

**ADVANCED MEAT RECOVERY
SYSTEM SURVEY PROJECT**

FINAL REPORT

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USDA, FSIS, OPPDE, LPTSD

ADVANCED MEAT RECOVERY SYSTEMS SURVEY PROJECT REPORT

Background:

In general, certain characteristics associated with product from mechanical deboning systems include increased calcium content, a paste-like end product, and a relatively short shelf life when compared to whole meat cuts or ground meat products. However, mechanical meat separation systems were recently introduced which utilize hydraulic pressure to detach the meat tissue from the bones without grinding the bone material. These recent advances in mechanical meat recovery technology have eliminated and/or reduced, to varying degrees, the characteristics associated with mechanically deboned meat systems. The new meat recovery systems are called "Advanced Meat Recovery Systems" (AMRS) and are being used by a steadily increasing number of meat processors to recover lean meat tissue which normally adheres to the bones to produce a meat product which resembles, and functions like, ground meat.

The Food Safety and Inspection Service (FSIS) published a final rule on December 6, 1994 for red meat entitled "Meat Produced by Advanced Meat/Bone Separation Machinery and Meat Recovery Systems" that went into effect January 5, 1995. This final rule amended the definition of "meat" (9 CFR 301.2 (rr)) to include, as "meat", product resulting from advanced meat/bone separation machinery that does not crush, grind, or pulverize bones to remove attached edible skeletal tissue and from which the bones that emerge from the system are similar and comparable to those resulting from hand-deboning (i.e., in a recognizable form similar to that of the original bones). Additionally, the final rule required that the meat from AMRS meet the criteria of a calcium limit of 0.15 percent (150 mg/100 g of product), with a tolerance of 0.03 percent (30 mg/100 g of product) for a single analysis, as a measure of the bone solids content of the product. These final rule criteria under which AMRS must operate were established in order for the product from these systems to be labeled as "meat" and not as mechanically separated meat (MSM). The fundamental difference between the AMRS and MSM processes is that in AMRS processes, the livestock bones are not purposefully incorporated into the meat prior to recovering the meat and that the bones are essentially intact and recognizable when they exit the AMRS, while the bones in the MSM system are purposefully incorporated into the meat and then removed in the process and exit the system in a ground form and are not recognizable. Also, the AMRS product has an appearance much like finely ground meat with filament structures.

In the fall of 1995, the Agency gave consideration to the developing and completing a directive which would provide guidance to field personnel related to the inspection tasks associated with verifying AMRS operations for compliance with the regulation. A field survey (REF, 1995) was conducted to evaluate how the final rule was implemented by processors, how compliance checks were being made, the appearance of the bones when they emerged from the ARMS machinery, and what the calcium levels were in the meat from the ARMS machinery. The information obtained from that survey was used to develop compliance checking procedures for assuring that the regulatory criteria would be met. The Agency published FSIS Directive 7160.1 entitled "Meat Produced by Advanced Meat/Bone Separation Machinery and Meat Recovery Systems" on September 13, 1996.

A consumer organization has raised, among other things, the concern that regulatory criteria for

meat were not being met consistently. The claim is that, operations using AMRS machinery, result in bones emerging from the systems which are not recognizable and in natural conformation. Thus, the product can have high calcium levels and possibly bone marrow

Purpose of Survey:

In order to ascertain a profile of the chemical composition of meat derived from beef bones by the press-type AMRS machinery, and to gather information concerning the morphology of the bones exiting the AMRS machinery, a chemical and histological survey was conducted.

Methods:

Since the main concern with the AMRS machinery centered around bone and bone material being expressed into the meat obtained from beef carcasses, this survey was limited to establishments utilizing beef neck bones in their AMRS processes. Beef neck bones were selected for the survey due to the fact that the vertebra are split, thus exposing tissue and forming bone dust. Using this criteria, qualifying establishments were randomly selected. Additionally, the survey was limited to operations producing AMRS "meat product" that is used in the same form as it exits the AMRS process, rather than AMRS product which is further processed, such as low temperature rendering the product into finely textured lean.

Samples were collected and handled from each of the randomly selected establishments for chemical and histological analysis following the AMRS survey sampling instructions included as Attachment 1. This survey only evaluated meat obtained from beef neck bones by AMRS equipment and did not include product produced from other types of bones or species of animal. Samples selected in the AMRS establishments consisted of meat derived primarily from neck bones as normally processed within the establishment. Samples selected in the hand deboned establishments were derived from only neck bones, because this type of bone is generally the major component used in AMRS operations surveyed.

Since all of the AMRS selected establishments pre-sized the bones prior to processing, whole neck bone samples (sample "A" in Attachment 1) were not collected. For chemical analysis, samples were collected from seven (7) establishments using AMRS equipment (1 Protocon and 6 Hydrosep) and two (2) establishments that were hand deboning (control). For histological analysis, samples were collected from seven (7) establishments using AMRS equipment (1 Protocon and 6 Hydrosep) and four (4) different establishments that were hand deboning (for comparative purposes). Chemical analyses were performed at the chemistry laboratories of the Agricultural Research Service (ARS) and the Food Safety and Inspection Service (FSIS), while the histological analyses were performed at the FSIS pathology laboratory.

The chemical analyses of AMRS samples conducted by ARS included fat, moisture, protein, pH, and ash were performed according to official methods as published by the Association of Official Analytical Chemists (15th Edition, 1990), color using a Minolta CR200C chroma meter set on illuminant C according to the procedure published by the International Commission on Illumination, Recommendations on Uniform Color-Difference Equations, Psychometric Color Terms, Supplement No. 2, and fatty acids according to the procedures of Morrison, W. H., Robertson, J. A., and Burdick, D., 1973 (JAOCS 50:440) and Waliking, A. E. and Zmachinski,

H., 1970 (JAOCS 47:530) using a Perkin Elmer Sigma 2000 gas chromatograph. The chemical (calcium, total iron, cholesterol, and bone residue) analyses conducted by the FSIS Eastern Laboratory were performed according to FSIS standard chemical methods as published in the FSIS Analytical Chemistry Laboratory Guidebook - Food Chemistry. Additionally, the FSIS Eastern Laboratory analyzed AMRS samples for heme and non-heme iron according to the procedure of Rhee, K. S. and Ziprin, Y. A., 1987 (J. Food Sci., 52:1174).

Histological examination of the AMRS samples was conducted by the FSIS Eastern Laboratory according to standard histological methods as published in the FSIS Histologic Staining Techniques and Laboratory Methods - A Manual of Procedures (Pathology Laboratories of Science, FSIS, USDA, 1986). All samples examined histologically were evaluated for type of tissue present, and if present, bone cartilage, nerve, and bone tissue were identified. Bone tissue was reported as an estimated percentage of the total amount of tissue examined. One American College of Veterinary Pathologists (ACVP) Board Certified pathologist performed all of the histological examinations to assure uniformity and reduce additional sources of variation in the project design. Three other ACVP pathologists reviewed a representative sample of cases for confirmation purposes (See Attachment 3 for further details). Additionally, a bone score evaluation of bone samples obtained both before and after the AMRS process were performed according to the procedure and criteria described in Attachment 2.

All data was statistically analyzed using PC-Statistical Analysis System[®] (SAS[®] Institute, Inc., version 6.10). The data tables which contain all of the AMRS survey data are appended to this report.

Results:

Because the food chemistry variables protein, moisture and fat are correlated and sum close to 100%, the usual practice for distinguishing meat through these variables is to consider the fat levels and either the ratio of moisture to protein (moisture protein ratio, MPR) or the concentration of protein in the fat free portion of the meat (protein fat free, PFF). While fat levels may vary by large amounts in similar cuts or types of meat, MPR or PFF values are nearly constant. It is for this reason these values are used by USDA for determining economic adulteration in meat.

Comparison of Results before and After Desinewing

The first examination concerns differences between AMRS product before and after desinewing. Table 1., presents, for each establishment and pooled over all establishments, the average of differences between results on paired samples collected before and after desinewing. Using an analysis of variance with establishment and day of sampling within establishment as random factors, there were statistical significant effects for the average difference for: protein, PFF, moisture protein ratio (MPR), calcium, and bone residuals. The ratios, *t*, of the means (of the differences) to the standard errors of these means for these variables are: for protein, *t*=3.75; for MPR, *t*= 3.66; for calcium, *t*=4.25; for bone residual, *t*=3.79. Of particular interest is the difference for calcium, where the average calcium level before desinewing is approximately 0.18%, while after desinewing the average level is approximately 0.11%. For the MPR, PFF, calcium, cholesterol, total iron, and ash variables there were significant establishment effects (*P*<0.05).

Comparison of Results by Type of Deboning

Food Chemistry

As a result of the significant differences between results before and after desinewing, only the results after desinewing will be used for further comparisons of the AMRS product with the hand deboned product. Table 2., presents the mean levels of the food chemistry variables for each establishment and for pooling results over establishments. An additional variable, labeled "summary average", is a weighted average of the seven variables: MPR, calcium, bone residue, total iron, ratio of non-heme to heme, ash, and ratio of saturated to unsaturated fat. Statistically the "summary average" variable is the first principal component using these seven variables, computed on the correlation matrix. The fat and cholesterol contents are determined by the incoming product and can be controlled, to some extent, by the manufacturer, and thus were excluded from the summary average measure. The weighting factors (after adjusting for the standard deviations) for MPR, calcium, bone residue, and total iron were approximately equal to 0.44, while the weighting factors for ash and the ratio of non-heme to heme iron were approximately 0.30, and the weight for ratio of saturated to unsaturated fats was approximately 0.21. The sum of this variable over all observations is zero, and the standard deviation of the values of this variable over all observations is 1.77.

The results from Table 2., show clearly the differences between products as a function of the type of deboning, "A" for machine (AMRS), and "H" for hand deboning. Using an analysis of variance (AOV) on the ranked values, with establishment and day of sampling as random effects, all variables had significant type of deboning effects ($P < 0.001$), except for the ratio of saturated to unsaturated fat. For the summary average variable, there is no overlap of the two distributions. Geometrically this means that in the 7-dimensional space defined by the seven variables, there are two galaxies (clusters of points) which are completely distinct from each other. Further analysis indicated that two distinct galaxies can be seen in the 2-dimensional space determined by calcium and MPR, and even greater separation is obtained in the three dimensional space defined by MPR, calcium, and total iron. Figure 1 presents a scatter-plot of sum versus difference of standard scores for the variables, which are obtained by subtracting the mean value for the variable from the results and dividing the difference by the variable standard deviation. From this figure, the separation between the AMRS and hand deboned results can be seen.

Of the seven (7) establishments that used the machine deboning, six (6) used the Hydrosep machine, and one (1) used the Protocon machine. Using an analysis of variance (AOV), treating establishment and day of sampling as random factors and ignoring the possible effects of machine pressure and dwell time, no variable was statistically significant for machine effect. As discussed below, it is possible that product characteristics are influenced by machine pressure and dwell time, and type of incoming product (steers, heifers, cows, and bulls). Of the six (6) establishments that used the Hydrosep machine, one had pressure and dwell time close to those of the establishment that used the Protocon machine. Both of these establishments used steers and/or heifers. Comparing these two establishments, treating day of sampling as a random factor, only MPR was statistically significant (P -value = 0.004). Among the other variables, total iron and cholesterol had low P -values of approximately 0.10. With only one establishment using the Protocon machine, it is not possible to conclusively determine a machine effect.

Figure 2., presents distributions of the results of the food chemistry variables, by type of deboning

(hand deboned, AMRS deboned), and by type of machine (Protocon, Hydrosep). The distributions reveal the occasional high values obtained for some of the variables for the machine deboned product. For the calcium variable there were 5% of the results that were above the regulatory limit of 0.18%. However, there were four (4) samples that exceeded 0.25%, with the two (2) highest near 0.40% from the same establishment on the same day. While these two results were quite distinct from the other values (the next highest value was 0.28%), because they were obtained from samples collected in the same day, these results can not be dismissed as exceptional. A further discussion of variability is presented in section *Estimation of Variability*.

Histology

Table 3., presents the mean results for the histological variables: presence of nerve tissue, the percentage of precursor blood cells (associated with bone marrow) among all cells found on a slide section of dimension of 0.88 in. by 0.5 in., and whether or not the spinal cord was removed, as determined by the inspector when selecting the sample, and the bone scores before and after deboning. Analysis of variances were performed on these variables to determine the statistical significance of differences between establishments, with day of sampling as a random factor for all variables. There were significant differences between establishment and product samples for the number of samples found to contain nerve tissue. The same was true for the proportion of blood precursor cells in relation to all cells within the product samples. However, for the variable difference of bone scores before and after deboning, the establishment effect was not statistically significant (P value =0.12). Table 4., presents cross tabulations of scores on sample bone scores before and after deboning for the 164 samples which had both scores. The results from this table indicate that 92% of the samples with bone score of "1" before deboning receive a bone score of "3" after deboning. Only three (3) samples had lower bone scores after deboning, and 18 samples had the same bone scores.

Relation of Product Composition as a Function of Pressure and Dwell Time of AMRS Machinery

Investigation of the establishment product profiles was made as a function of the characteristics of the deboning machine used. The pressure and the dwell time were used as independent variables. Figure 3., present plots of "standardized values" for the average establishment values for the variables on Tables 2., and 3., versus the pressure. Standardized values for a variable are obtained by subtracting the mean of the establishments' average values for the variable from the establishment average value and dividing the differences by the standard deviation of the establishments' average values. However, it is expected that high pressure would create a product that has a higher water content and a higher amount of nerve tissue. The plot of Figure 3., are consistent with this expectation. Most of the variables' average values for the establishment using the highest pressure (5500 PSI) are above the corresponding mean of establishments' average values (points above the x-axis in Figure 3) ; three of the average values (for MPR, presence of nerve tissue, and ratio of non-heme to heme iron) are the highest averages, and the average value for total iron is the second highest. On the other hand, most of the average values for the establishment using the lowest pressure (2011 PSI) are below the mean of establishments' average values (points below the x-axis of Figure 2). A similar graph (Figure 4) also shows a possible relationship between product characteristics and dwell time. The larger dwell times seem to be associate with lower values. It does appear that, at least, large differences

of pressure (between 2000 PSI and 5500 PSI) and differences in dwell time may effect product composition. However, the small number of establishments and the confounding of the variables dwell time and pressure (for example, the establishments with the highest pressure had among the lowest dwell time), make definitive conclusions from this study difficult.

Examination of Variability

This section presents summary results of various measures of variability of results associated with the type of deboning. These results reflect product sample variability. Comparing measures of variability of results obtained for AMRS machine deboned product to those obtained by hand deboned product can provide a relative measure of process control for AMRS machine versus hand debone processing. To develop efficient quality control procedures, it is important to know what is the process variability and what are the correlations among the variables.

Three (3) samples were collected a day. On the average, there appears to be no trend with time of sampling, with the possible exception for the ratio of non-heme to heme iron. An analysis of variance (AOV), treating establishment as a random factor did not reveal a significant time of sampling effect for this variable, with P- value equal to 0.31. However, for this variable, average values decreased with increasing time for six (6) of the seven (7) establishments, where the highest (average) value is associated with the first sample of the day. Deleting the one exceptional establishment, the AOV did reveal a significant time of sampling trend effect for the non-heme to heme iron ratio variable (P-value = 0.05). However, for simplicity of analysis, the models used for estimating variances assume that sample results within a day are strictly random.

Tables 5a., and 5b., present summary measures describing the variation of results within establishments for AMRS machine and hand debone processing, respectively. The tables include: 1) the mean; 2) the within day standard deviation; 3) the between day standard deviation; 4) the total within establishment standard deviation, which is the square root of the sum of the between and within day variances; 5) the coefficient of variation (CV), which is 100% times the ratio of the within establishment standard deviation divided by the mean; and 6) and the intra-day correlation, which is 100% times the ratio of the between day to total within establishment standard deviation. The estimates were derived by computing an analysis of variance within each establishment, and then taking a weighted average of the components, with the weights were the total number of samples for the establishments. The estimates of variances and the intra-day correlation measure can be influenced greatly by outlier values. For example, the intra-day correlation measure for calcium for the AMRS deboning decreases by approximately 50% (from 39% to 18%) when two high results are discarded.

As can be seen, for all the variables presented on Tables 5a., and 5b., the within day standard deviations for the AMRS machine deboning are higher than those for the hand deboned processing. On the other hand, the between day standard deviations for the machine deboning are sometimes lower and sometimes higher than those for the hand deboning. After taking into account the possible relationship between expected levels and variation of results, for the variables: MPR, total iron and ash, the total within standard deviations for machine deboning are higher than those for the hand deboning. For the MPR variable, the estimated CV for AMRS machine deboning is 5.7%, with a mean of 3.9, while the estimated CV for hand deboning is 4.3% with a mean of 3.3. For the total iron, the estimated CV for AMRS machine deboning is 25%,

with a mean of 2.8 mg/100g, while the estimated CV for hand deboning is 17% with a mean of 1.4 mg/100g. For the ash variable, the estimated CV for AMRS machine deboning is 11.7%, with a mean of 1.17 mg/100g, while the estimated CV for hand deboning is 8.9% with a mean of 1.0 mg/100g. Thus, the processing variability using AMRS machine deboning appears to be higher than that for hand deboned. However, with only two (2) establishments, for which results for hand deboned product were collected, further validation is needed to become more confident of the above relation.

The regulation uses calcium as a control variable, requiring a level of 0.15% or less, with a 0.03% tolerance for a single measurement. The MPR variable, which provides useful measure of process control does not appear to be highly correlated with calcium. The pooled within establishment correlation between these two variables was 0.13 (P value =0.11) and the between establishment correlation was 0.08, which is clearly not statistically significant. When the two high calcium results are included in the calculations of correlations, there is a significant pooled within establishment correlation of 0.21 (P value =0.0080) between bone residue and calcium. By examining Table 2., it is clear that over establishments there is a significant correlation between bone residue and calcium. The establishments with the lowest mean bone residual (0.007 g/10g) had also the lowest mean calcium values (< 0.10%). In general, the calcium variable had statistically significant non-zero correlations with the total iron and ash variables (P value <0.05). This limited analysis of correlations does suggest that calcium and moisture protein ratio represent independent aspects of the product composition and thus might be useful measures for process control.

Discussion:

An evaluation of the AMRS survey data indicated that there were wide variations between the operations in the establishments surveyed. These variations included differences in the food chemical composition of the raw meat/bone material (neck bones) processed and the AMRS equipment operating pressures and dwell times. Due to the limited number of establishments surveyed and the degree of variation for each of the variables examined, further controlled studies are needed to thoroughly evaluate the effects of these parameters on the final AMRS meat product.

Statistical analysis of the AMRS survey data indicated that the desinewer significantly affected the composition of the AMRS meat product. In particular, protein, MPR, calcium, and bone residue contents of the final product were reduced by the desinewer. Since the function of the desinewer is to remove extraneous bone not associated with the AMRS processing operation (such as carcass splitting and bone fragments resulting from size reduction) and connective tissue from the product, these differences are not unexpected. Therefore, the location at which samples are taken for bone or calcium content could have a significant effect on establishing whether or not the product is in compliance with the regulations. Additionally, the lower protein content in the final AMRS meat product also indicated that connective tissue level, which is higher in protein, with corresponding lower fat and moisture, than meat tissue, was also reduced.

Statistical analysis of the chemistry data indicate that the composition of the AMRS final product is significantly different from that of the hand deboned product. The AMRS final product had lower protein values and higher fat, MPR, calcium, bone residue, total iron, non-heme to heme

ratio, cholesterol, ash, and saturated to unsaturated fatty acid ratio values than those of the hand deboned product. The low protein and high MPR and fat level found for Establishment 3 (Table 2) may be due to the fact that it processed bone sections with higher levels of fat than did the other establishments in the survey, or possibly some plate bones, even though this was not observed by the FSIS inspector. However, when the other food chemical components were compared, the difference between the hand deboned and AMRS final product samples was quite evident.

Lean meat that is free of bone contains approximately less than 20 mg of calcium per 100 g of tissue (0.020%), depending on the cut of meat, meat grade, and age of the animal at time of slaughter (USDA, 1990, *Agriculture Handbook No. 8 Series*). Generally, fatter samples and samples from younger animals have lower levels of calcium (Field, R. A., 1981, *Adv. Food Research*, 27:23). The results of this survey indicate that the calcium content of the hand deboned meat is in agreement with that reported in the literature, and that the calcium level of the AMRS final product was higher than that of the hand deboned meat. For the most part, the calcium values for the AMRS machine deboned product were within the regulatory limit of 0.18%, however, 5% of the sample results were above 0.18%, with a few sample results approximately 0.4%.

The calcium level in mechanically separated meat and that from AMRS is affected by the type of bone, processing operations, processing pressure, and the age of the animal (Goldstrand, R. E., 1975, *Proc. Recip. Meat Conf.*, 28:116; Field, R. A., 1976, *Food Tech.*, 30(9):38; Kruggel, W. G. and Field, R. A., 1977, *J. Food Sci.*, 42:190). However, the results of this survey provided no evidence that the beef class (steer/heifer or cow/bull) would significantly affect the calcium levels, or any other variable levels of the AMRS finished product. However, the small number of establishments make it difficult to detect significant differences. More study would be needed. Because the estimation of bone content in mechanically recovered meat systems by calcium content must be based on bones with the same degree of calcification as those providing the mechanically separated meat (Field, R. A., 1988, *Advances in Meat Research, Volume 5, Chapter 4, Edible Meat By-products*, A. M. Pearson and T. R. Dutson, (Ed.), 83 -126, Elsevier Applied Science, New York, NY), it is possible that differences among establishments are due to difference in bone calcification. However, high variability of food chemical variable results within the establishments suggest that better process control procedures are needed.

The iron content of muscle and bone tissues is in several different forms which include heme compounds such as myoglobin and hemoglobin, storage complexes, and low molecular weight molecules (Hazell, T., Ledward, D. A., and Neale, R. J., 1978, *Br. J. Nutrition*, 39:631). There are numerous reports in the literature related to the iron content of mechanically deboned meat. Generally, it has been shown that the iron content of mechanically deboned meat is two to three times that of hand deboned meat. The results of this survey approximate those reported in the literature in that the total iron content of the AMRS final meat products was approximately 2 times that of the hand deboned product. However, the increase in the non-heme to heme iron ratio results would indicate that the increase in the iron level was not due to increases in heme compounds such as hemoglobin. Schricker, B. R., Miller, D. D., and Stouffer, J. R., 1982 (*J. Food Sci.*, 47:740) reported that 62% of the iron in meat was heme iron, and Underwood, E. J., 1971 (*Trace Elements in Human and Animal Nutrition*, 3rd Edition, Academic Press, New York, NY) reported that the iron which exceeds the requirements for hemoglobin, myoglobin, and other essential cellular functions is mainly stored in the form of hemosiderin and ferritin, which are non-

heme compounds. However, Blum, J. W., and Zuber, U., 1975 (Res. Vet. Sci., 18:294) reported that hemosiderin was not found in bone tissue. Further studies would be required to determine the component sources of the increased iron levels in the AMRS product.

The cholesterol level of the AMRS finished product was found to be approximately 2 times that of the hand deboned product. These data are less than those reported by Kunsman, J. E., Collins, M. A., Field, R. A., and Miller, G. J., 1981 (J. Food Sci. 46:1785) who found that the cholesterol content of mechanically deboned meat to be approximately 3 times that of hand deboned meat. However, since the bones used in mechanically deboned meat are ground and incorporated into the meat before the meat is separated from the bone, the tissue material within the bone would probably be incorporated into the meat. The increase in the cholesterol level in the AMRS final product is also less than the approximate 2.4 times increase in the fat content of the AMRS final product over that of the hand deboned product in this survey. These results would indicate that the increased cholesterol level in the AMRS final product could well be associated with the increased total fat level of the product, rather than from bone tissue. The slight increase in the saturated to unsaturated fatty acid ratio for the AMRS product also appears to be the result in higher levels of saturated fat generally found in adipose tissue rather than in bone tissue. Again, further controlled studies are needed to determine the source(s) of these materials.

Using the histological examination criteria for nervous tissue described in Attachment 3, two (2) AMRS product samples were found to contain CNS tissue (spinal cord). Both samples contained fragments of nervous tissue consisting of gray matter surrounding a central canal lined by ependymal cells, which are only seen lining the ventricles of the brain and the central canal of the spinal cord. Additional samples contained tissue that was compatible with CNS tissue, but the tissue was so fragmented that the larger structural features specific to spinal cord were not identifiable by routine histological examination. Other samples contained tissue that most likely originated in the spinal nerve root/ganglia, which is a transitional tissue with characteristics of CNS tissue and peripheral nerve tissue that is embedded in the vertebra and not removed with the spinal chord. Many of the nerve fragments found in the AMRS product samples were peripheral nerve fibers typically associated with skeletal muscle. Only peripheral nerve tissue was found in the hand deboned product. These data would indicate that some of the nerve tissue present in the neck bone was incorporated into the AMRS final product.

Based on the histological examination criteria for bone marrow tissue described in Attachment 3, many, if not most, of the AMRS product samples were found to contain hematopoietic cells, while only rarely did the hand deboned samples contain hematopoietic cells. These results would indicate that some of the bone tissue was, in fact, being incorporated into the AMRS final product.

Table 1. Average Differences Between Results Before and After Desinewing

Est. number	beef class	Number of Samples	protein (%)	moisture (%)	fat (%)	Moist/Protein Ratio (MPR)
1	1	27	0.797	-1.531	0.704	-0.239
2	1	24	0.422	-0.377	0.228	-0.112
3	2	16	0.949	2.446	-2.644	-0.113
4	2	27	0.377	0.608	-0.527	-0.048
5	2	26	0.116	-0.414	0.141	-0.052
6	2	25
7	2	18	0.284	-0.770	0.537	-0.104
Average	.	163	0.472	-0.141	-0.136	-0.112

Est. number	protein fat free (%)	cal-cium (%)	total iron (mg/100g)	heme iron (mg/100g)	non-heme iron (mg/100g)	ratio non-heme/heme
1	1.164	0.147	-0.245	-0.122	-0.123	-0.030
2	0.569	0.055	-0.333	-0.290	-0.043	0.051
3	0.685	0.051	0.089	0.017	0.072	0.024
4	0.333	0.037	0.259	0.201	0.058	-0.044
5	0.179	0.078	-0.459	-0.372	-0.088	0.006
6
7	0.476	0.060	0.046	0.032	0.013	0.031
Average	0.567	0.074	-0.125	-0.099	-0.027	0.002

Est. number	Chol-esterol (mg/100g)	bone residue (g/10g)	ash (mg/100g)	ratio saturated/unsaturated
1	-8.889	0.028	0.273	-0.028
2	-1.292	0.017	-0.004	0.033
3	7.875	0.004	-0.136	0.002
4	2.963	0.007	0.082	-0.001
5	1.769	0.031	0.129	-0.003
6
7	-12.056	0.010	0.113	0.032
Average	-1.710	0.017	0.092	0.004

For Beef class: 1 = steers/heifers, and 2 = cows/bulls
 This establishment did not use a desinewer.

Table 2. Mean of Results for Food Chemistry Variables

Est. number	Type Debone	deboning machine	pres sure PSI	dwel l time (sec)	Number of Samples	Summary Average
Average	A		3238	10	191	0.840
Average	H		0	0	54	-2.598
1	A	Protocon	2466	2	29	0.584
2	A	Hydrosep	2796	4	29	1.113
3	A	Hydrosep	3300	4	20	1.040
4	A	Hydrosep	2915	20	28	-0.212
5	A	Hydrosep	3500	10	28	0.963
6	A	Hydrosep	5500	3	30	2.301
7	A	Hydrosep	2011	28	28	0.109
8	H		0	0	27	-2.443
9	H		0	0	27	-2.747

Est. number	pro- tein (%)	fat (%)	Moist Protein Ratio (MPR)	cal- cium (%)	bone residue (g/10g)	total iron (mg/100g)
Average	16.359	20.011	3.890	0.108	0.013	2.795
Average	21.645	8.352	3.307	0.017	0.001	1.445
1 A	17.321	18.422	3.713	0.125	0.013	2.600
2 A	16.277	19.497	3.906	0.127	0.013	3.238
3 A	11.893	38.654	4.071	0.116	0.017	2.719
4 A	17.047	18.422	3.755	0.075	0.007	2.460
5 A	17.529	17.562	3.722	0.128	0.019	2.877
6 A	15.512	17.434	4.317	0.099	0.013	3.195
7 A	17.342	16.301	3.836	0.084	0.007	2.427
8 H	21.436	9.846	3.270	0.024	0.001	1.454
9 H	21.854	6.857	3.343	0.011	0.001	1.436

Est. number	ratio non-heme /heme	Chol- esterol (mg/100g)	ash (mg/100g)	ratio saturated /unsaturated
Average	0.486	115.048	1.174	1.127
Average	0.227	69.444	1.004	1.036
1 A	0.339	119.250	1.188	1.107
2 A	0.323	106.917	1.189	1.092
3 A	0.544	104.063	1.069	1.036
4 A	0.451	96.111	1.113	1.148
5 A	0.262	121.346	1.217	1.063
6 A	1.145	121.360	1.177	1.301
7 A	0.335	138.368	1.245	1.109
8 H	0.250	74.185	1.031	1.075
9 H	0.204	64.704	0.976	0.997

A = AMRS Processed, H = Hand Deboned
 Not all samples had results for each variable.

Table 3. Mean Results of Histological Results

Est. number	Type Debone	deboning machine	Pressure (PSI)	Dwell Time (SEC)
Average	A		3238	10
Average	H		0	0
1	A	Protocon	2466	2
2	A	Hydrosep	2796	4
3	A	Hydrosep	3300	4
4	A	Hydrosep	2915	20
5	A	Hydrosep	3500	10
6	A	Hydrosep	5500	3
7	A	Hydrosep	2011	28

Est. number	Number of Samples	Percent Spinal Cord Removed	Percent Nerve Tissue Present	Percent Bone Material Cells per slide	Bone Score	
					Before	After
Average A	191	100%	58.0%	1.750	1.40	2.86
Average H	108	7.4%	40.7%	0.074		
1 A	29	100%	57.7%	1.231	1.37	3.00
2 A	29	100%	66.7%	2.926	1.19	2.80
3 A	20	100%	57.9%	1.158	1.47	2.60
4 A	28	100%	51.9%	0.630	1.44	2.88
5 A	28	100%	60.7%	2.250	1.32	2.84
6 A	30	100%	76.9%	2.038	1.76	2.76
7 A	28	100%	33.3%	1.815	1.29	3.00
10 H	15	0.00	26.7%	0.000		
11 H	16	0.00	62.5%	0.000		
8 H	27	0.00	.	.		
12 H	15	0.00	33.3%	0.267		
9 H	27	0.00	.	.		
13 H	8	100%	37.5%	0.000		

A = AMRS Processed, H = Hand Deboned

Not all samples had results for each variable

Table 4. Cross Classification on Matched Samples of Bone Scores Before and After Deboning

Bone Score Before		Bone Score After			Total
		1	2	3	
Frequency	Percent				
Row Pct	Col Pct				
1		2	7	105	114
		1.22	4.27	64.02	69.51
		1.75	6.14	92.11	
		50.00	70.00	70.00	
2		1	2	31	34
		0.61	1.22	18.90	20.73
		2.94	5.88	91.18	
		25.00	20.00	20.67	
3		1	1	14	16
		0.61	0.61	8.54	9.76
		6.25	6.25	87.50	
		25.00	10.00	9.33	
Total		4	10	150	164
		2.44	6.10	91.46	100.00

Table 5a. Within Establishment Standard Deviations, Coefficient of Variation (CV) and Intra-day Correlations

Type of Deboning: AMRS machine Deboning

Variable	Mean Value	Between Day Standard Deviation	Within day Standard Deviauton	Total Within Estab. Stan. Dev.	CV(%)	Intra-day Correlation (%)
Protein	16.36	0.431	1.03	1.12	6.8	14.90
Fat	20	0.457	3.18	3.21	16.04	2.02
MPR	3.89	0.125	0.19	0.22	5.7	31.30
Calcium	1.08e-01	2.81e-02	3.52e-02	4.50e-02	41.7	38.9
Calcium ¹	1.05e-01	1.24e-02	2.66e-02	2.93e-02	28.0	17.8
Bone residue	1.30e-02	1.70e-03	5.18e-03	5.45e-03	41.9	10.40
Total iron ²	2.79	0.404	0.56	0.69	24.9	33.90
Non-Heme/Heme ²	0.486	0.0889	0.21	0.23	47.5	14.80
Cholesterol	115	4.953	17.53	18.22	15.8	7.39
Ash	1.17	0.0503	0.13	0.14	11.7	13.40
Saturated to unsaturated fatty acids	1.127	0.08	0.12	0.14	12.7	31.10

¹ two high values of 0.39% and 0.43% were deleted

² one high total iron result of 8 mg/100g was deleted

Table 5b. Within Establishment Standard Deviations, Coefficient of Variation (CV) and Intra-day Correlations

Type of Deboning: Hand Deboning

Variable	Mean Value	Between Day Standard Deviation	Within day Standard Deviation	Total Within Estab. Stan. Dev.	CV(%)	Intra-day Correlation (%)
Protein	21.64	1.042	0.676	1.24	5.7	70.40
Fat	8.35	2.13	1.67	2.71	32.4	61.90
MPR	3.31	0.089	0.11	0.14	4.3	38.80
Calcium	1.70e-02	7.40e-03	6.24e-03	9.68e-03	56.9	58.35
Bone residue	9.31e-04	1.38e-03	1.16e-03	1.80e-03	193.7	58.80
Total iron	1.44	0.1654	0.19	0.25	17.4	43.36
Non-Heme/Heme ¹	0.227	0.115	0.06	0.13	57.2	78.27
Cholesterol	69.4	6.11	7.56	9.72	14	39.45
Ash	1	0.068	0.06	0.09	8.88	58.20
Saturated to unsaturated fatty acids	1.036	0.126	0.08	0.15	14.3	72.36

¹ one high ratio of non-heme to heme iron result of 2.5 was deleted

Figure 2. Box-plots of Chemical Variables by Type of Deboning

Variable=Protein (%)

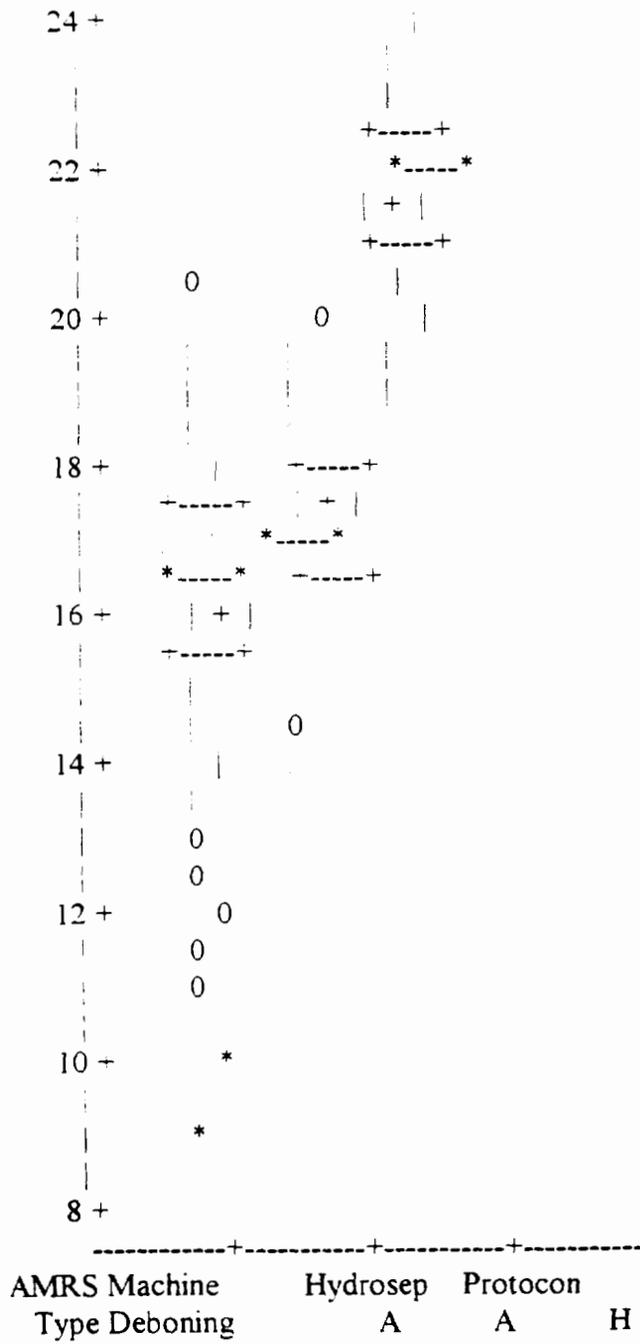


Figure 2(cont). Box-plots of Chemical Variables by Type of Deboning

Variable=Fat (%)

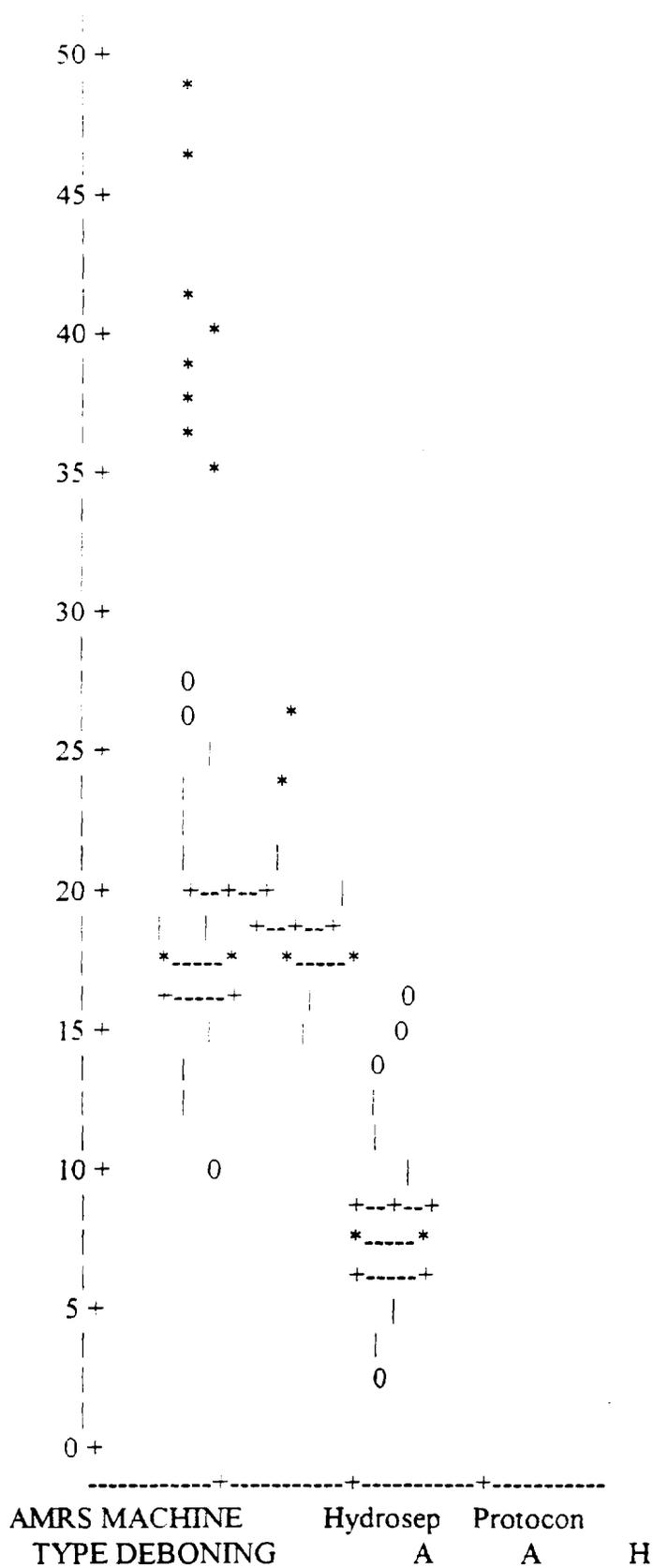


Figure 2(cont). Box-plots of Chemical Variables by Type of Deboning

Variable=Moisture Protein ratio (MPR)

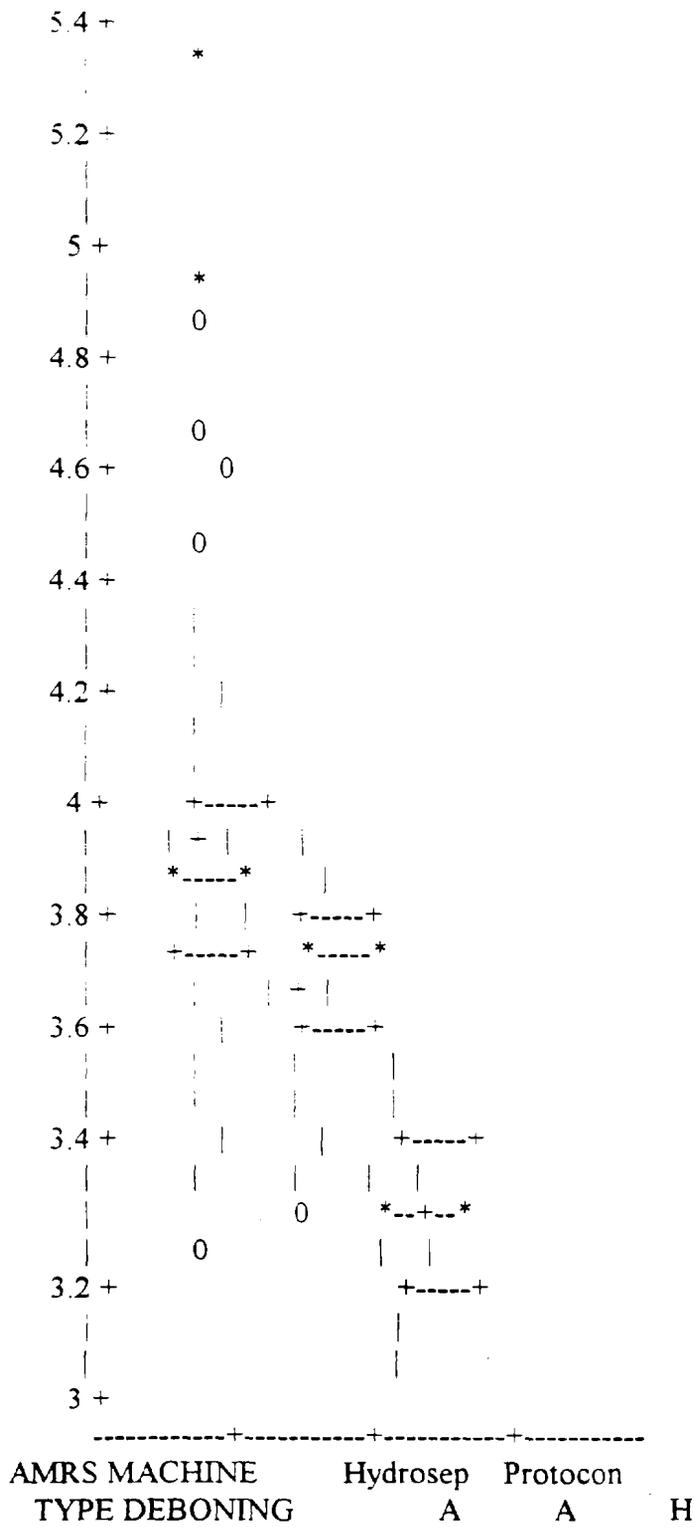
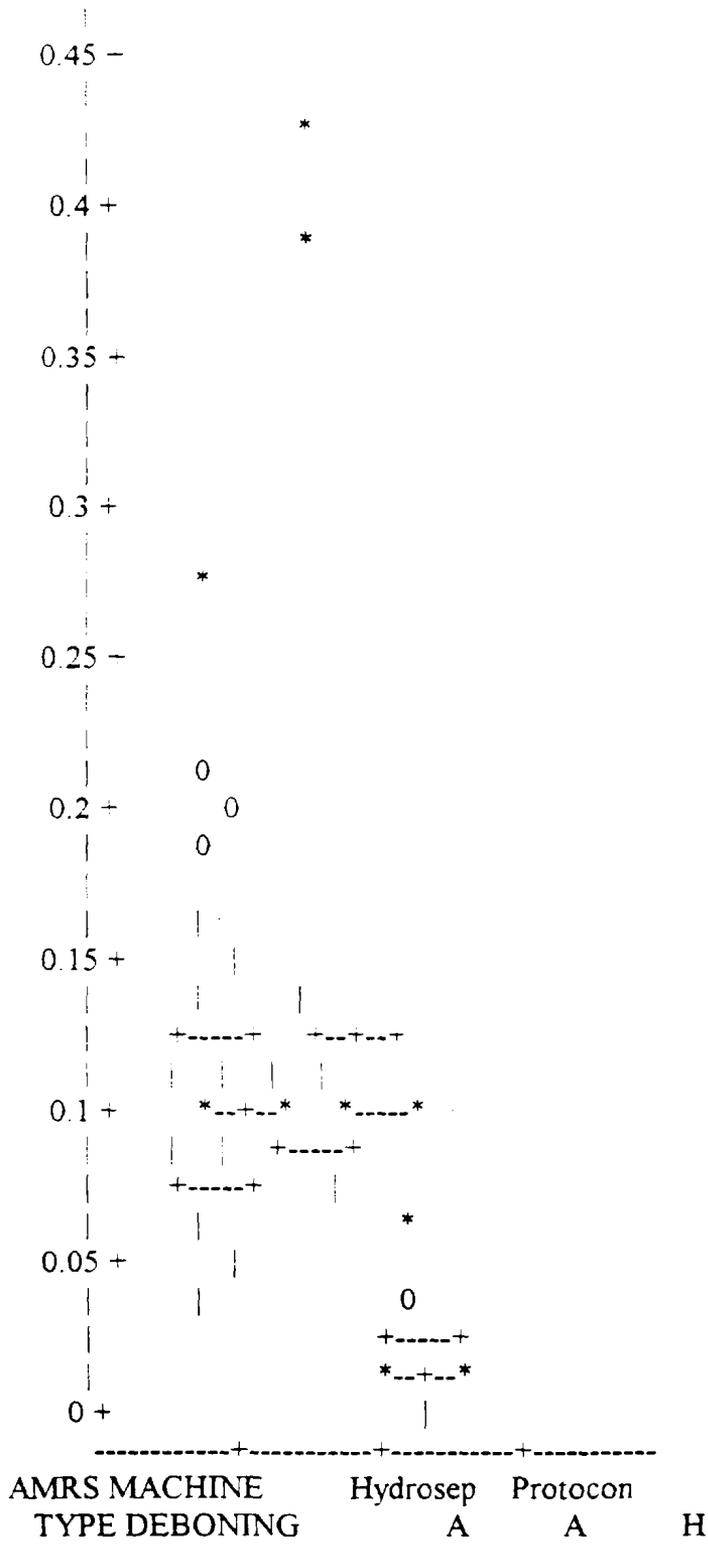


Figure 2(cont). Box-plots of Chemical Variables by Type of Deboning

Variable=Calcium(%)



Variable=Bone residue (g/10g)

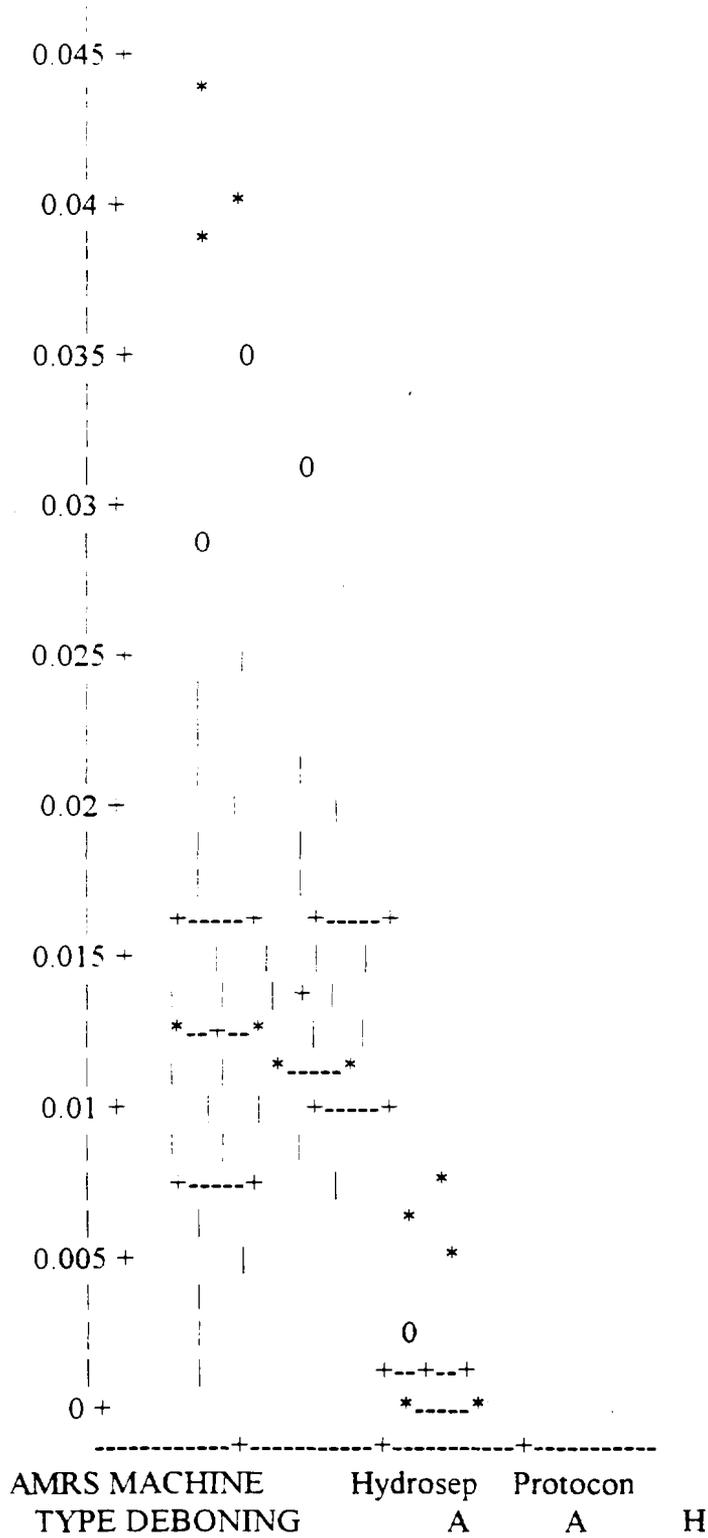
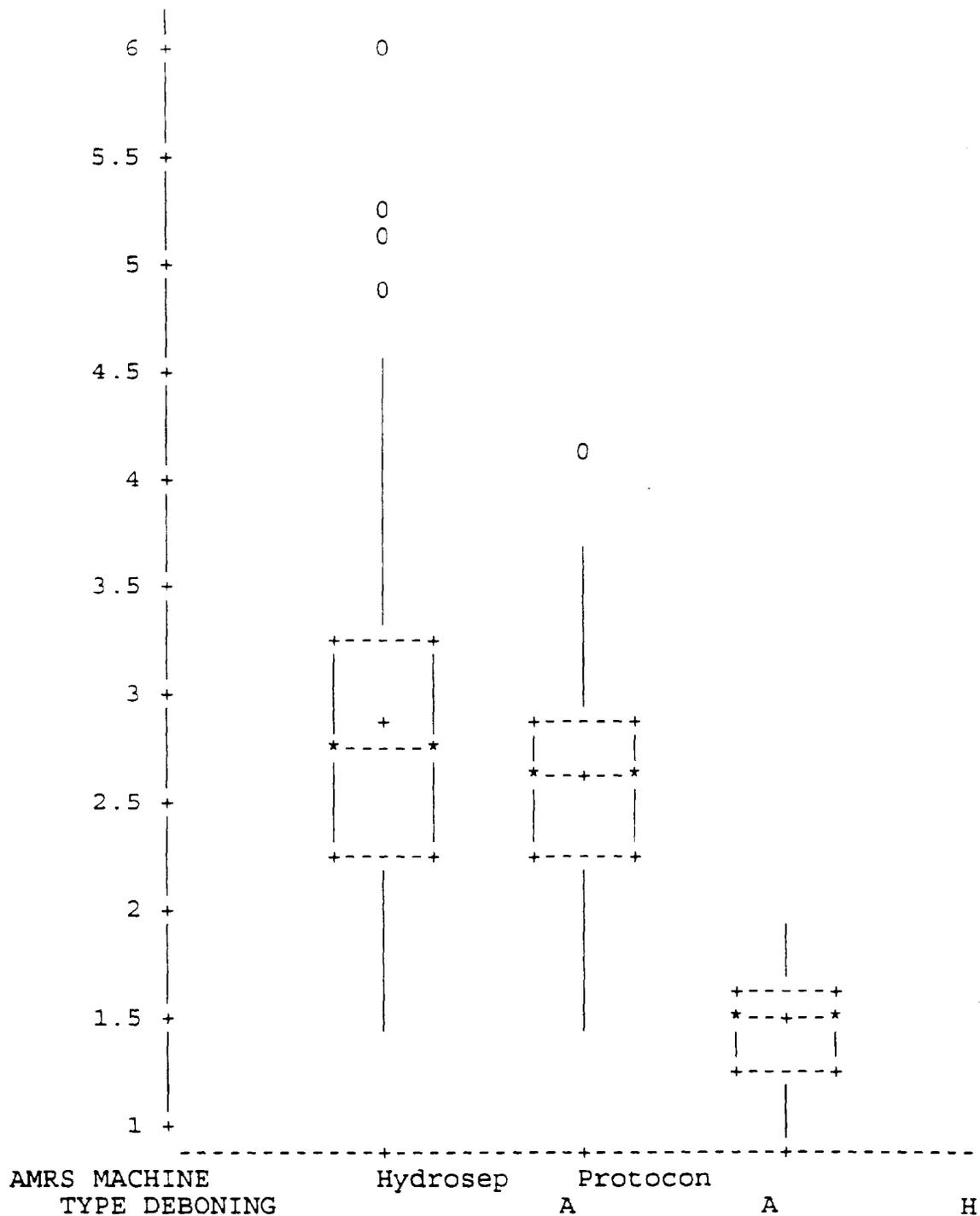


Figure 2 (cont): Box-plots of Chemical Variables by Type of

Variable=Total Iron (mg/100g)



One AMRS Hydrosep machine results of 8 mg/100g is not included

Figure 2(cont). Box-plots of Chemical Variables by Type of Deboning

Variable=Ratio of Nonheme to heme iron

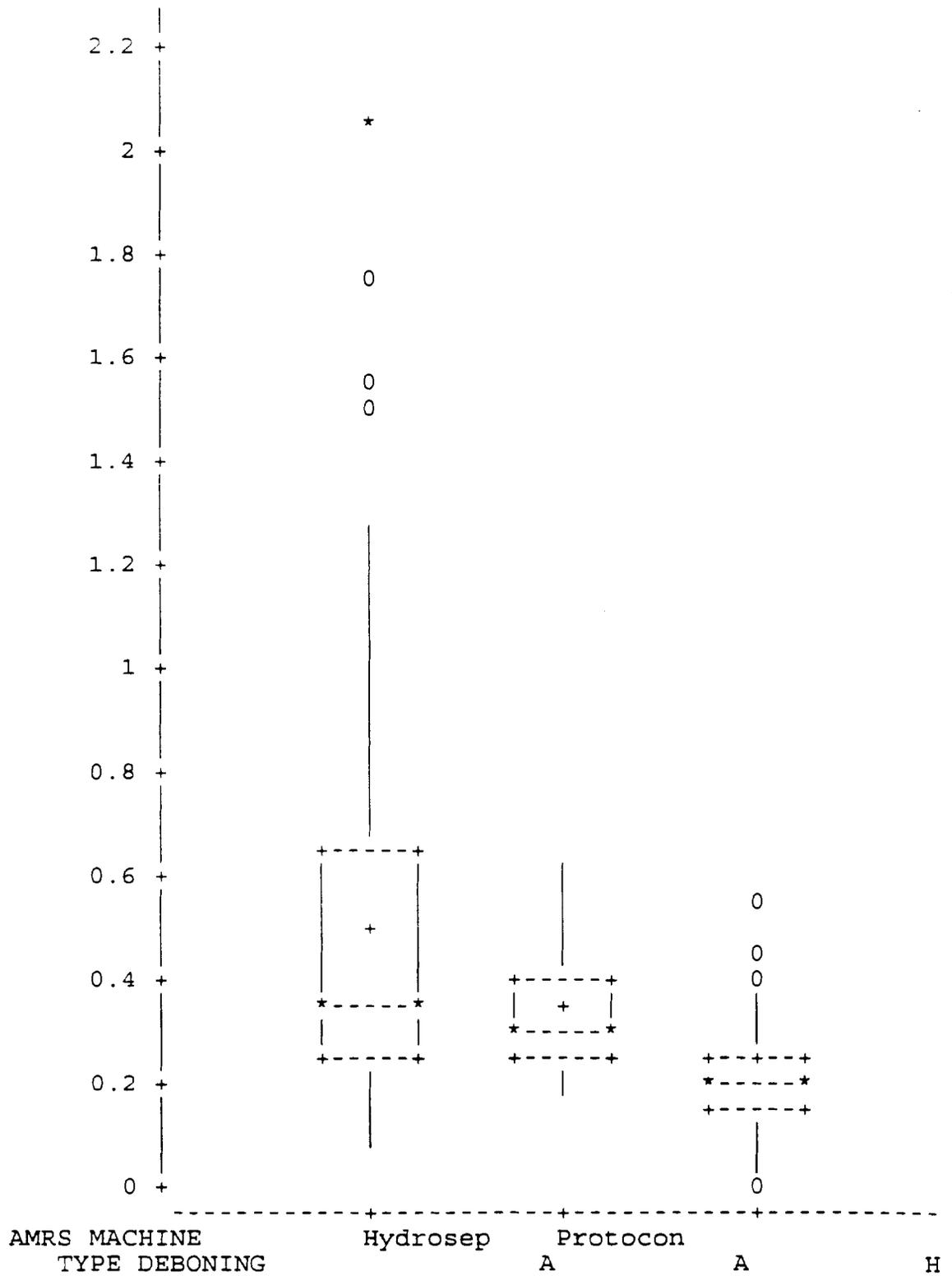


Figure 2 (cont). Box-plots of Chemical Variables by Type of Deboning

Variable=Cholesterol (mg/100g)

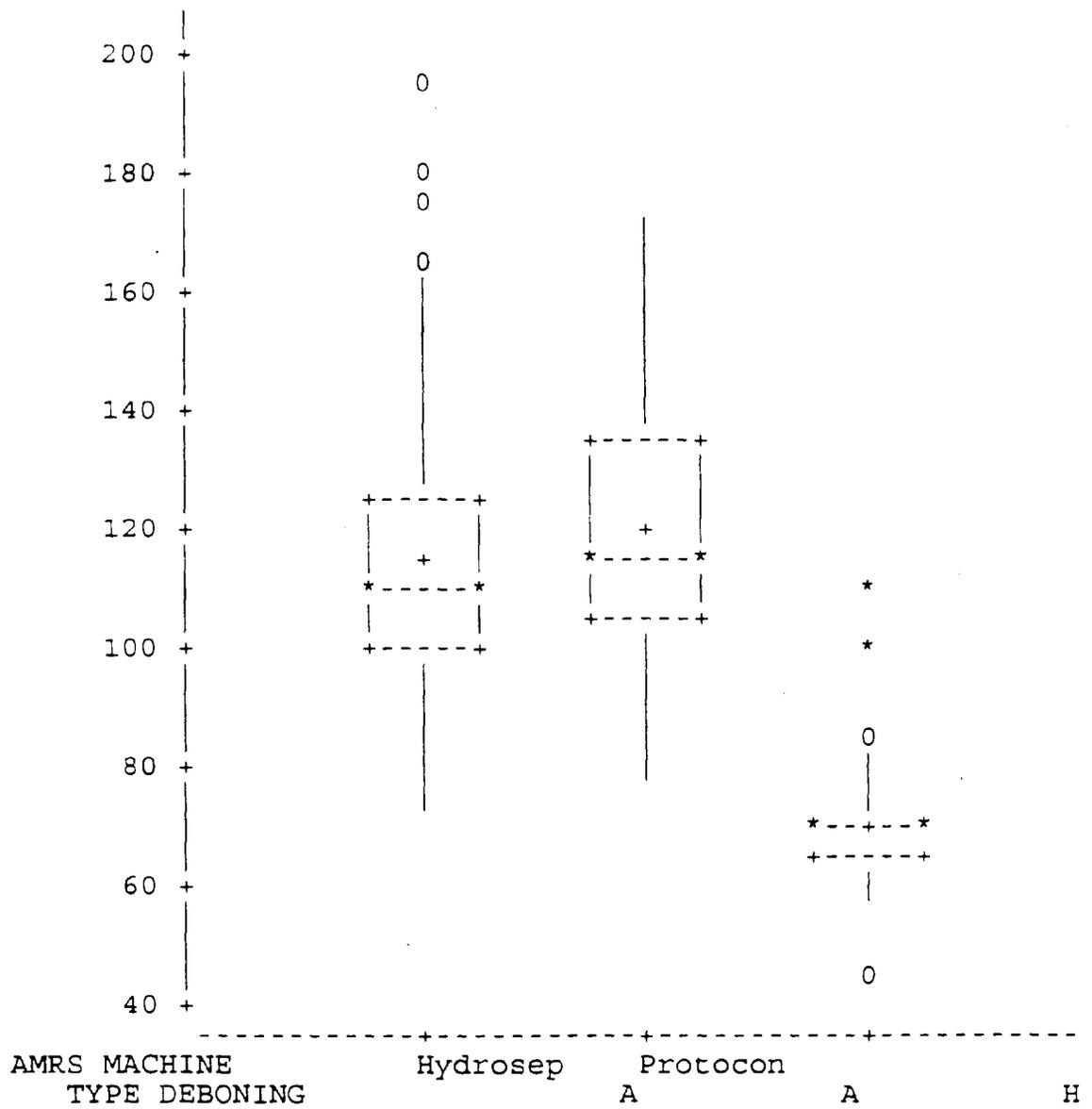


Figure 2 (cont). Box-plots of Chemical Variables by Type of Deboning

Variable=Ash (mg/100g)

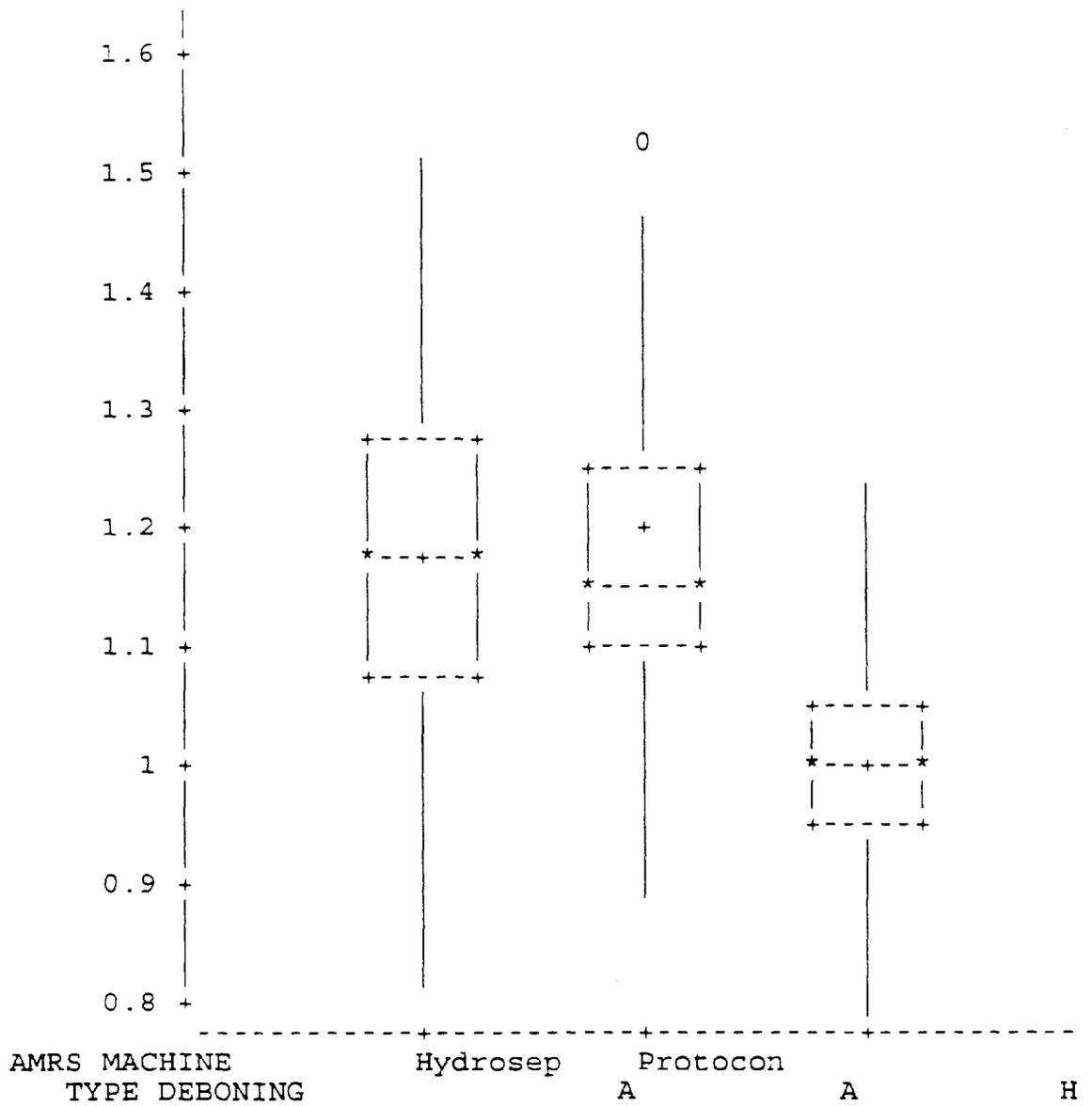


Figure 2(cont). Box-plots of Chemical Variables by Type of Deboning

Variable=Ratio of saturated to non-saturated fat

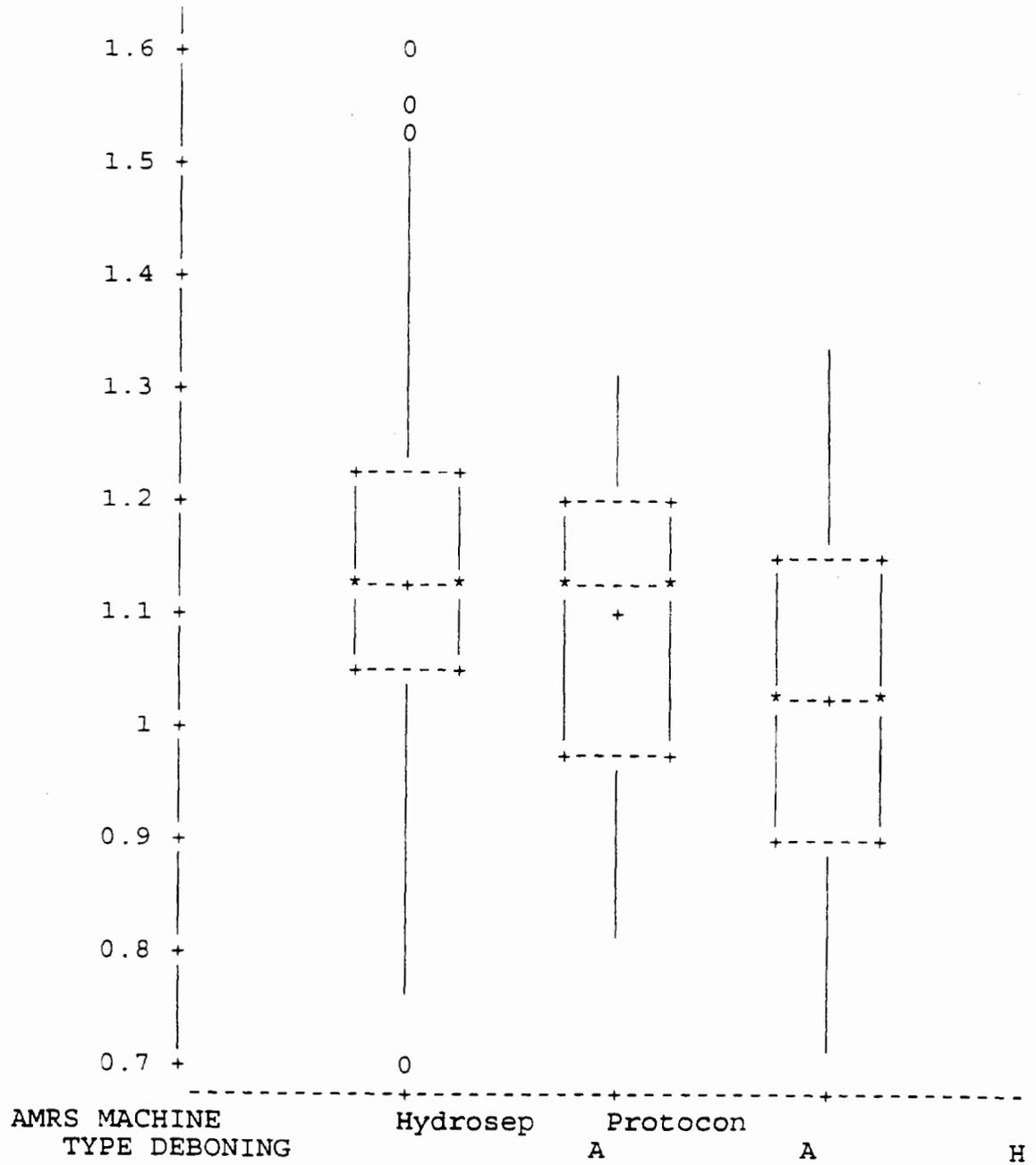
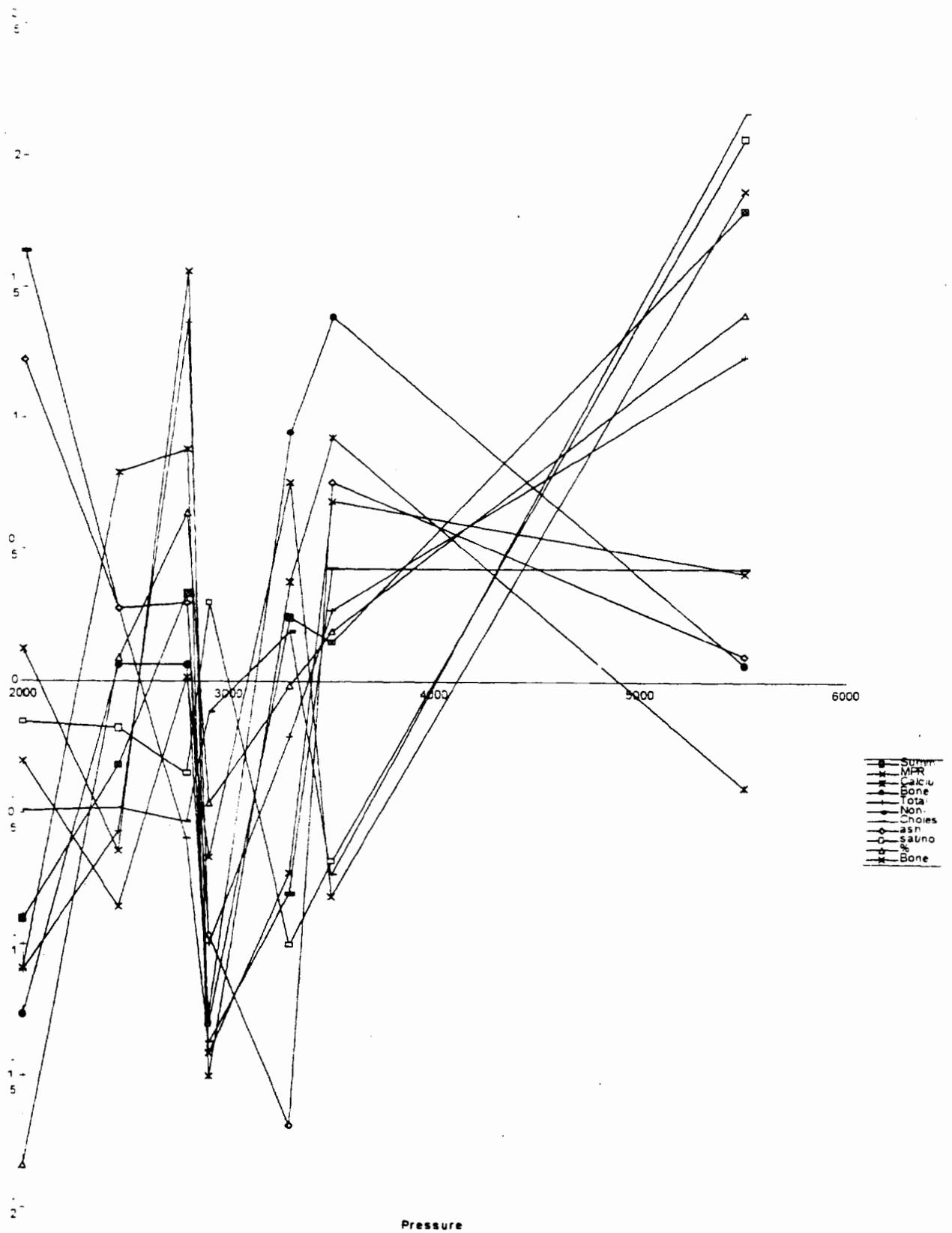
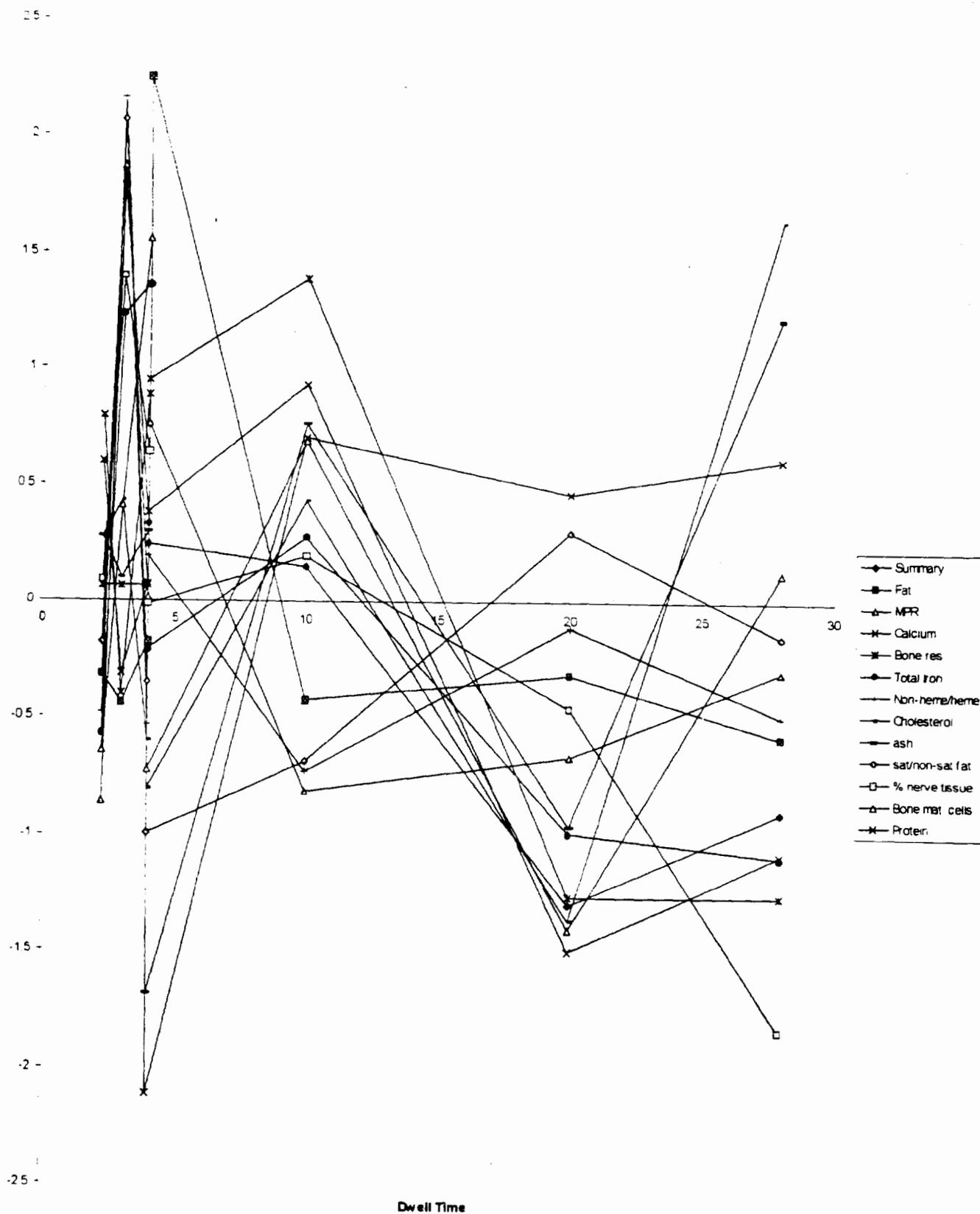


Figure 3 Standardized Scores¹ Versus AMRS Machine Pressure



¹ Standardized Score = (result - mean) / std. deviation

Figure 4 Standardized Scores¹ Versus AMRS Machine Dwell Time



¹ Standardized Score = (result - mean)/std. deviation

**Complete Advanced Meat Recovery Systems (AMRS)
Survey Sampling Instructions**

Background Information.

Science and Technology is conducting a survey on Advanced Meat Recovery Systems (AMRS). The survey is intended to provide analytical data on two main issues: compliance with the FSIS regulations on AMRS and the nutrient composition of the AMRS product. Comparison product will be hand-boned beef neck meat.

A variety of samples will be collected at sample points before and throughout the AMRS to be submitted to the Eastern Laboratory. Bone samples will be evaluated by FSIS pathologists to determine whether the bones are being damaged during processing. FSIS pathologists will analyze formalin-fixed samples of product. FSIS and ARS chemists will analyze product samples for nutrient composition.

Veterinary Medical Officers will verify that all spinal cord tissue has been removed from neck bones prior to processing and complete an unofficial PPID/HACCP supplemental AMRS Survey Instrument.

Sample Identification Procedures.

Sticky labels will be attached to all sample bags (before freezing) and jars. Product will be enclosed in special slotted plastic cassettes to prevent its dispersion in the formalin. Label information will include the FSIS laboratory form **serial number** for that sample (not the form number 10,300-2 or 10,600-1), the code letter for the sampling point (A, B, C, D, or E) where the sample was collected (see Quick Reference Coding Chart), and the date and time the sample was collected.

Sampling Instructions for the Collection of Bones to be sent to the Pathologists.

A. General Instructions.

1. The sampling period will last for three consecutive weeks (if the holiday week over Labor Day is a problem, discuss alternatives during conference call).
2. Samples will be collected on three randomly selected days per week. **Note:** Samples can be taken on any three days during the week. If possible, do not collect samples on the same three days each week.
3. Collect one complete set of samples at three different, randomly-selected times during one shift of operations. **Note:** When determining the times to take samples, try to sample throughout the shift. (i.e., take the first set of samples soon after the shift begins, a second set midway through the shift, and the final set of samples towards the end of the shift). However, if the establishment

does not process neck bones through the entire shift, collect the samples at three different times whenever neck bones alone are being processed. Do not collect samples from other than neck bones or mixed bones.

B. Collecting Bone Samples.

1. Bone samples will be obtained at two of the following three sites (A&C or B&C):

a. one whole bone(s) (vertebra) section if no pre-sizing is done before bones enter the AMRS machinery or,

b. one 4 to 6 inch section of bone(s) (vertebra) after pre-sizing and before the bones enter the AMRS, and,

c. one piece of bone(s) residue equivalent to either one whole or one pre-sized section of bone(s) upon exiting the AMRS machinery.

Each sample set (A&C or B&C depending on the system's configuration) will be separately bagged, labeled, and collected three times during the shift. **Note:** Depending on the type of system used, the establishment may pre-size the bone(s) sections (cut into smaller individual pieces) before placing them into the AMRS pressing machinery. If the establishment does not perform this operation before placing the bones into the pressing machinery, one whole bone(s) section will be collected before they are placed into the AMRS machinery.

2. Collecting bone samples will be done at one of two sites depending on whether or not the establishment includes pre-sizing in their process. Samples will be collected before entering the AMRS machinery, either as whole bone(s) or as a pre-sized bone(s) sections.

a. Complete a sample form (FSIS Form 10,300-2, Pathology Specimen Submission) and the corresponding sample labels. **Note:** A separate sample form will be used for each sample.

b. Obtain a plastic sample bag large enough to accommodate the sample being taken.

c. Collect a representative sample of one complete bone(s) or pre-sized bone(s) section before entering the AMRS machinery.

d. Identify the sample with a stick-on label containing the FSIS form's serial number, sampling point (A or B), and time/date of collection. The forms will be found either inside the sample boxes supplied by the Eastern Laboratory of from PPID/HACCP.

3. Collecting processed bone residue samples exiting the AMRS machinery after the meat has been harvested.

a. Obtain a separate plastic sample bag large enough to accommodate the sample being taken. **Note:** One FSIS Form 10,300-2 will be used per sample.

b. Collect the equivalent of at least one whole bone(s) or pre-sized section of bone(s) residue exiting the AMRS machinery. **Note:** Some AMRS machines process the bones more heavily than others. When collecting the samples, please do your best to obtain the equivalent of a whole or pre-sized bone(s) section. The sample does not need to be in one piece as long as it represents one bone(s) section. The sample should also represent the physical condition of the bones that normally exit the AMRS machinery.

c. Identify the sample on the bag with a stick-on label containing the FSIS form's serial number, sampling point code C, and time/date of collection. The forms will be found either inside the sample boxes supplied by the Eastern Laboratory or sent from PPID/HACCP.

4. Place each bone sample in a freezer immediately after collection. Freeze completely. Ship the entire week's bone samples in a single container, if possible, to the Eastern Laboratory. Do not ship samples in Friday.

C. Collecting Formalin Fixed Samples.

1. Complete a sample form (FSIS Form 10,300-2, Pathology Specimen Submission) and the corresponding sample labels. **Note:** A separate sample form will be used for each sample (a sample in this case is two cassettes of product from sampling points D and/or E).

2. Obtain a formalin shipping kit with formalin jars and plastic cassettes for this survey. **Note:** Each formalin kit will contain two vials of formalin and four small plastic sample cassettes, as well as the form.

3. Collect and place a small amount of sample from sample points D and E (probably less than one teaspoon per cassette) into each of two cassettes per sample point (two D and two E).

4. Close the cassettes and place the two cassettes of the same sample type into one vial of formalin. Close the formalin jar lids. Do not agitate or shake the formalin jars for approximately 12-24 hours to allow adequate fixation of the tissues.

5. Identify the sample with the FSIS form's serial number, sampling point code (D or E), and time/date of collection with the stick-on label provided by PPID/HACCP on the formalin jar.

D. Completing FSIS Forms 10,300-2, and the PPID/HACCP Supplemental Survey Form.

1. On FSIS Form 10,300-2 Pathology Specimen Submission, fill in the blocks as follows:

a. Project name - "AMRS Survey";

b. Complete other blocks as for a routine pathology sample - Species, Age, Date Collected, Date Mailed, Establishment No., Name of Veterinarian, Telephone No., Region, and State;

c. In the block "Please Check Tissues Submitted", under "Other" write the appropriate sampling point code letter.

2. Obtain one PPID/HACCP Supplemental Survey Form and fill it out completely. You will receive a set of pre-addressed FedEx air bills to send an entire week's forms to Dr. Bob Hasiak, PPID/HACCP.

E. Shipping Samples.

1. Once the samples are ready to be shipped, place the frozen samples into an appropriate insulated shipping container and the formalin-fixed samples into their formalin kit box.

2. Attach all forms to the sample that correspond to the sample before placing the sample in the shipping container. **Note:** Remember that each sample will be identified with the laboratory form serial number. Be sure that the form serial number and sample numbers of the samples in the shipping container match.

3. Ship samples to:

Attn: W. W. Kosciński, Chemist-in-Charge
USDA/FSIS/S&T
Eastern Laboratory
950 College Station Road
Athens, GA 30605
Phone number: (706) 546-3571

4. All samples will be sent through Federal Express - the Fed Ex air bills will be provided. If you run out of any supplies, contact either your Regional contact or Lynvel Johnson at (202) 501-7319.

5. **Only** ship samples to the laboratory on Monday, Tuesday, Wednesday, or Thursday. **Do not ship any samples on Friday.**

Sampling Instructions for the Collection of Intermediate and Finished Product to be sent to the Chemists.

A. General Instructions.

1. The sampling period will last for three consecutive weeks (if the holiday week over Labor Day is a problem, discuss alternatives during conference call).
2. Samples will be collected on three randomly selected days per week. **Note:** Samples can be taken on any three days during the week. If possible, do not collect samples on the same three days each week.
3. Collect one complete set of samples at three different, randomly-selected times during one shift of operations. **Note:** When determining the times to take samples, try to sample throughout the shift. (i.e., take the first set of samples soon after the shift begins, a second set midway through the shift, and the final set of samples towards the end of the shift). However, if the establishment does not process neck bones through the entire shift, collect the samples at three different times whenever neck bones alone are being processed. Do not collect samples from other than neck bones or mixed bones.

B. Collecting Recovered Meat Product Samples.

1. Collecting intermediate finished meat product before it enters the desinewer.
 - a. Obtain and complete a sample form (FSIS Form 10,600-1, Domestic Chemical Laboratory Report) and corresponding sample stick-on labels. **Note:** Complete one FSIS Form 10,600-1 for each set of two bags of product from this sampling point. Forms and sheets of stick-on labels will be provided by PPID/HACCP.
 - b. Obtain two plastic sample bags for this sampling point.
 - c. Before the meat from the ARMS machinery enters the desinewer, collect two 1-pound samples.
 - d. Label each 1-pound bag with the FSIS form's serial number, sampling point D, and time/date of collection. **Note:** The serial number to be used is found in block 6.
 - e. Freeze all samples as soon as possible after collection and before shipping to the laboratory.
2. Collecting finished meat product.
 - a. Obtain and complete a sample form (FSIS Form 10,600-1, Domestic Chemical Laboratory Report) and corresponding sample stick-on labels. **Note:** Complete one FSIS Form 10,600-1 for each set of two bags of product from this sampling point. Forms and sheets of stick-on labels will be provided by PPID/HACCP.

- b. Obtain two plastic sample bags for this sampling point.
- c. Collect two 1-pound samples of finished meat product as the product exits the desinewer into a box or combo bin.
- d. Label each 1-pound bag with the FSIS form's serial number, sampling point D, and time/date of collection. **Note:** The serial number to be used is found in block 6.
- e. Freeze all samples as soon as possible after collection and before shipping to the laboratory.

C. Completing FSIS Form 10,600-1, Domestic Chemical Laboratory Report

- 1. On FSIS Form 10,600-1, complete boxes 1, 7, 8, 11, 16, 17, 21, 22, 23, and 24. In block 11, fill in the product code letter E. In block 21, fill in "**AMRS Survey**".
- 2. Obtain one PPID/HACCP Supplemental Survey Form and fill it out completely for all samples collected during one sample period. Mail these forms only once each week to Dr. Bob Hasiak, PPID/HACCP, using the pre-addressed FedEx air bills.

D. Shipping Samples.

- 1. Freeze all samples as soon as possible after collection and ship the frozen samples to the Eastern Laboratory.
- 2. Attach the appropriate form to the samples before placing the samples in the shipping container. **Note:** Remember, each sample will be identified with a serial number found in block 6 on the FSIS Form 10,600-1. Before sealing the shipping container, make sure the numbers on the form and sample match.
- 3. FedEx the frozen samples in the shipping container via Government Overnight Delivery to:

Attn: W. W. Koscinski, Chemist-in-Charge
USDA/FSIS/S&T
Eastern Laboratory
950 College Station Road
Athens, GA 30605
Phone number: (706) 546-3571

- 4. All samples will be sent through Federal Express. The FedEx air bills will be provided. If you run out of any supplies, contact your Regional contact or Lynvel Johnson at (202) 501-7319.
- 5. Only ship samples to the laboratory on Monday, Tuesday, Wednesday, or Thursday. **Do not ship any samples on Friday.**

Quick Reference

Advanced Meat Recovery System (ARMS) Sample Collection Point Coding Chart

General: Because there are different configurations of AMRS, identify and select the appropriate sample series for the system in the establishment. Possible combinations are: A-C-D, A-C-D-E, A-C-E, B-C-D-E, or B-C-E. Each time samples are collected, complete the appropriate FSIS forms. Also complete one PPID/HACCP Supplemental AMRS Survey Form to send to PPID/HACCP once a week. Other forms are to be sent with samples to the Eastern Laboratory. Ship all samples, forms, etc., by Federal Express using Government Overnight Delivery. All samples should be collected at random times and days, three times per day during one shift, three days per week, for each of the three weeks of the survey. Do not collect samples other than beef neck bones.

Sampling Point A: Whole bone(s) (vertebra) entering the AMRS machinery. **Note:** If pre-sizing is done, skip this sampling point.

- Sample type: whole bone(s) (vertebra) in bag; complete Pathology form 10,300-2; label; freeze completely; ship to Eastern Laboratory.

Sampling Point B: Pre-sized bone(s) (vertebra) prior to entering the AMRS machinery.

- Sample type: one pre-sized section of bone(s) (vertebra) in bag; complete Pathology form 10,300-2; label; freeze completely; ship to Eastern Laboratory.

Sampling Point C: AMRS bone residue (only from the AMRS machinery, not from desinewer).

- Sample type: quantity of bone residue equivalent to one whole bone(s) or pre-sized section in bag; complete Pathology form 10,300-2; label; freeze completely; ship to Eastern Laboratory.

Sampling Point D: Intermediate finished product (only if a desinewer is part of the process).

- Sample type: two 1-pound bags of product; complete Chemistry form 10,600-1; label; freeze completely; ship to Eastern Laboratory.
- Sample type: partially fill two cassettes with product; gently put both cassettes in one jar of formalin (see detailed instructions); complete Pathology for 10,300-2; label jars; do not disturb for 12 to 24 hours; ship in formalin kit boxes to Eastern Laboratory.

Sampling Point E: Final finished product (all AMRS).

- Sample type: two 1-pound bags of product; complete Chemistry form 10,600-1; label; freeze completely; ship to Eastern Laboratory.
- Sample type: partially fill two cassettes with product; gently put both cassettes in one jar of formalin (see detailed instructions); complete Pathology for 10,300-2; label jars; do not disturb for 12 to 24 hours; ship in formalin kit boxes to Eastern Laboratory.

PPID/HACCP Survey Sampling Instructions for Hand Deboned Meat

General Instructions.

1. Samples of hand deboned beef neck meat will be collected and submitted to:

Attn: W. W. Koscinski, Chemist-in-Charge
USDA/FSIS/S&T
Eastern Laboratory
950 College Station Road
Athens, GA 30605
Phone number: (706) 546-3571

2. All samples will be sent through Federal Express; the FedEx air bills will be provided. If you run out of any supplies, contact either your Regional contact or Lynvel Johnson at (202) 501-7319.
3. Double bag all samples.
4. Hand deboned beef neck meat samples will be collected during three consecutive weeks.
5. Samples will be collected during one shift of operations on three randomly selected days of the week.

Note: Samples can be taken on any three days during the week, however, try not to sample on the same days each.

6. Collect two 1-pound bags of hand deboned meat three different times throughout the shift. Label the samples and complete FSIS Form 10,600-1 boxes 1, 7, 11, 15, 16, 17, 21, 22, 23, and 24. In block 11, fill in the product code letter E. In block 21, fill in **Controls for AMRS Survey**.

Note: When deciding the times to take samples, spread them throughout the shift; i.e., take the first set of samples soon after the shift begins, a second set midway through the shift, and a final set of samples towards the end of the shift. However, if the establishment does not process neck bones throughout the entire shift, try to collect the samples at three different times while the neck bones are being processed.

Sampling Procedure.

1. Obtain two plastic sample bags (should be found in sample shipping containers).
2. Collect two 1-pound samples of hand deboned meat.
3. Place each 1-pound sample into a separate sample bag.
4. Label both sample bags with the stick-on labels provided with the following information: form serial number from block 6, time/date sample collected, and establishment number.

5. Freeze the samples overnight or until they are hard frozen as soon as possible after collection.

Shipping Samples.

1. Either before or after the samples have been collected, fill out the laboratory form that corresponds to the labeled samples. Attach the form to the sample prior to freezing and shipping.
2. Place the samples and attached forms in the insulated shipping container. Ship an entire day's samples in one shipping container, if possible. No coolant bottles are needed if the samples are hard frozen prior to shipping.

Note: Before sealing the shipping container, make sure that the sample numbers on each label matches the corresponding laboratory serial number.

3. FedEx the samples to the following address using the pre-addressed FedEx air bills for Government Overnight Delivery:

Attn: W. W. Kosciński, Chemist-in-Charge
USDA/FSIS/S&T
Eastern Laboratory
950 College Station Road
Athens, GA 30605
Phone number: (706) 546-3571

Attachment 2

Criteria and Methodology for Scoring Waste Bone from Advanced Meat Recovery System Equipment

Each sample of bone collected from sample points B and C for the AMRS Survey will be thawed, examined grossly by a pathologist and scored from one to three using the criteria listed below. Once examined, a sample of tissue will be collected from representative cases for possible future histological examination.

Criteria for Scoring Bones Subjected to the ARMS Process.

1 = Vertebra - the bone is recognizable as a vertebra and is essentially intact; the vertebral processes are intact; there is no dislocation of intervertebral joints or loss of joint capsule integrity; external cartilage is intact; the periosteal surface is not abraded/lacerated or "polished"; the spongy bone containing red marrow is a deep red color and bony spicules are not crushed or fractured; there is no evidence of crushing on external surfaces; there is minimal bone dust on external surfaces.

Ribs/plates - the bone is recognizable as a rib/plate bone and essentially intact; the bone is not fractured; there is no loss of cartilage; the periosteal surface is not abraded/lacerated or "polished"; the spongy bone containing red marrow is a red color and shows no evidence of crushing or fracture of bony spicules; there is minimal bone dust on external surfaces.

Long bones - the bone is recognizable as to identity (femur, humerus, tibia, etc.); the bone is not fractured; there is no loss of cartilage; the bone or cartilage is not easily detached at the edges of the tissue; the periosteal surface is not abraded/lacerated or "polished"; there is no loss of fatty marrow from the marrow cavity in the center of the long bone shaft; there is no evidence of crushing on external surfaces; there is minimal bone dust on external surfaces.

2 = Vertebra - the bone is recognizable as vertebra; occasionally there is fracture of the vertebral processes; there is occasional dislocation of intervertebral joints or loss of joint capsule integrity; external cartilage is intact; there is abrasion/laceration or "polishing" of the periosteal surface of the bone; the spongy bone containing red marrow is red in color and there is only occasional fracturing of bony spicules in the spongy bone; there is no evidence of crushing on external surfaces; there is minimal bone dust on external surfaces.

Ribs/plates - the bone is recognizable as a rib/plate bone; occasionally there is a fracture of the bone; there is only occasional loss of cartilage; the bone or cartilage is not easily detached at the edges of the tissue; there is abrasion/laceration or "polishing" of the periosteal surface of the bone; any spongy bone containing red marrow is red in color and there is only occasional fracturing of bony spicules in the spongy bone; there is no evidence of crushing on external surfaces; there is minimal bone dust on external surfaces.

Long bones - the bone is recognizable as to identity (femur, humerus, tibia, etc.); occasionally is fractured; there is only occasional loss of cartilage; the bone or cartilage is not easily detached at the edges of the tissue; there is abrasion/laceration or "polishing" of the periosteal surfaces; there is occasional loss of part of the fatty marrow from the marrow cavity in the center of the long bone shaft; there is no evidence of crushing on external surfaces; there is minimal bone dust on external surfaces.

3 = Vertebra - the bone is not intact; the vertebral processes are fractured; there is dislocation of intervertebral joints and loss of joint capsule integrity; external cartilage is not intact; the spongy bone containing red marrow is a pale pink or tan in color or bony spicules are crushed or fractured; there is evidence of crushing on the external surfaces; there is accumulation of bone dust on external surfaces.

Ribs/plates - the bone is not intact; the bone is routinely fractured; there is loss of cartilage; the periosteal surface is abraded/lacerated or "polished"; the spongy bone containing red marrow is a pale pink or tan in color and shows evidence of crushing or fracture of bony spicules; there is evidence of crushing on external surfaces; there is accumulation of bone dust on external surfaces.

Long bones - the bone is not intact; the bones are routinely fractured; there is loss of cartilage; the bone or cartilage is easily detached at the edges of the tissue; the periosteal surface is abraded/lacerated or "polished"; there is loss of fatty marrow from the central marrow cavity in the shaft of the long bone; there is evidence of crushing on external surfaces; there is accumulation of bone dust on external surfaces.

Attachment 3

HISTOLOGICAL EXAMINATION OF AMRS PRODUCT AND HAND DEBONED MEAT

The FSIS AMRS Survey included microscopic examination hand deboned comparison samples, product that had been subjected to AMRS equipment before going through a desinewer, and product that had gone through both AMRS equipment and a desinewer. All samples were evaluated for types of tissue present. If present, bone, cartilage, and nerve were identified, counted, and sized on a qualitative basis. Bone marrow was reported as an estimated percent of the total amount of tissue examined. A pathologist certified by the American College of Veterinary Pathologists (ACVP) performed all of the histological examinations to assure uniformity and minimize variation in the project design.

In addition, three (3) other ACVP certified pathologists reviewed a representative sample of the cases. Below is a brief description covering the presence of nervous tissue and bone marrow, and a summary of criteria used to identify each in the histological sections.

Nervous Tissue.

The nervous system can be divided into two components, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain and spinal cord. The PNS consists of the nerve fibers throughout the remainder of the body. Spinal nerve root and spinal nerve root ganglia are the transitional tissue with characteristics of both the CNS tissue and peripheral nerves. These spinal nerve roots are attached to, and link, the spinal cord (CNS) to the PNS. These spinal nerve roots are located in the small channels in the bone of the vertebra (intervertebral foramina).

Histologically, peripheral nervous tissue and spinal nerve roots can be differentiated from spinal cord tissue. These tissues can be identified on routine histological examination by their specific structural features.

Peripheral nervous tissue is a normal component of skeletal muscle and should be present in products containing skeletal muscle. Spinal nerve root ganglia and spinal cord are found in the vertebral column and therefore, are not normal components of skeletal muscle.

Samples were reported in the FSIS AMRS Survey database as having nervous tissue present (Y) or not present (N). Spinal cord was specifically identified in the histology report only if the tissue fragments contained a structural feature that only would be present in spinal cord. These structural features include:

- a. gray matter surrounding a central canal lined with ependymal cells; or
- b. dorsal, lateral, and/or ventral horns of gray matter; or
- c. three (3) meningeal layers covering the spinal cord.

The Pathology Section has recently reviewed samples of both the hand deboned comparative samples and the AMRS samples using additional standard histological techniques to further separate out which samples contained nervous tissue of CNS origin and which samples contained no nervous tissue or only peripheral nervous tissue.

A series of 24 samples (7 hand deboned and 15 AMRS product) from the original survey were reviewed in an attempt to estimate the prevalence of CNS tissue, spinal nerve ganglia, and peripheral nervous tissue in the samples of hand deboned and AMRS products. The two (2) samples with demonstrated spinal cord were included in this study. An attempt was made to balance the subset of survey samples by establishment by selecting 1-3 samples from each of the 11 establishments. One sample previously reported as containing nervous tissue, and one sample not containing nervous tissue (in the original examinations) were selected from each establishment. If only positive or negative results were found for a particular establishment, then only one (1) sample was selected from that establishment. This resulted in a total of 22 net samples.

These samples were examined by a second ACVP board certified pathologist. This review confirmed that all 22 samples contained peripheral nerve tissue. None of the seven (7) hand deboned samples contained spinal nerve root ganglia or CNS tissue. Eleven (11) of the 15 AMRS samples contained spinal nerve root ganglia or spinal nerve root, and two (2) of the 11 AMRS samples contained tissue identified as originating in the CNS (most likely spinal cord).

In addition to routine histological examination and review, immunohistochemical techniques are being used to confirm, delineate, and possibly quantitate the amount of CNS tissue present. Immunohistochemical staining techniques use antibodies directed toward specific antigens. Once the antibody is bound to a specific antigen, the antigen-antibody complex can be selectively stained and identified in routinely sectioned, paraffin embedded tissue sections.

The Pathology Section looked at a battery of test that used three different antibodies specific for components of nervous tissue. The presence, amount, and specific pattern of staining found in tissues with these antibodies confirms whether a tissue is CNS, spinal nerve root ganglia, peripheral nervous tissue, or non-neural in origin. Each of these highly specific monoclonal antibodies is commercially available from BioGenix Laboratories. The antibodies that were used were for the following antigens:

1. synaptophysin is a 38 kD transmembrane glycoprotein of presynaptic vesicles localized in the brain, spinal cord, retina, neuromuscular junctions, small vesicles of adrenal medulla, and pancreatic islets;
2. neurofilaments and microtubules comprise the main structural elements of neuronal axons, dendrites, and perikarya;
- 3.
4. glial fibrillary acidic protein is a 51 kD protein found in astroglial cells, which are cells only found in the supporting structures of the CNS.

Each of these widely accepted antibody techniques have been previously used in bovine tissues. The antibodies interact well with the bovine antigens with a minimum of background staining. These three tests, when used together, give a high degree of specificity for differentiating CNS

tissue from peripheral nervous tissue and spinal nerve roots. Even when the tissue is fragmented to the point that the pieces do not contain one of the structural features specific to spinal cord, these procedures may be able to identify the tissue as nervous tissue in origin. Immunohistochemical techniques must be used in conjunction with standard histological evaluation to be fully effective.

Bone Marrow

Bone marrow is not a tissue normally associated with or found in skeletal muscle products. Bone marrow is found in the spongy bone or central cavity in the center of most bones in the carcass, and is made of predominantly two types of tissue - hematopoietic (meaning producing blood cells) marrow, or red marrow, and nonhematopoietic marrow (fatty marrow). Hematopoietic marrow in mature animals is concentrated in the spine, sternum, ribs, head, pelvis, and portions of the long bones of legs. In fetal, very young, or some diseased animals there may be a small amount of splenic or extramedullary hematopoiesis (blood cell production), however the number of precursor cells is much smaller than in the red marrow centers of hematopoiesis, and the cells are limited mostly to the red blood cell series.

Intact bone marrow can be identified using routine histological examination by several structural features. These include:

- a. nonhematopoietic marrow consisting of nerves, fat cells, and bone marrow stroma (which may include bone fragments);
- b. hematopoietic marrow consisting of colonies of hematopoietic cells (blood precursors) from multiple cell lines (erythroid, myeloid, megakaryoid, and lymphoid), fat cells, and bone marrow stroma.

Of these two, only the characteristics of the hematopoietic cells would be specific to bone marrow. The hematopoietic cells in bone marrow show a progression from undifferentiated (pluripotent stem cells) to nearly mature or mature cells. The presence of hematopoietic cells from several cell lines should only be present in bone marrow tissue.

Other conditions might mimic the cell population found in hematopoietic bone marrow, but usually can be differentiated during histological examination. Extramedullary hematopoiesis, found in some diseased/severely anemia animals, may be found in parenchymatous organs (such as liver, spleen, lymph nodes), but would be predominantly erythroid (red blood cell precursors) in nature. These organs should not be found in AMRS product, and if they were present, they could be detected histologically. Large numbers of myeloid cells (both mature and precursor cells) could be a feature of purulent material (pus) from abscesses, but would lack erythroid or megakaryocytic precursors. The most primitive, undifferentiated cells in the myeloid series would remain in the bone marrow and not be present in the purulent material.

ACVP board certified pathologists reviewing the 22 samples previously discussed were of the opinion that none of the seven (7) hand deboned samples reviewed contained bone marrow tissue. All of the 15 AMRS samples reviewed contained hematopoietic tissue consistent with bone marrow.

Additional techniques are being investigated by the Pathology Section to confirm, delineate, and possibly quantitate the amount of CNS tissue and hematopoietic bone marrow present. These techniques include (1) immunohistochemical procedures specific for CNS tissues, hematopoietic cells, and bone marrow stroma, (2) transmission electron microscopy, and (3) scanning electron microscopy of AMRS product.

Glossary.

Adrenal medulla - The central portion of the adrenal gland. This portion of the organ originates from the neuroectoderm.

Antibody - A type of globulin protein synthesized by B lymphocytes in response to a specific antigenic stimulus.

Antigen - A high molecular weight substance (usually protein or protein-polysaccharide complex) which, when foreign to the tissues of an animal, stimulates the formation of a specific antibody.

Astroglial cells - star shaped neuroglial cells found in the central nervous system.

Axon - A cytoplasmic process of nerve cells generally carrying impulses away from the cell body.

Dendrites - Branching, tree-like cytoplasmic processes of nerve cells that carry impulses toward the cell body.

Ependymal cell - The cell that form the membrane that lines the ventricles of the brain and the central canal of the spinal cord.

Erythroid - Pertaining to the red blood cell series of cells.

Extramedullary hematopoiesis - The production of blood cell components outside the medullary or marrow cavities of the bones.

Glial/neuroglial - Pertaining to the supporting structure of nervous tissue. This consists of a fine web of tissue made up of modified ectodermic elements which contain distinctive branched cells (glial/neuroglial cells). There are three types of glial cells - astroglia, oligodendroglia, and microglia.

Hematopoietic - pertaining to the production of red blood cells, granulocytes (myeloid cells), lymphocytes, monocytes, and platelets from pluripotent (undifferentiated) stem cells. In the normal adult animal, hematopoiesis takes place in the red bone marrow.

Hematopoiesis - The production of blood cells from pluripotent stem cells.

Immunohistochemical - The application of antibodies to tissue sections for the purpose of localizing specific antigens in tissue.

Kilodalton (kd) - A molecular weight designation.

Lymphoid - Pertaining to lymphocytes, one of the cells in the progression of cells from pluripotent stem cells to mature lymphocytes.

Megakaryocytic/megakaryoid - pertaining to a precursor cell line that forms platelets.

Monoclonal - Antibody produced from a single clone of B lymphocytes, producing a single, specific antibody for a specific portion of an antigen.

Myeloid - Pertaining to the granulocytic line of white blood cells which, when mature and differentiated, are one of three types - neutrophils, eosinophils, or basophils.

Pancreatic islets - Small clusters of cells contained within the pancreas which produces insulin.

Parenchymatous - Referring to the essential elements of certain organs, as differentiated from the supporting elements (stroma) of those organs.

Perikarya - The main protoplasmic mass of nerve cell bodies, the cell bodies as opposed to the nucleus and cytoplasmic processes.

Polypeptide - A peptide made up of more than two amino acid molecules.

Synaptophysin - A protein present in synaptic vesicles of nervous tissue.