The 25-SCP or 50-SCP were lower in percentage of N retention because the total dietary CP in these groups were higher than NC[d] or PC[d] diets. The digestibility coefficient of ash was highest in PC[b] (46.8%); this result was expected and it was in agreement to previous results in trial 1 and 2 in which higher amounts of CSM (25%) used in the diet ingredients tended to increase the digestibility of ash.

Digestibility coefficients of EE among the treatments were not different (P>.05) and they were all in the 90% range. The digestibility coefficient of GE were not significant among the DT[c]. The 50-SCP[f] tended to be similar to PC[d] and 25-SCP[e] was 1.6% lower than NC[d], although the total amount of GE in 50-SCP (4.6) was higher than PC[d] (4.5) and 25-SCP (4.4) was higher than NC[d] (4.3), (table 7).

Estimated digestibility coefficients (EDC) for DM, CP, OM and GE of supplemental proteins are shown in table 9. EDC of CP for CSM calculated using negative control by Individual^b (I^b), Group^c (G^c) and Group^d (G^d) were 78.8, 78.9 and 71.1%, respectively. When I^b was compared to G^c, an almost identical estimated value of CP digestibility of CSM was shown to have an almost similar SD. This result indicated that the calculations of EDC by G^c was equally as accurate as using I^b. However, when EDC was calculated using G^d method, the value was lower than I^b or G^c. These differences could be explained by; (1) the same number of lambs (12) were used in I^b and G^c, however, a different set of lambs (4) were used in G^d; (2) the 12 lambs were adapted to metabolism crate environment longer than the G^d and the G^d lambs were somewhat older than the former groups. The EDC of CP from CSM from I^b and G^c was similar to the data presented by Aderibigbe (1981), however EDC of CP from G^d was slightly lower. However, the CP digestibility coefficient of the NC^d diet was higher than NC^a by .3%, therefore the differences observed in EDC of these components may have resulted from the reasons stated earlier. The EDC of DM, OM and GE of CSM calculated as I^b, G^c and G^d followed a similar trend to CP.

The EDC of 25-SCP and 50-SCP derived from I^b, G^c and G^d were 69.3, 88.1, 83.4%, and 99.4, 93.6, 91.4%, respectively. EDC derived from I^b was highest in variability and lowest and highest in the EDC of CP values for 25-SCP and 50-SCP, respectively. Higher variability/SD within the lambs (6) using I^b may have been caused by lower amounts of SCP used in the diets of 25-SCP and the smaller number of lambs (6) which were used. Since these lambs were subjected to three dietary changes and shorter adaptation periods, these factors probably accounted for the higher variability. Greater variability in CP digestibility was observed in G^c and G^d of 25-SCP compared to 50-SCP. The differences between 25-SCP and 50-SCP may have been caused by the different levels of SCP used in the diets; the level of SCP in 50-SCP was twice as much of 25-SCP. The EDC of CP for SCP derived from 6 lambs of I^b of 25-SCP may be a little lower than that of G^c and G^d and a little higher in 50-SCP. However, in estimating the CP digestibility of SCP in this study, it appeared that the values derived from G^c and G^d were better since the variability observed was lower than by using I^b. The calculations of estimated values of digestibility of CP arrived at from I^b and G^c involved more work and time, therefore G^d was believed to be reliable, although possibly less accurate than G^c. The results further suggested that estimating digestibility coefficients of CP using individual lambs as their own control was accurate provided that adequate adaptation time and larger number of lambs were used.

Estimated digestibility coefficients calculated using the positive control was not a satisfactory method to estimate digestibility of the various components for supplemental protein.

CHAPTER I

TABLE 1. INGREDIENTS OF THE BASAL DIET FOR DIGESTIBILITY TRIAL WITH LAMBS IN TRIAL 1 AND 2[a]

Ingredients	IFN	Percent of diet[b]	
		Trial 1	Trial 2
Corn, ground	4-02-931	53.2	65
Ryegrass straw, chopped	2-04-073	21.8	28
Molasses, cane	4-04-695	5.9	6
Cottonseed meal, 41%	5-01-621	17.1	
Limestone	6-02-632	2	1

[a]Dry matter basis.

[b]Vitamin A was added at 800 IU per kg of diet.

TABLE 2. INGREDIENTS OF THE DIETS FOR DIGESTIBILITY TRIAL WITH LAMBS IN TRIAL 3[a]

Ingredients	IFN	Treatments: Negative control	Positive control	25-SCP[b]	50-SCP[c]
Corn, ground	4-02-931	45	32	33	39
Ryegrass straw, chopped	2-04-073	20	20	20	20
Molasses, cane	4-04-695	7	7	7	7
Cottonseed hulls	1-01-599	15	15	15	15
Cottonseed meal, 41%	5-01-621	12	25	18	11
Single cell protein				6	12
Limestone	6-02-632	1	1	1	1

[a]Percent of dry matter; vitamin A and D were added at 800 IU and 18 IU per kg of diet, respectively.

[b]Twenty-five percent of the cottonseed meal protein was replaced by pulp mill SCP.

[c]Fifty percent of the cottonseed meal protein was replaced by pulp mill SCP.

TABLE 3. CHEMICAL COMPOSITION OF DIETS AND PROTEIN SUPPLEMENTS IN DIGESTIBILITY TRIAL WITH LAMBS IN TRIAL 1[a].

Composition	Basal diet	Protein supplements		Complete diets[b]			
		CSM[c]	SCP[d]	Control	CSM-50	SCP-40	SCP-50
Dry matter, DM	92.6	92.5	94.3	92.6	92.6	93.0	93.1
Crude protein, CP	13.8	45.9	38.5	13.8	21.3	18.6	20.4
Acid detergent fiber, ADF	23.8	23.1	39.7	23.8	23.6	26.9	28.0
Organic matter, OM	94.5	92.8	68.4	94.5	94.1	89.4	87.6
Ash	5.4	7.2	31.6	5.4	5.8	10.5	12.4
Ether extract, EE	2.3	1.3	.7	2.3	2.1	2.0	1.9
Gross energy, GE Kcal/g	4.5	4.8	3.6	4.5	4.5	4.3	4.2

[a]Percent dry matter basis.

[b]Total consumptions of basal diets were 9264 and 7411 g in Control and the other three treatments plus 2247 g of CSM, 1788 and 2682 g of SCP in CSM-50, SCP-40 and SCP-50, respectively.

[c]Cottonseed meal.

[d]Single cell protein was grown on potato waste provided by Orelda Inc..

TABLE 4. APPARENT DIGESTIBILITY COEFFICIENTS OF DIETS AND ESTIMATED APPARENT DIGESTIBILITY COEFFICIENTS OF PROTEIN SUPPLEMENTS (CALCULATED BY DIFFERENCE) AND N RETENTION OF DIGESTIBILITY TRIAL WITH LAMBS IN TRIAL 1

Item	Apparent digestibility and N[a] retention of dietary treatments				SEM	Estimated digestibility and N retention of protein supplements		
	Control	CSM-50	SCP-40[b]	SCP-50		CSM-50	SCP-40[b]	SCP-50
Dry matter**, %	71.3^{e}	72.1^{e}	67.4^{d}	64.5^{c}	.79	74.5	50.9	45.8
Crude protein**, %	66.8^{d}	74.6^{e}	60.5^{c}	59.0^{c}	1.54	82.8	51.3	51.2
Acid detergent fiber, %	54.3	58.7	53.6	59.7	1.13	73.9	51.8	68.6
Organic matter**, %	72.9^{d}	73.5^{d}	$72.0^{c,d}$	70.4^{c}	.40	75.5	66.9	61.0
Ash**, %	43.9^{d}	48.9^{d}	28.6^{c}	22.7^{c}	2.71	61.4	17.7	12.7
Ether extract, %	85.4	83.9	82.2	83.9	1.11	74.7	40.3	70.7
Gross energy**, %	72.7^{d}	73.0^{d}	70.6^{c}	69.1^{c}	.48	73.8	59.7	57.0
N intake, g	204.6	328.8	273.8	328.9		165.2	110.2	165.2
Fecal N**, g	68.0^{c}	28.5^{d}	53.7^{e}	80.6^{f}	6.28	28.5	53.7	80.6
Urinary N, g	59.1	107.6	78.1	93.9	5.04	60.3	30.9	46.7
N retention**, g/Kg $BW^{.75}$	6.2^{d}	6.1^{d}	2.2^{c}	3.0^{c}	.58	6.1	2.1	3.0
N retention, % of N intake	37.9	58.6	51.9	46.9	3.97	46.2	23.3	23.0

[a]Statistical analyses valid for this group only; *$P<.05$, **$P<.01$.

[b]Average of 4 lambs, 1 lamb was sick and data were not used for analyses.

[c,d,e,f]Means in the same row with different superscripts differ ($P<.05$).

TABLE 5. CHEMICAL COMPOSITION (%) OF DIETS AND PROTEIN SUPPLEMENTS IN DIGESTIBILITY TRIAL WITH LAMBS IN TRIAL 2[a]

Composition	Basal diet	Protein supplements			Complete diets[b]				
		CSM[c]	SCP[d]	Urea	Control	100SCP	50SCP:50CSM	50SCP:50UREA	30SCP:70UREA
Dry matter, DM	89.6	89.2	95.7	98.0	89.6	90.7	90.1	90.3	90.1
Crude protein, CP	8.5	44.5	35.1	281.0	8.5	13.6	13.8	14.1	15.1
Acid detergent fiber, ADF	14.8	24.0	14.2		14.8	14.7	15.4	14.6	14.5
Organic matter, OM	95.7	93.3	95.8		95.7	95.7	95.5	94.6	94.0
Ash	4.3	6.8	4.2		4.3	4.3	4.5	4.2	4.2
Ether extract, EE	1.3	.8	42.4		1.3	9.1	5.3	5.5	4.0
Gross energy, GE Kcal/g	4.3	4.8	6.8	2.5	4.3	4.8	4.6	4.5	4.4

[a]Dry matter basis.

[b]Total consumption of basal diets were 8510 and 7170 g in Control and the other four treatments, respectively; plus 1680 g of SCP, 840 & 640 g of SCP & CSM, 840 & 98 g of SCP & Urea and 510 & 140 g of SCP & Urea in 100SCP, 50SCP:50CSM, 50SCP:50UREA and 30SCP:70UREA, respectively.

[c]Cottonseed meal

[d]Single cell protein from a product dried in a pilot plant using the carver Green-Field process. It was supplied by ITT Rayonier Inc..

TABLE 6. APPARENT DIGESTIBILITY COEFFICIENTS OF DIETS AND ESTIMATED APPARENT DIGESTIBILITY COEFFICIENTS OF PROTEIN SUPPLEMENTS (CALCULATED BY DIFFERENCE) AND N RETENTION OF DIGESTIBILITY TRIAL WITH LAMBS IN TRIAL 2

Item	Apparent digestibility and N[a] retention of dietary treatments					SEM	Estimated digestibility and N retention of protein supplements			
	Control	100SCP	50SCP:50CSM	50SCP:50UREA	30SCP:70Urea		100SCP	50SCP:50CSM	50SCP:50UREA	30SCP:70UREA
Dry matter, %	73.6	73.1	72.7	72.5	75.2	.49	71.2	68.6	64.4	92.7
Crude protein**, %	61.5^b	67.0^{c,d}	66.2^c	70.0^d	76.2^e	1.23	72.6	71.2	78.9	92.1
Acid detergent fiber, %	36.2	33.9	34.6	34.9	38.1	1.40	23.2	28.5	23.2	66.9
Organic matter, %	74.8	74.4	75.5	73.3	76.1	.55	72.7	68.7	61.2	94.9
Ash, %	47.1	45.0	51.0	47.0	46.0	.80	35.4	66.5	41.4	28.9
Ether extract**, %	60.6^b	91.7^c	88.1^c	88.2^c	85.2^c	2.99	95.9	95.3	95.6	96.1
Gross energy, %	73.6	74.5	73.7	73.3	75.7	.46	77.0	74.3	72.1	92.8
N intake, g	115.6	191.9	190.4	183.6	187.2		94.6	93.0	91.2	90.0
Fecal N**, g	44.5^b	63.4^{c,d}	63.7^d	56.7^c	44.6^b	2.19	26.0	26.8	19.3	8.3
Urinary N**, g	27.7^b	50.4^c	60.2^c	82.6^d	97.5^e	5.69	12.8	22.6	45.0	59.8
N retention**, g/kg $BW^{.75}$	2.8^b	5.0^d	4.2^{c,d}	3.3^{b,c}	3.0^b	.24	3.5	2.8	1.8	1.4
N retention**, % of N intake	37.2^c	40.9^c	34.9^c	26.1^b	24.2^b *	1.04	59.0	46.9	29.5	24.3

*P < .05 (applicable to a only).

**P < .01 (applicable to a only).

b,c,d,eMeans in the same row with different superscripts differ (P < .05).

TABLE 7. CHEMICAL COMPOSITION OF PROTEIN SUPPLEMENTS AND DIETS FOR DIGESTIBILITY TRIAL WITH LAMBS IN TRIAL 3[a]

Composition	Protein supplements		Complete diets			
	CSM	SCP	Negative control	Positive control	25-SCP[b]	50-SCP[c]
Dry matter, DM	91.1	97.5	89.1	89.0	89.3	89.3
Crude protein, CP	48.2	50.6	14.6	17.2	18.3	19.4
Acid detergent fiber, ADF	23.7	20.6	19.2	21.6	24.3	22.3
Organic matter, OM	93.3	93.6	95.4	95.0	95.1	94.9
Ash	6.8	6.4	4.6	5.0	4.9	5.1
Ether extract, EE	2.9	13.4	2.6	2.2	3.1	4.2
Gross energy GE, Kcal/g	4.8	5.4	4.3	4.5	4.4	4.6

[a]Percent dry matter basis.

[b]Twenty-five percent of the cottonseed meal protein was replaced by pulp mill SCP.

[c]Fifty percent of the cottonseed meal was replaced by pulp mill SCP.

TABLE 8. APPARENT DIGESTIBILITY COEFFICIENTS OF DIETS AND N RETENTION IN DIGESTIBILITY TRIAL WITH LAMBS FOR TRIAL 3

Item	Negative control[a]	SD	Positive control[b]	SD	Dietary treatments[c]: Negative control[d]	Positive control[d]	25-SCP[e]	50-SCP[f]	SEM
Dry matter, %	69.1	1.30	69.9	1.82	69.4	67.7	67.1	68.7	.36
Crude protein, %	63.3	2.31	67.2	2.72	63.6	65.5	65.8	67.0	.49
Acid detergent fiber**, %	20.7	.97	24.2	.92	18.6[g]	27.3[h,i]	27.7[i]	23.7[h]	1.05
Organic matter, %	70.4	1.29	71.1	1.88	70.7	69.7	68.3	69.0	.38
Ash, %	42.3	3.06	46.8	4.26	43.3	45.9	44.7	44.5	.92
Ether extract, %	90.2	2.17	88.5	4.92	90.6	88.7	89.6	90.3	.62
Gross energy, %	67.7	1.22	69.6	2.25	67.7	68.4	66.1	68.5	.42
N intake, g	97.0	4.89	114.2	5.76	117.8	137.6	140.1	140.6	2.53
Fecal N, g	35.6	2.70	37.4	4.02	42.8	47.5	47.9	46.4	.85
Urinary N, g	24.6	9.14	33.0	6.64	33.7	43.4	49.8	48.0	3.34
N retention, g/Kg $BW^{.75}$	3.0	.70	3.6	5.26	3.0	3.4	3.1	3.6	.24
N retention, % of N intake	37.9	8.62	38.3	5.20	35.0	34.0	30.0	32.9	2.25

[a]Average of 12 lambs; average total dry matter dietary intake was 4661 g, each lamb was fed premixed negative control diet at 3.5% of body weight (dry matter); these lambs were used in b later.

[b]Average of 12 lambs; average total dry matter dietary intake was 4653 g, each lamb was fed premixed positive control diet at 3.5% of body weight (dry matter); these lambs were randomly alloted in to two groups of 6 lambs each to be used in c for 25-SCP and 50-SCP treatments.

[c]Average total dry matter dietary intake were 5658, 5607, 5355 and 5082 g of Negative control, Positive control, 25-SCP and 50-SCP diets, respectively; diets were fed at 3.5% of body weight (dry matter).

[d]Average of 4 lambs; these groups were fed their respective diets before this experiment while the other 12 lambs were used in a and b.

[e]Twenty-five percent of the CSM protein was repalced by SCP in the diet; lambs in this group were previousely fed wit Negative control and Positive control diets; average of 6 lambs.

[f]Fifty percent of the CSM was replaced by SCP in the diet; lambs in this group were previousely fed with Negative control and Positive control diets; average of 5 lambs, 1 lamb was sick and data from this lamb were excluded from analyses.

[g,h,i]Means in the same row within each item having different superscripts differ (P .05).

**P .01 (applicable only in c).

TABLE 9. ESTIMATED APPARENT DIGESTIBILITY COEFFICIENTS OF SELECTED COMPONENTS OF SCP AND CSM CALCULATED BY DIFFERENCE USING NEGATIVE CONTROL AND POSITIVE CONTROL WITH INDIVIDUALS OR GROUPS OF LAMBS FOR TRIAL 3[a]

Components	Estimated digestibility coefficients of supplemental protein								
	CSM			25-SCP			50-SCP		
	Individual[b]	Group[c]	Group[d]	Individual[b]	Group[c]	Group[d]	Individual[b]	Group[c]	Group[d]
Using Negative Control[e]									
Dry matter, %	72.3 ± 6.3	72.7 ± 7.1	65.8 ± 3.8	29.1 ± 14.6	36.5 ± 15.2	30.9 ± 15.2	63.9 ± 9.6	58.0 ± 10.9	55.3 ± 10.9
Crude protein, %	78.8 ± 10.6	78.9 ± 10.8	71.1 ± 4.3	69.3 ± 37.4	88.1 ± 17.3	83.4 ± 17.3	99.4 ± 20.0	93.6 ± 8.4	91.4 ± 8.4
Organic matter, %	73.3 ± 6.5	73.3 ± 7.6	66.8 ± 4.0	24.0 ± 21.6	29.8 ± 16.8	24.5 ± 16.8	64.0 ± 10.0	58.7 ± 11.7	56.3 ± 11.7
Gross energy, %	75.4 ± 8.9	75.4 ± 9.0	70.5 ± 6.0	35.3 ± 20.9	41.1 ± 20.6	40.9 ± 20.6	80.6 ± 9.8	74.9 ± 4.2	74.8 ± 4.2
Using Positive Control[f]									
Dry matter, %				19.5 ± 16.4	23.8 ± 15.2	44.9 ± 15.2	55.8 ± 9.1	52.0 ± 10.9	61.9 ± 10.9
Crude protein, %				12.1 ± 45.6	26.8 ± 17.3	54.3 ± 17.3	70.0 ± 12.4	64.9 ± 8.4	77.8 ± 8.4
Organic matter, %				17.8 ± 30.2	18.6 ± 16.8	40.0 ± 16.8	57.4 ± 8.6	53.4 ± 11.7	63.5 ± 11.7
Gross energy, %				8.8 ± 17.7	11.0 ± 20.6	31.1 ± 20.6	64.2 ± 18.4	60.8 ± 4.2	70.2 ± 4.2

[a]Calculated using formula in appendix 1; digestibility coefficients (percent) ± standard deviation.

[b]Using individual lamb as their own control; in CSM total number of lambs were 12, in SCP-25 and SCP-50 total number of lambs were 6 of which they were the same lambs used in Negative control[a] and Positive control[b] (refer table 8); for further detail refer to text.

[c]Digestibility coefficients in this group were calculated using group average of which Negative control[a] and Positive control[b] were used as a basis of calculation (please refer to text for further explaination).

[d]Digestibility coefficients in this group were calculated using group average of which Negative control[d] and Positive control[d] were used as a basis of calculation (please refer to text for further explaination).

[e]Negative control used were Negative control[a] (table 8) in calcualtion of digestibility coefficients for Individual[b] and Group[c]; Negative control[d] (table 8) was used to calculate the digestibility coefficients for Group[d].

[f]Positive control used were Positive control[b] (table 8) in calculation of digestibility coefficients for Individual[b] and Group[c]; Positive control[d] (table 8) was used to calculate the digestibility coefficients for Group[d].

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Running Head: Performance and tissue composition of heavy metals in steers.

Effect of Pulp Mill Single Cell Protein when Fed to Feedlot Steers on Performance, Carcass Quality, Sensory Evaluation and Tissues Residue of Selected Heavy Metals[1]

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Key Words: Feedlot Steers, Single Cell Protein, Performance, Palatability, Heavy Metals, Tissues Residue

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SUMMARY

Eighty crossbred beef steers were randomly allotted to eight experimental groups, utilizing two groups (replicates) per treatment. The steers were fed a concentrate basal diet plus .91 kg/head/day of a four different supplements for an average period of 106.5 days. The dietary treatments consisted of a positive control supplement in which cottonseed meal was a major ingredient. In experimental supplements, single cell protein (SCP) from a pulp mill replaced 20, 40 or 60% of the cottonseed meal protein. At termination of the trial, three steers from each treatment were slaughtered and tissue samples were taken for heavy metals analyses and histological evaluations, and rib steaks were obtained for organoleptic evaluation. There were no differences (P>.05) due to treatments in animal performance or carcass quality. There were differences (P<.05) in juiciness and overall desirability of the steaks among the treatment groups. Cr, Cu and Cd concentrations in dietary supplements were increased by increased levels of SCP. There were differences in concentration of Cr in liver and heart (P<.01) and kidney (P<.05) among the treatments. Cr concentration in liver and heart were higher (P<.05) in the control groups. However, kidney Cr concentrations were higher (P<.05) in SCP-40 and SCP-60 groups. Cu concentration in liver, heart and spleen were similar (P>.05) among the treatments. Concentration of Cu in kidney was lowest (P<.05) in the SCP-60 treatment. Concentration of Zn was found at similar levels (P>.05) among the

treatments in liver and spleen. Lowest (P<.05) concentration of Zn was found in the kidney and heart of the SCP-60 treatments. There were increased (P<.05) Cd concentrations in the liver and spleen from steers consuming the dietary SCP supplements but there were no differences in kidney, bone and heart tissues (P>.05). Pb concentration in liver and bone were very low (P>.05). Histological examinations revealed no uncommon pathological signs in the liver, kidney and muscle of the twelve steers.

INTRODUCTION

Higher cost of waste water treatment (as enforced by the EPA) in the pulp mills industries of the Pacific Northwest region has lead the industries to produce single cell protein (SCP) from its cellulose-rich effluents. This novel product was produced by methods previously described by Aseltine (1979) and Church et al. (1982). However, the region has potential daily production of this SCP on the order of 300 dry tons (D. C. Church, personal communication). The SCP of this type was comparable in its crude protein (CP) content to cottonseed meal (CSM) (Aseltine, 1979; Kellems et al., 1981; Atil, 1982). Several studies evaluating this product by laboratory techniques (Aseltine, 1979; Kellems et al., 1981) showed good promise that the SCP can be fed successfully to ruminant animals. In vivo digestibility trials (Aseltine, 1979; Kellems et al., 1981; Atil, 1982) using sheep revealed that digestibility of the CP from SCP was ca. 70% of CSM. SCP of this type were also tested with growing cattle fed on diets containing 30% roughage (Aseltine, 1979) and liquid supplements (LS) (Kellems and

Church, 1979). There were no differences in performance of the steers (P .05) fed SCP diets or a basal CSM diet (Aseltine, 1979). However, Kellems and Church (1980) showed that the cattle fed on LS containing SCP tend to gain more weight.

Laboratory analyses of the major and trace elements (Kellems et al., 1981; Church et al., 1982; Atil, 1982) from the SCP showed that some of the potentially toxic heavy metals tend to be higher than in conventional protein sources such as CSM. However, heavy metals contents such as Cu, Zn and Pb of the SCP is much less than the Chicago digested sludge or Pensacola liquid digested sludge (Bertrand et al., 1980; 1981). Two other heavy metals, Cd and Cr, in the SCP were found to be at similar levels to that found in the Pensacola sludge.

The objective of this study was to evaluate the effects of feeding finishing beef steers SCP produced from pulp mills on performance, carcass quality, palatability of the meat and the concentrations of selected potentially toxic heavy metals (Cr, Cu, Zn, Cd and Pb) in liver, kidney, spleen, heart and bone tissues.

MATERIALS AND METHODS

Eighty crossbred beef steers (average 444 kg) of British breeding were weighed and allotted at random to eight experimental groups of ten steers each. The eight experimental groups, utilizing two groups (replicates) per treatment, were assigned to four pelleted supplement diets (table 2): (1) Control (supplement diet containing 50% CSM); (2) SCP-20, (3) SCP-40 and (4) SCP-60; supplement diets in which 20, 40 and 60% of the CSM protein was replaced by SCP, respectively. All steers were fed a concentrate basal diet (table 1) ad libitum plus .91 kg/head/day of their respective pelleted supplement diet (table 2) for 110 days and 103 days for replicate 1 and 2, respectively. Before the experimental period the steers were adapted to feedlot conditions through feeding of alfalfa (IFN: 1-00-063) pellets and rolled barley (IFN: 4-07-939) for a period of 3 weeks.

Ear tagging, deworming and vaccination were done during the adaptation period. After an overnight shrink off feed, individual animal weights were obtained at the beginning and end of the experiment. Individual animal weights were also obtained at biweekly intervals to check on individual performance and health of the steers on each of the four diet supplements. Samples of the concentrate basal diet and supplement diets were collected routinely on a weekly basis. One steer had to be removed from replicate 2 of the control group during the experimental period due to sickness; the data from this steer were not used in analysis of the results. At day 80 of the experimental period, a local veterinarian took fat biopsies near the tail head of three animals (randomly selected) from each treatment.

At the termination of experiment, 68 steers were slaughtered at a commercial packing plant and 12 steers representing 4 treatments which had been biopsied were slaughtered at the Meat Science Laboratory of Oregon State University (OSU). Carcass data on the 68 cattle were compiled by a USDA grader, and carcass quality data for the 12 cattle killed at OSU were taken by personnel at the Meat Science Laboratory.

From the 12 cattle which had been biopsied, samples of liver, muscle and kidney were obtained for histological examination by the

OSU Veterinary Diagnostic Laboratory. Steak samples of muscle (Longissimus dorsi) from the 11th to 13th rib were taken, frozen and stored at -34 C until subsequent organoleptic (taste panel) evaluations were made by the OSU Department of Food Science and Technology. 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 steaks which were cooked to a final 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.

Samples of tissues from liver, kidney, spleen, heart and bone (thoracic vertebra) were also taken at slaughter. They were freeze dried and kept in precleaned polyethylene bottles (Nalgene) until analyzed for heavy metals (Cr, Cu, Zn, Cd and Pb) contents. All glassware and polyethylene bottles used in the analysis of heavy metals were soaked in 5N HNO_3 for at least 24 h and rinsed five times with glass double distilled water. About .5 g freezed dried samples (in duplicate) and 1 g of supplement diets (in duplicate) were subjected to wet ashing in 15 ml of HNO_3 (reagent grade) and 3 ml of $HClO_4$ (reagent grade). After the digestion was completed, the residue was dissolved and diluted with 0.1 N HNO_3 to 10 ml. Digests were stored until analysis in precleaned polyethylene bottles (Nalgene). Standard solutions were made by diluting 1000 ppm stock solution (J. T Baker Chemical Co., Phillipsburg, NJ 08865) to appropriate working ranges. National Bureau of Standards (NBS), standard reference material (SRM) 1577 from bovine liver, NBS SRM 1566 oyster tissues and NBS SRM 1571 orchard leaves were used to calibrate instrument and working standards and also to double check contamination of the glassware and reagents used in the digestion process. The analytical results for all of the heavy metals analyzed from NBS SRM samples were within the error limits of certified values (Appendix 2). Samples were analyzed using flame atomic absorption (Perkin-Elmer Model 4000). Samples containing elements which were not detectable by flame technique were analyzed with a graphite furnace (Perkin-Elmer Model 2100) as outlined by Davidson and Secrest (1972) for Cr and Poldoski (1980) for Pb and Cd.

The data on performance, carcass quality and selected heavy metals content of the tissues were analyzed statistically using one-way analyses of variance as outlined by Steel and Torrie (1980). Means were compared using the LSD procedure as described by Snedecor and Cochran (1967). Sensory evaluation data were analyzed by use of 3 factor analysis of variance and treatment means were compared using the LSD as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Animal Performance

Data on the performance of the steers are shown in table 3. No differences (P>.05) were found among treatments in average daily gain (ADG), average feed consumed (AFC) or feed conversion (FC). The ADG (1.32 to 1.35 kg/day) observed were normal because the initial body weight (IBW) of the steers were heavy (444 kg). Thus, these data provide evidence of no problems in using SCP as a CP supplement in finishing steer rations. Data reported by Aseltine (1979) showed a slight reduction in ADG for SCP treatment groups compared to a CSM control. He also reported that the steers began to reject the ration fines from SCP diets toward the end of the trial. However, the SCP used by Aseltine were from different sources and were dried with different procedures than the product used in our trial.

In the present study, the steers readily consumed the SCP-supplemented diets. The dry feed consumption in this trial was almost identical (P>.05) among treatment groups, but there was a trend (P>.05) for an improved FC as the level of SCP increased in the supplements. This may have been a reflection of an increase in energy consumption because of the fat content of the SCP. Similar findings were reported by Kellems and Church (1980) on improved FC (P>.05) by steers fed on liquid supplements containing SCP.

The carcass data of the treatment groups are shown in table 4. There were no differences (P>.05) in quality grade, yield grade, hot carcass weight, dressing percentage, percentage kidney fat, back fat thickness or marbling score among the treatment groups. These data were in agreement with the data reported by Aseltine (1979). There was a trend for a slight reduction of quality grade and marbling score as SCP increased in the supplement diets. However, the carcass grades averaged low choice, being lowest in the SCP-60 group (7.2) and highest in the control group (7.6).

Sensory evaluation data are summarized in table 5. There were no differences (P>.05) in aroma, tenderness and flavor of the steaks among treatment groups. There were differences (P<.05) in juiciness and overall desirability of the steaks among the treatment groups. Steaks from the control treatment were juicier (P<.05). For overall desirability, there were no differences (P>.05) between the control and the SCP-60 treatment. Differences (P<.05) in overall desirability was observed among control to SCP-20 and SCP-40; and SCP-60 to SCP-20 treatment. Overall steak desirability data indicate that 60% CSM replacement by SCP tend to be better than 20 or 40%. SCP-60 steaks was rated highest for flavor (5.44) in spite of high fat content of the SCP. This suggests that the fat present in the SCP produced no detrimental effect on the flavor of the meat.

Heavy Metal Concentrations

Data on concentrations of selected heavy metals in SCP and supplement diets are presented in table 6. Increased levels of SCP in the supplement diets tended to increase the concentration of Cr, Cu, Zn and Cd. Pb was found at highest concentration in the following order; SCP-40 > SCP-20 > Control > SCP-60. Since the Pb concentration in the control diet was similar to that in the SCP-20 and SCP-40 diets and lowest in SCP-60, this indicates that Pb content was not increased by SCP content or that some contamination may have occurred in manufacture of the feed.

Selected heavy metals concentrations found in liver are presented in table 7 . There were differences (P<.01) in the concentrations of liver Cr and Cd among the treatments. Liver Cr from steers fed SCP-supplemented diets were normal when comparison was made against the NBS SRM 1577 bovine liver. These data suggest that the Cr in this SCP was not well absorbed or, if absorbed, it was not retained. The highest liver Cr was found in the control group (.127 ppm) even though the control diet contained much less Cr. The concentrations of Cd in liver between Control and SCP-20 cattle were less than ($P<.05$) from SCP-40 and SCP-60 treatments. Even though the level of liver Cd in SCP-40 (.375 ppm) and SCP-60 (.389 ppm) treatments were highest, they still were within normal values as reported by Stable-Taucher et al. (1975), NBS (1977) and Doyle and Spalding (1978). Cd concentration in the liver of lambs from the United Kingdom was .14 ppm of dry weight (Mills and Dalgarno, 1972). Lee and Jones (1976) from Australia reported Cd concentration in the liver of lambs was .35 ppm of dry weight. Concentration of Cd in the liver of broiler chickens and pigs (Doyle and Spalding, 1980) was .5 ppm (dry weight) and .24 ppm (wet weight), respectively. Therefore, although there were higher ($P<.05$) concentrations of Cd in the liver of SCP-40 and SCP-60 steers, the differences among diets were much less than those among species. The concentrations of Cu, Zn and Pb in liver were similar ($P>.05$) among the treatments. However, Cu and Zn concentrations in the liver of the steers in this study were slightly higher than the reported values (NBS, 1979; Doyle and Spalding, 1980; NRC, 1980). Concentrations of Zn within the liver of control steers were less variable than the SCP groups. Since SCP consumption increased Zn intake with little effect on liver Zn concentration, it may be that Zn in the SCP is less available to the animal. Pb concentration in the livers was much less than the reported values (Stable-Taucher et al., 1975; NBS 1977; Doyle and Spalding, 1978; NRC, 1980). Data in this study indicated that the concentrations of Pb found in major tissues such as liver and bone were very low since liver was the major tissue which stored Pb, accumulation of Pb in other edible tissues such as muscle is not likely.

Concentrations of selected metals in the kidney and bone are shown in tables 7 and 8, respectively. Cr concentrations were similar (P>.05) among the kidney of steers consuming SCP supplements. These data suggested that the inclusion of SCP up to 60% CP replacement of CSM will not increase the retention of Cr in the kidney. This agrees with data of Bertrand et al. (1980; 1981) who fed sewage sludge to growing beef steers and reported no accumulation of Cr in the kidney. The values reported were less than .10 ppm (Bertrand et al., 1980) and .73 ppm (Bertrand et al., 1981) of the wet weight. Kidney Cr levels in this study were much less than the reported values by Bertrand et al. (1980; 1981); even though the concentrations of Cr in the bone ranges from .631 to .814 ppm, there were no differences (P>.05) among the treatment groups. Cu and Zn concentrations in kidney tissues were higher (P<.01) among the treatment groups. However, the data did not suggest any significant contribution of SCP supplement toward increased level of kidney Cu and Zn. The concentration of kidney Cd was within the range reported by Powell et al. (1964) and Doyle et al. (1974). There was a trend (P>.05) towards a gradual increase in kidney Cd as the dietary Cd level increased. Cd concentrations found in the bone were very low (P>.05) among the dietary groups.

Concentrations of selected heavy metals in heart and spleen are shown in table 8. Dietary treatments had no effect (P>.05) on concentrations of Cu and Cd of heart and Cr, Cu and Zn of spleen, respectively. Even though there were differences (P<.01) in Cr and Zn concentrations in heart among the groups, the differences were not attributed to dietary SCP since the SCP-60 group was lowest ($P<.05$) in Cr and Zn. However, there was a trend increasing levels of the dietary SCP tended to decrease the concentrations of Cr and Zn in liver and heart. Cd concentrations in spleen increased ($P<.01$) among the groups that received the SCP supplements.

Results of a histological examination of liver, kidney and muscle from the 12 animals were reported by Dr. J. Schmitz. There were moderate inflammatory cell infiltrates in 11 of the livers, and 1 liver from the control group was normal. This finding was rather normal and it was not due to dietary treatments (Schmitz, written report). Suppurative nephritis of the kidney were observed in one of the control, two of the SCP-20, none in SCP-40 and two in SCP-60. Since nephritis was present in approximately equal numbers among the treatment groups, the findings did not suggest any chronic inflammatory change in the kidney. Histological samples of the muscle from SCP treatments were all normal. However, there were 2 muscles from the control group that showed degenerating myofibers. These findings were not uncommon and were not significant (Schmitz, written report).

CHAPTER II

TABLE 1. CONCENTRATE BASAL DIET FED TO STEERS FINISHING TRIAL

Ingredients	IFN	Percentage
Alfalfa, pellets	1-00-063	5
Barley, rolled	4-07-093	40
Beet pulp, ground	4-00-672	10
Corn, ground	4-02-931	15
Wheat, rolled	4-05-210	30

TABLE 2. SUPPLEMENT DIETS FED WITH THE CONCENTRATE DIET (PERCENT) IN STEERS FINISHING TRIAL

Ingredients	IFN	Treatments[a]			
		Control	SCP-20	SCP-40	SCP-60
Cottonseed meal, 41%	5-01-621	50	40	30	20
Single cell protein			15.5	31.5	47
Urea	5-05-670	4	4	4	4
Limestone	6-02-632	9.75	9	8.5	8
Molasses, cane	4-04-696	5	5	5	5
Barley, ground	4-07-093	29.9	25.15	19.95	14.65
TM-10		.35	.35	.35	.35
Vitamin A premix[b]		1	1	1	1

[a]Control treatment containing 50% CSM; in SCP-20, SCP-40 and SCP-60, 20, 40 and 60% of crude protein from CSM was replaced by SCP, respectively.

[b]1,500,000 IU/g.

TABLE 3. PERFORMANCE OF STEERS FED VARYING AMOUNTS OF SCP IN THE FINISHING TRIAL

Item	Treatments				SEM
	Control	SCP-20	SCP-40	SCP-60	
No. of steers	19[a]	20	20	20	
Initial weight, kg	433.98	445.6	444.4	441.9	8.83
Final weight, kg	583.9	586.1	583.1	584.9	10.63
Days on feed	106.1	106.5	106.5	106.1	.40
Total weight gain, kg	139.9	140.7	140.2	143.0	4.58
Avg. daily gain, kg	1.32	1.32	1.32	1.35	.04
Avg. feed consumed, kg/day	9.4	9.3	9.2	9.3	
Basal	9.4	9.3	9.2	9.3	
Supplement	.84	.85	.85	.89	
Feed conversion[b];					
Basal	3.23	3.19	3.18	3.14	
Supplement	.29	.30	.30	.30	

[a]One steer died at an early stage of the experiment due to sickness.

[b]Kilogram of feed consumed per kg of body weight gain.

TABLE 4. CARCASS DATA OF STEERS FED VARYING AMOUNTS OF SCP IN THE FINISHING TRIAL

Item	Treatments				SEM
	Control	SCP-20	SCP-40	SCP-60	
Avg carcass quality grade[a]	7.6	7.4	7.5	7.2	.14
Avg carcass yield grade[b]	2.75	2.60	2.82	2.79	.10
Avg hot carcass wt, kg	353.73	353.20	354.25	349.18	7.82
Avg dressing percent	61	60	61	60	
Kidney fat, %	2.52	2.34	2.48	2.49	.06
Back fat thickness[c], cm	1.76	1.53	1.69	1.80	.06
Marbling score[d]	13.84	13.25	13.20	12.90	.42

[a]Based on USDA quality grade standard; standard = 4, good = 6, choice = 8

[b]Yield grades range from 1 to 5 with 1 having the most lean meat.

[c]Back fat is measured over the ribeye at the 12th rib of the carcass.

[d]Intramuscular fat in L. dorsi which is a visual estimate where small- is = 11 (minimum for choice grade).

TABLE 5. MEANS OF SENSORY EVALUATION SCORES OF COOKED STEAKS FROM STEERS OF THE FINISHING TRIAL

Item[a]	Treatments				SEM
	Control	SCP-20	SCP-40	SCP-60	
Aroma	5.76	5.75	5.78	5.61	.61
Tenderness	5.44	5.44	5.08	5.61	.55
Juiciness*	6.17[b]	5.19[d]	5.33[c,d]	5.69[c]	.44
Flavor	5.33	5.14	5.36	5.44	.76
Overall desirability*	5.92[b]	5.28[d]	5.31[c,d]	5.75[b,c]	.44

[a]Score range based on a scale of 1 to 8; with 8 being the highest and 1 the lowest value.

[b,c,d]Means in the same line with different superscripts differ ($P<.05$).

*$P<.05$.

TABLE 6. CONCENTRATIONS OF SELECTED HEAVY METALS IN SCP AND SUPPLEMENT DIETS FED TO FEEDLOT STEERS, PPM

Metals	SCP	Treatments[a]			
		Control	SCP-20	SCP-40	SCP-60
Cr	46.9	2.60	6.50	10.97	14.90
Cu	16.1	16.69	19.00	18.42	21.57
Zn	73.4	77.9	52.29	63.07	87.32
Cd	6.7	2.10	3.44	4.79	4.62
Pb	8.7	19.91	20.15	21.32	13.61

[a]Samples were analyzed in duplicate.

TABLE 7. CONCENTRATIONS OF SELECTED HEAVY METALS IN LIVER AND KIDNEY OF FEEDLOT STEERS FED VARING AMOUNTS OF SCP[a]

Metals	Treatments			
	Control	SCP-20	SCP-40	SCP-60
No. of steers	3	3	3	3
	Liver[b], ppm dry weight			
Cr*	.127[e] ± .007	.095[d] ± .007	.085[d] ± .009	.072[d] ± .072
Cu	262.37 ± 38.22	197.68 ± 16.20	201.74 ± 11.54	218.21 ± 37.95
Zn	157.01 ± 7.50	158.53 ± 26.12	150.42 ± 25.89	177.30 ± 20.16
Cd**	.250[d] ± .021	.221[d] ± .018	.375[e] ± .042	.389[e] ± .064
Pb	.031 ± .329	.039 ± .538	.052 ± .756	.046 ± .745
	Kidney[b], ppm dry weight			
Cr*	.121[d] ± .007	.143[d,e] ± .008	.161[e] ± .011	.155[e] ± .013
Cu**	19.69[e] ± .76	20.36[e] ± .45	19.32[e] ± .45	14.35[d] ± 1.48
Zn**	93.68[d] ± 5.80	125.29[e] ± 9.53	97.63[d] ± 3.22	92.17[d] ± 2.23
Cd	2.08 ± .12	2.27 ± .14	3.07 ± .50	2.37 ± .07

[a]Mean ± standard error.

[b]From duplicate samples.

[d,e]Means in the same row with different superscripts are different ($P<.05$)

*$P<.05$; **$P<.01$

TABLE 8. CONCENTRATIONS OF SELECTED HEAVY METALS IN HEART, SPLEEN AND BONE OF FEEDLOT STEERS FED VARING AMOUNTS OF SCP[a]

Metals	Treatments			
	Control	SCP-20	SCP-40	SCP-60
No. of steers	3	3	3	3
	Heart[b], ppm dry weight			
Cr**	.565[d] ± .050	.529[d] ± .015	.485[d] ± .019	.108[c] ± .003
Cu	15.70 ± .36	16.42 ± .18	15.75 ± .20	15.59 ± .52
Zn**	84.64[d] ± 1.15	83.52[d] ± 3.38	82.38[d] ± 1.39	75.07[c] ± 1.59
Cd	.022 ± .789	.106 ± .149	.137 ± .197	.140 ± .442
	Spleen[b], ppm dry weight			
Cr	.112 ± .014	.249 ± .137	.162 ± .005	.141 ± .032
Cu	4.02 ± .24	4.09 ± .11	4.13 ± .13	4.08 ± .27
Zn	112.89 ± 3.19	110.70 ± .56	107.31 ± 2.79	107.00 ± 2.17
Cd**	.026[c] ± .078	.032[d] ± .217	.034[d] ± .184	.039[d] ± .283
	Bone[b], ppm dry weight			
Cr	.767 ± .154	.814 ± .057	.711 ± .139	.631 ± .203
Cd	.083 ± 1.984	.083 ± 2.621	.022 ± .603	.091 ± 3.787
Pb	.027 ± .325	.057 ± 1.027	.069 ± 2.028	.037 ± .714

[a]Mean ± standard error.

[b]From duplicate samples.

[c,d]Means in the same row with different superscripts are different ($P<.05$)

*$P<.05$; **$P<.01$.

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Running Head: Heavy metals residue and performance of lambs.

Heavy Metals Residue in Tissues and Performance of Growing Lambs Fed High Levels of Pulp Mill Single Cell Protein[1]

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Key Words: Heavy Metals, Tissue Residues, Single Cell Protein, Sheep Performance, Polyacrylamide

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SUMMARY

Twenty-five wether lambs were randomly allotted to five group treatments to study the long term effects of feeding high levels of pulp mill single cell protein (SCP) on concentration of selected potentially toxic heavy metals in liver, muscle, kidney, heart, spleen and bone tissues and on animal performance. One group of five lambs (Negative control; NC) were killed at the beginning of the study. The other four groups were assigned to (1) Positive control (PC) diet containing cottonseed meal (CSM) as the major supplemental crude protein (CP); (2) Grays Harbor (GH) diet in which 33.3% of the total CP was supplied by GH SCP; (3) Port Angeles (PA) diet in which 33.3% of the total CP was supplied by PA SCP; (4) polyacrylamide (P) diet which contained 2.5% polyacrylamide polymer (Calgon's WT 2580). Concentrations of Cr in liver ($P<.01$), spleen ($P<.01$) and kidney ($P<.05$) were different among the treatment groups. Pb in liver, muscle and kidney were different ($P<.01$) among the treatments. Concentrations of Pb in the liver (1.42 ppm) and muscle (.26 ppm) of the PA group were higher ($P<.05$) than for other treatments. Concentrations of Cd in liver and kidney of GH and PA groups were higher ($P<.01$) than other treatment groups. Dietary Cd from SCP tended to increase ($P<.05$) the Cd level in the liver and kidney of GH and PA groups. Concentrations of Cu in tissues from higher to lower were liver > heart > kidney > bone > spleen > muscle. Concentrations of Cu in various tissues were normal. Zn was found at highest

level in liver with decreasing amounts in spleen, kidney, bone, heart and muscle. Concentrations of Zn in tissues were normal. Inclusion of GH and PA SCP had no effect on the performance of the growing sheep. The high level of polymer used in the P diet caused some diarrhea during the first 3 weeks of the experimental period but did not affect the performance of the sheep.

INTRODUCTION

Pulp mill single cell protein (SCP) produced in pilot plants have been used successfully as supplementary protein sources for feedlot cattle (Aseltine, 1979; Kellems and Church, 1979; Atil, 1982) and sheep (Aseltine, 1979; Kellems et al., 1981; Atil, 1982). However, the pulp mill SCP used in a growing broiler chicken feeding trial and a rabbit trial (Atil, unpublished data) was less successful. Data from reports by Aseltine (1979), Kellems and Church (1979), Kellems et al. (1981) and Atil (1982) clearly suggest that this novel crude protein source was very suitable as a feed ingredient for ruminant animals.

Analyses of heavy metals on various samples of SCP revealed variations in the contents of potentially toxic heavy metals from one batch to another. SCP derived from Grays Harbor and Port Angeles sources appeared to contain high amounts of Cr (284 and 219 ppm, respectively) which was comparable to the level present in Pensacola municipal sludge (Bertrand et al., 1980). Other potentially toxic heavy metals (Cu, Zn, Cd and Pb) in these SCP were found to be higher than conventional protein sources such as cottonseed meal (CSM) and soybean meal (SBM). However, those elements were present in much lower concentrations than found in Pensacola (Bertrand et al., 1980), Chicago (Bertrand et al., 1981) or Denver (Keinholz et al., 1979) sewage sludge.

Short term feeding of SCP supplements to finishing beef steers has not caused any detrimental effect on the performance or the quality grade or palatability of the meat from these steers (Aseltine, 1979; Atil, 1982). In a study of a similar type using sewage sludge fed to growing steers (Bertrand et al., 1980; 1981), there was no evidence of detrimental effects on performance and carcass quality. However, ingestion by cattle of heavy metals via sewage sludge tends to increase the accumulation of various heavy metals in the liver and kidney (Keinholz et al., 1979; Bertrand et al., 1980; 1981). It has been shown that Cd tends to accumulate in the liver and spleen of steers fed on pulp mill SCP supplements (Atil, 1982).

The purpose of this study was to evaluate the long term effects on animal performance of feeding high levels of pulp mill SCP sources to growing wether lambs and if some selected potentially toxic heavy metals accumulated in selected tissues.

MATERIALS AND METHODS

Twenty-five crossbred wether lambs were assigned randomly (according to initial body weight) to five groups of lambs/group. One group of lambs were killed at the initiation of the trial. Dietary treatments for the remaining four groups were: (1) Positive control (PC) (CSM was a major supplemental crude protein (CP) in the diet); (2) SCP from Grays Harbor (GH) (about 33.3% of the total CP in the diet came from GH SCP);

(3) SCP from Port Angeles (PA) (about 33.3% of the total CP in the diet came from PA SCP); and (4) Polymer (P) (a diet which contained 2.5% polyacrylamide polymer (Calgon's WT 2580 manufactured by Calgon Co. Inc., Pittsburgh, PA 15230). The Calgon WT 2580 polymer was added at about 10 times the level of polymer estimated to be in the SCP diets.

The diets were formulated to be isonitrogenous and isocaloric with respect to digestible nutrient contents (table 1). All diets were ground, mixed and pelleted (3.5 mm). They were fed for a period of 169 days. Diets and water were available to the lambs throughout the experimental period ad libitum. Records were kept on the amount of feed consumption, body weight gain and general conditions of the lambs during the experimental period. Body weights were obtained monthly at 1700 h for 3 consecutive days, and the gain recorded was based on an average of these three measurements.

Five sheep were slaughtered at the time when the trial was initiated in order to obtain initial levels of selected heavy metals in the tissues. Eighteen lambs were slaughtered at the end of 169 days. One lamb was slaughtered at day 100 because of severe rectal prolapse; and one lamb died due to undetermined causes. Lambs were slaughtered at the Oregon State University Meat Science Laboratory. Liver, kidney, spleen, heart, muscle (L. dorsi) and bone (tibia) tissues were collected at slaughter. Tissue samples were freeze-dried and kept for later analyses.

All glassware and polyethylene bottles used in the analyses were soaked in 5N HNO_3 for at least 24 h and rinsed 5 times with glass double distilled water. About .5 g of freeze-dried samples/tissue and 1 g of diets were subjected to wet ashing in 15 ml of HNO_3 (reagent grade) and

3 ml of $HClO_4$ (reagent grade). After the digestion was completed, the residue was dissolved and diluted with 0.1N HNO_3 to 10 ml. Digests were stored until analyses in precleaned polyethylene bottles (Nalgene). Standard solutions were made by diluting 1000 ppm stock solution (J. T. Baker Chemical Co., Phillipsburg, NJ 08865) to appropriate working/calibration ranges. National Bureau of Standards (NBS), standard reference material (SRM) 1577 bovine liver, NBS SRM 1566 oyster tissue and NBS SRM 1571 orchard leaves were used to calibrate instrument and working standards and also to check contamination of the glassware and reagents used in the digestion process. The analytical results for all of the selected elements analyzed from NBS SRM samples were within the error limits of certified values (Appendix 2). Samples were analyzed using flame atomic absorption (Perkin-Elmer Model 4000). Samples containing elements which were not detectable by the flame technique were analyzed by graphite furnace methods (Perkin-Elmer Model 2100) as outlined by Davidson and Secrest (1972) for Cr and by Poldoski (1980) for Pb and Cd.

The data on performance and selected heavy metals content of the selected tissues were analyzed statistically using oneway analyses of variance as outlined by Steel and Torrie (1980). Means were compared using the LSD procedure as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Heavy Metals in Tissues

Concentrations of the heavy metals in SCP and diets is presented in table 2. Cr was the heavy metal present in highest concentrations in the

SCP. The Cr levels of GH and PA SCP were 284.0 and 219.5 ppm, respectively. Inclusion of GH and PA SCP in GH and PA diets increased the Cr levels by 5 and 3 times, respectively compared to PC diets. Cr level in the P diet was 1.59 ppm, the lowest among the treatments. Cu levels in GH and PA SCP were 27.67 and 29.18 ppm, respectively. Using SCP in GH and PA diets did not have much affect on Cu levels in the diets. Concentrations of Zn in GH and PA SCP were similar. Zn was highest in the PC diet (105.33 ppm), high in GH (77.46 ppm) and PA (72.11 ppm) diets and lowest in the P (48.70 ppm) diet. However, the levels of Zn in all diets were much less than maximum tolerable values (300 ppm) as suggested by NRC (1980). The Cd levels in GH and PA SCP were 3.4 and 6.5 ppm, respectively. Dietary Cd in GH (.51 ppm) and PA (.76 ppm) diets was higher than in PC (.23 ppm) and P (.27 ppm) diets. In the GH and PA diets, Cd level slightly exceeded the maximum tolerable limit of .50 ppm (NRC, 1980). Levels of Pb in diets were much lower than maximum tolerable values of 30 ppm (NRC, 1980).

Data on the selected heavy metals found in liver, muscle and kidney are summerized in table 3 and for heart, spleen and bone in table 4. There were differences in concentrations of Cr found in liver ($P<.01$), kidney ($P<.05$) and spleen ($P<.05$) among the treatments. Liver and kidney Cr were highest ($P<.05$) in lambs from the negative control (NC) group and those fed the positive control (PC) diets. These results indicate that dietary Cr from SCP did not affect the retention of liver Cr even though the highest amount of dietary Cr was found in GH and PA diets. This finding was not expected and suggests that the Cr from SCP sources either was not absorbed or, if so, was excreted quantitatively, or that the values determined were not true values of the tissues. Some Cr salts are essentially indigestible, allowing their use as external markers in digestibility trials (Church, 1979). The type of Cr compounds present in these SCP is not known. Tissue levels of Cr reported by NRC (1980) were in the range of 10 to several ppb. Average Cr concentrations among the control and treated groups in liver, muscle and kidney of cattle fed sewage sludge were .73, .73 and .73 ppm of wet weight (Bertrand et al., 1981).

Maximum tolerable levels for Cr were set at 3000 ppm as oxide and 1000 ppm as chloride (NRC, 1980). Since there were high levels of Cr in tissues of NC and PC sheep, therefore the Cr present in the GH and PA SCP does not appear to be a problem with respect to accumulation in animal tissues or human food.

Liver Cu ranged from 148.0 ppm ($P<.05$) in P sheep to 264.1 ppm ($P<.05$) in GH sheep, but there were differences ($P>.05$) in liver Cu levels in GH sheep if compared to NC and PC sheep. The level of Cu found in this study was normal and similar to NRC (1980) values, but much less than the reported values in sheep reviewed by Doyle and Spalding (1978). The concentrations of Cu in various tissues in this study ranging from highest to lowest are in the following order: Liver > heart > kidney > bone > spleen > muscle. Since Cu is one of the essential trace elements the pulp mill SCP should provide enough Cu and should not pose any ill effect when fed to ruminant animals.

Concentrations of Zn in the tissues of sheep in this study from highest to lowest were: liver (123.55 - 148.45 ppm) > spleen (86.43 - 114.08 ppm) > kidney (86.80 - 101.45 ppm) > bone (84.12 - 107.23 ppm) >

heart (80.79 - 85.85 ppm) > muscle (67.98 - 88.85 ppm). Spleen and muscle ($P<.01$) and liver ($P<.05$) levels were different among the treatments. Even though dietary Zn was highest in PC diets, there was no difference ($P>.05$) in Zn accumulation in the liver, spleen or muscle tissues of PC, when compared with GH, PA or P. Liver Zn levels from NC sheep were higher ($P<.05$) compared to PC, PA and P, but were not different ($P>.05$) from GH sheep. However, the levels of Zn in spleen and muscle of NC sheep were lower ($P<.05$) than PC, GH, PA and P sheep. This indicates that spleen and muscle Zn levels tend to increase as the sheep were exposed to dietary Zn. Zn levels in liver, kidney and muscle were similar to those summerized by Doyle and Spalding (1978).

Our data showed some trend for slightly higher levels of Zn in the tissues of P sheep. Even though the dietary Zn in PC, GH and PA diets were higher than probable dietary requirements, they were much lower than the maximum tolerable level of 300 ppm as suggested by NRC (1980). The dietary level of Zn in this study ranged from 48.7 ppm in the P diet to 105.3 ppm in the PC diet. Church (1979) summerized the probable dietary requirement and toxic level of Zn at 30 - 40+ ppm and > 100 ppm, respectively.

The levels of Cd in kidney, liver and heart were different ($P<.01$) among the treatments. Concentrations of Cd in various tissues in the order of highest to lowest were: kidney > liver > heart > muscle > spleen. Cd levels in kidney were highest ($P<.05$) in GH (1.286 ppm) and PA (1.236 ppm) sheep, and thus the dietary Cd from SCP was a contributing factor. There were no differences ($P>.05$) in Cd levels of the kidney between PC, NC and P sheep. Higher concentrations of Cd in kidney tissues were expected because it is the major organ which retains Cd. Lee and Jones (1976) reported that Cd concentrations in kidney, liver and muscle of lambs were 1.93, .35 and .07 ppm of dry weight, respectively. When dietary Cd concentrations of .2 to .7 ppm were fed to growing calves and lambs, Cd concentrations of 1 to 4 ppm accumulated in the dry liver, and concentrations of 2 to 5 ppm accumulated in the kidney (Powell et al., 1964; Doyle et al., 1974). Even though dietary Cd concentrations of our study were similar, the Cd accumulation in the dry kidney and liver of sheep in this study were much lower. The concentrations of Cd in liver was highest ($P<.05$) in PA and higher ($P<.05$) in GH when compared to NC, PC or P sheep. Concentrations of Cd in the liver of PA and GH sheep further indicated that dietary Cd from SCP tends to accumulate in kidney and liver tissues. However, the levels of Cd in kidney and liver of this study were within the normal values as discussed earlier, but much lower than the values summerized by Doyle and Spalding (1978) in the kidney and liver of sheep. Concentrations of Cd in heart tissue were higher ($P<.05$) in NC than PC, GH, PA and P sheep. Cd concentrations in heart of PC, GH, PA and P sheep were similar to values reported by Doyle and Pfander (1975). There were no differences ($P>.05$) in Cd concentration in muscle among the treatments. Cd concentration in muscle of NC, PC, GH, PA and P were .086, .082, .082, .074 and .073 ppm, respectively. These values were slightly higher than .03 ppm reported by Doyle and Pfander (1975) and .0093 ppm reported by NRC (1980). Concentrations of Cd in spleen were .048, .055, .055, .046 and .046 ppm from NC, PC, GH, PA and P sheep, respectively. F.A.O/W.H.O (1972) recommended that the dietary Cd intake for humans should not exceed 1 mg per kilogram of body weight or 57 to 71 mg/day. Therefore, the results from this study did not suggest any serious harmful effects of transfering Cd into the human food chains via feeding SCP to animals.

Pb concentrations in the liver, kidney and muscle were different ($P<.01$) among the treatments. Pb found in the liver and muscle of PA sheep was higher ($P<.05$) than the remaining treatments. However, the concentrations of Pb in liver (1.421 ppm) and muscle (.261 ppm) of PA sheep were very similar to that of the Pb concentrations in liver (1.30 ppm) and muscle (.20 ppm) as reported by Fick et al. (1976) (dry weight basis). Doyle and Spalding (1978) reported that Pb concentrations from 442 sheep's liver, kidney and muscle were .53, .44 and .25 ppm, respectively. Average dry matter content of most tissues was 22 to 26% (Doyle and Spalding, 1978); the concentration of Pb in dry liver, kidney and muscle would be equivalent to 2.04, 1.68 and .96 ppm, respectively. The NRC (1980) suggested that 0 to 3 ppm (dry weight) of Pb in liver was a normal value. Pb concentrations in heart, spleen and bone were not different ($P>.05$) among treatments. Concentrations of Pb in heart ranged from .123 ppm from PC to .189 ppm for GH sheep. Fick et al. (1976) reported that the Pb concentration of dry sheep's heart was .20 ppm. The concentrations of Pb found in tissues ranging from highest to lowest were: liver > kidney > muscle > heart > spleen > bone.

Animal Performance Data

Performance data of the sheep in this study are shown in table 5. Daily feed consumptions for PC, GH, PA and P groups were 2.78, 2.48, 2.73 and 2.61 kg per head per day. The GH group had the lowest feed consumption because two of the sheep were prematurely terminated from the experiment and the feed consumptions were computed up to the date of termination.

Sheep on the P diet had relatively severe diarrhea during the first three weeks of the experiment. Average daily gains for the PC, GH, PA and P groups were .22, .22, .20 and .22 kg/head/day ($P>.05$), respectively. The daily gains in this study (.22 kg) were slightly lower than that reported by Shqueir (1981) of .36 kg, possibly because Shqueir used lighter weight lambs. Feed efficiencies for PC, GH, PA and P groups were 5.80, 5.16, 6.13 and 5.45 kg of feed/kg body weight gain, respectively. The GH had the lowest feed efficiency because two of the sheep from this group were terminated from the experiment earlier. The P group tended to have a slightly better feed efficiency than PC and PA groups, and poor feed efficiency was observed from PA group. In general, the efficiencies are relatively poor because the lambs were excessively fat when slaughtered. Since the lambs were over finished, data on gain and efficiency are not very meaningful except to demonstrate that long term feeding of SCP or polyacrylamide did not appear to have any depressing effects.

CHAPTER III

TABLE 1. COMPOSITION OF THE DIETS[a]

Ingredients	IFN	Treatments: Positive Control	Grays Harbor	Port Angeles	Polyacrylamide
Cottonseed meal	5-01-621	8.30			7.30
Alfalfa, ground	1-00-063	16.75	16.60	16.60	16.30
Wheat mill run	4-05-206	16.75	16.60	16.60	16.30
Ryegrass, ground	2-04-073	38.00	37.30	39.00	37.70
Tallow	4-08-127	3.30			3.30
Bentonite		8.25	8.30	8.30	8.10
Molasses, cane	4-04-696	8.25	8.30	8.30	8.10
Grays Harbor SCP			12.50		
Port Angeles SCP				10.80	
Polymer[b]				2.50	
Salt		.40	.40	.40	.40

[a]Percent as fed; diets were prepared on a ton basis.
[b]Polymer used was Calgon WT-2580.

CHAPTER III

TABLE 2. CONCENTRATIONS (PPM) OF SELECTED HEAVY METALS IN THE SCP AND DIETS

Metals	SCP		Diets			
	Grays Harbor	Port Angeles	Positive Control	Grays Harbor	Port Angeles	Polyacrylamide
Cr	284.0	219.5	2.10	12.56	8.89	1.59
Cu	27.67	29.18	10.00	12.39	9.96	12.92
Zn	88.75	94.12	105.33	77.46	72.11	48.70
Cd	3.4	6.5	.23	.51	.76	.27
Pb	9.2	6.6	1.20	1.60	1.60	1.32

CHAPTER III

TABLE 3. CONCENTRATIONS OF SELECTED HEAVY METALS IN LIVER, MUSCLE AND KIDNEY OF THE SHEEP FED GRAYS HARBOR AND PORT ANGELES SCP FOR 169 DAYS[a]

	Treatments				
Metals	Negative control	Positive control	Grays Harbor	Port Angeles	Polyarcylamide
No. of lamb	5	5	5	5	5
			Liver, ppm dry weight		
Cr**	8.021^{d} ± .633	$7.311^{c,d}$ ± .382	6.528^{c} ± .614	5.149^{d} ± .333	4.359^{b} ± .318
Cu**	249.84^{d} ± 15.14	$233.73^{c,d}$ ± 24.84	264.15^{d} ± 15.38	199.25^{c} ± 8.27	148.05^{b} ± 15.47
Zn*	148.45^{c} ± 5.35	123.55^{b} ± 4.61	$134.69^{b,c}$ ± 9.07	129.72^{b} ± 6.04	126.61^{b} ± 2.20
Cd**	$.213^{b}$ ± .017	$.177^{b}$ ± .008	$.445^{c}$ ± .064	$.614^{d}$ ± .022	$.162^{b}$ ± .007
Pb**	$.927^{b,c}$ ± .165	$.845^{b}$ ± .113	$1.289^{c,d}$ ± .134	1.421^{d} ± .074	$1.008^{b,c}$ ± .140
			Muscle, ppm dry weight		
Cr	5.050 ± .211	5.793 ± .300	5.523 ± .306	5.169 ± .324	4.701 ± .208
Cu*	3.30^{c} ± .26	$2.87^{b,c}$ ± .17	3.27^{c} ± .15	3.40^{c} ± .16	2.64^{b} ± .12
Zn**	67.98^{b} ± 1.41	83.71^{c} ± 1.97	82.93^{c} ± 4.45	88.85^{c} ± 3.74	90.25^{c} ± 3.83
Cd	.086 ± .002	.082 ± .003	.082 ± .005	.074 ± .005	.073 ± .005
Pb**	$.168^{b}$ ± .007	$.166^{b}$ ± .005	$.209^{b}$ ± .008	$.261^{c}$ ± .025	$.210^{b}$ ± .024
			Kidney, ppm dry weight		
Cr*	7.821^{c} ± .338	7.904^{c} ± .377	$6.858^{b,c}$ ± .309	6.037^{b} ± .842	$6.632^{b,c}$ ± .343
Cu**	13.31^{b} ± .33	16.85^{e} ± .53	$13.73^{b,c}$ ± .43	$14.69^{c,d}$ ± .25	15.32^{d} ± .52
Zn	86.80 ± 2.54	101.10 ± 3.41	94.59 ± 4.97	101.10 ± 2.99	101.45 ± 7.54
Cd**	$.877^{b}$ ± .018	$.958^{b}$ ± .039	1.286^{c} ± .174	1.236^{c} ± .064	$.821^{b}$ ± .033
Pb**	$.444^{b,c}$ ± .046	$.819^{d,e}$ ± .119	$.627^{c,d}$ ± .069	$.886^{e}$ ± .056	$.393^{b}$ ± .026

[a] Means ± standard error.

[b,c,d,e] Means in a row with different superscripts differ ($P<.05$).

$^{*}P<.05$; $^{**}P<.01$.

CHAPTER III

TABLE 4. CONCENTRATIONS OF SELECTED HEAVY METALS IN HEART, SPLEEN AND BONE OF THE SHEEP FED GRAYS HARBOR AND PORT ANGELES SCP FOR 169 DAYS[a]

Metals	Treatments				
	Negative control	Positive control	Grays Harbor	Port Angeles	Polyarcylamide
No. of lamb	5	5	5	5	5
	Heart, ppm dry weight				
Cr	5.877 ± .466	6.081 ± .375	5.804 ± .397	6.050 ± .184	6.652 ± .336
Cu**	14.80[b] ± .28	16.81[c] ± .63	17.02[c] ± .31	17.17[c] ± .50	16.96[c] ± .24
Zn	85.85 ± 3.23	82.83 ± 1.82	80.79 ± 2.27	81.25 ± 1.75	85.27 ± .84
Cd**	.156[c] ± .018	.099[b] ± .006	.089[b] ± .012	.080[b] ± .004	.072[b] ± .003
Pb	.159 ± .012	.123 ± .014	.189 ± .030	.163 ± .026	.165 ± .028
	Spleen, ppm dry weight				
Cr*	.535[b] ± .070	.606[b,c] ± .072	.819[d] ± .046	.711[c,d] ± .049	.735[c,d] ± .059
Cu	3.80 ± .22	4.39 ± .17	4.65 ± .67	3.88 ± .04	4.29 ± .15
Zn**	86.43[b] ± 4.48	109.42[c] ± 5.57	109.42[c] ± 6.20	111.23[c] ± 7.48	114.08[c] ± 1.63
Cd	.048 ± .003	.055 ± .008	.055 ± .002	.046 ± .004	.046 ± .003
Pb	.048 ± .008	.091 ± .021	.076 ± .018	.049 ± .005	.054 ± .004
	Bone, ppm dry weight				
Cr	20.40 ± 1.86	21.80 ± 2.33	21.00 ± 3.76	14.80 ± 1.02	18.00 ± 1.52
Cu	8.76 ± 1.67	7.54 ± .20	7.50 ± .45	7.32 ± .38	8.74 ± 1.13
Zn	84.12 ± 4.71	98.96 ± 6.08	107.23 ± 8.87	94.06 ± 6.99	97.97 ± 8.30
Pb	<1.0 ± 0	<1.0 ± 0	<1.0 ± 0	<1.0 ± 0	<1.0 ± 0

[a] Means ± standard error; analyses were made from duplicates sample; symbol (<) placed before means denote below the detectable limit of the instrument by flame technique, graphite furnace technique was used without success.

[b,c,d] Means in a row with different superscripts differ ($P<.05$)

*$P<.05$; **$P<.01$

CHAPTER III

TABLE 5. PERFORMANCE OF THE SHEEP FED GRAYS HARBOR AND PORT ANGELES SCP FOR 169 DAYS.

Items	Treatments			
	Positive Control	Grays[a] Harbor	Port Angeles	Polyacrylamide
No. of sheep	5	5	5	5
Average initial weight, kg	42.09	41.14	42.14	42.14
Average daily gain, kg	.22	.22	.20	.22
Daily feed consumption, kg	2.78	2.48	2.73	2.61
Feed efficiency[b], kg	5.80	5.16	6.13	5.45

[a]One lamb was sick and died on a weekend and was not posted. Another lamb was slaughtered earlier due to rectal prolapse. Data in this group were given as June 3rd due to the problems mentioned, resulting in somewhat improved values of feed efficiency and lower feed consumption.

[b]Feed consumed per unit of body weight gain.

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CONCLUSION

Data generated from this study has led to the following conclusions:

1. SCP from potato waste have very similar digestibility coefficient of CP to pulp mill SCP; and higher ash contents of SCP from potato waste caused a lower overall nutrients digestibility.

2. Urea supplementation to SCP tended to improve the dietary digestibility.

3. SCP supplementation at 100% and 50% of CSM replacement showed good CP digestibility and support good growth in lambs.

4. SCP from pulp mill was a very viable protein supplementation for feedlot cattle.

5. Cr, Cu, Zn and Pb in the SCP were not causing any toxicity effect to cattle and sheep and neither were they retained substantially in the major tissues.

6. Feeding SCP to steers do not cause any pathological problems.

7. Cadmium tissue levels tended to increase as SCP were used in the diet; but its accumulation is not considered to be severe for the safety of the animals as well as a protein source for human consumption.

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APPENDICES

APPENDIX 1. FORMULA FOR CALCULATION OF ESTIMATED DIGESTIBILITY COEFFICIENTS OF NUTRIENTS COMPONENTS FROM CRUDE PROTEIN SUPPLEMENTS FOR TRIAL 3, OF TABLE 9.

$$EDC = \frac{A - (B \times \frac{C}{100})}{D}$$

where EDC = Estimated digestibility coefficients of nutrients/components from supplemental protein.

A = Digestibility coefficients of a specific nutrient/component from experimental diets.

B = Digestibility coefficients of basal diet of a specific nutrient/component.

C = Percent of basal in the experimental diet.

D = Total amount of tested crude protein supplement used in the diet.

and C = 100 - D.

D = 100 - C.

APPENDIX 2. COMPARATIVE VALUES OF VARIOUS ELEMENTS IN NBS SRM MATERIALS (CERTIFIED VALUES VS THIS DETERMINATION[a])

Elements	NBS	This study
Bovine Liver SRM 1577:		
Cr	.088 ± .012	.054 ± .022
Cu	193 ± 10	187 ± 1
Zn	130 ± 13	137 ± 5
Cd	.27 ± .04	.26 ± .02
Pb	.34 ± .08	.38 ± .04
Oyster Tissue SRM 1566:		
Cu	63 ± 3.5	62.6 ± 3.0
Cd	3.5 ± .4	3.53 ± .01
Pb	.48 ± .04	.45 ± .04
Orchard Leaves SRM 1571:		
Cr	2.6 ± .3	2.01 ± .08
Cu	12.11 ± 1	11 ± .05
Zn	25 ± 3	27.7 ± .5
Pb	45 ± 3	44 ± .6

[a]All values were ± ppm.