DETERMINATION OF THE PHYSIOCHEMICAL AND MICROBIOLOGICAL QUALITY OF CARCASS, BONE AND BLOOD MEAL

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ABSTRACT


The pH value and the moisture, fat and protein content of abattoir by-products which are commercially available in the Republic of South Africa were examined, and the total bacterial count and the extent of Salmonella spp., Escherichia coli, Bacillus spp., yeasts and fungus contamination were determined. In this survey of by-product plants by means of a standardized tubular sampler, 500 g specimen was taken within 5 days of collection. As far as possible, specimens were examined at the laboratory within 5 days of their collection. In this investigation 100 carcass meal, 61 blood meal and 59 bone meal specimens were analysed.

INTRODUCTION

The world-wide shortage of animal protein has created an increasing demand for abattoir by-products for animal feed but, since these products are often contaminated by micro-organisms, they constitute a health hazard and may also affect the quality of the feeds. Keil & Keller (1961) reported that the total bacterial count of 9 specimens of carcass meal ranged from 9 x 10^8 to 2 x 10^10 organisms per gram, while Morehouse & Wedman (1961) and Keil & Keller (1961) reported that the total bacterial count of 59 bone meal specimens was 1 x 10^9 to 1 x 10^10 organisms per gram. Tittiger (1971), who examined the by-products of 5 different sterilizing plants in the USA, was unable to obtain sterile material from the continuous rendering process, but found that the degree of bacterial contamination was indirectly related to the maintenance of a high level of hygiene in the plant.

In the RSA, animal by-products are rendered in registered sterilizing plants from carcasses and parts of carcasses which are unfit for human consumption, as required by Schedules 3 and 4 under the standing regulations of the Animal Slaughter and Animal Products Hygiene Act, 1967 (Act 87 of 1967). The total production of carcass meal, including blood meal and meat meal in the RSA for 1977, was 13,649 metric tons, while that of bone meal was 15,565 metric tons (Anon., 1979).

The large amount of available material and the extent of its application in the animal feed industry as a whole emphasize the need for good nutritional and bacteriological quality. The variation in quality can be determined by an analysis of presently available products from which improved but attainable quality standards can be derived.

The pH value, the moisture, fat and protein content and the total bacterial count, the presence of Salmonella spp., Escherichia coli, Bacillus spp., yeasts and fungus were determined in this survey of by-product meals. Mean and highest frequency values of these variables were calculated to find standards which are both reasonably attainable by industry and for comparison with existing legal requirements. In an attempt to simplify the full-scale examination of these products for quality, further statistical analyses were performed to establish whether a reliable mathematical model exists for the prediction of total bacterial counts.

MATERIALS AND METHODS

Collection of specimens

Specimens of processed material were collected at by-product plants by means of a standardized tubular sampler immediately after they had been packed into bags. Three per cent of the bags or a minimum of 5 bags per batch of by-product meal were sampled, of which a representative 500 g specimen was sent to the laboratory. As far as possible, specimens were examined at the laboratory within 5 days of their collection. In this investigation 100 carcass meal, 61 blood meal and 59 bone meal specimens were analysed.

Biological test

The 500 g specimen was mixed thoroughly and of this material a 10 g sample was suspended in 50 ml of physiological saline which, after being well-shaken,
was placed in a water-bath at 60 °C for 60 min. After
the specimen had been cooled for 10 min, 2 ml of the
supernatant was injected intraperitoneally into each
of 2 guinea pigs. These were kept under observation
for 10 days and, if one or both died, a post-mortem
examination was performed. Blood smears of heart
blood were examined for the presence of Bacillus
anthracis. Specimens of liver and spleen were cultured
for Bacillus anthracis and Clostridium spp. In addition,
the latter were identified on impression smears of liver
and spleen by a fluorescent antibody test.

Microbiological examination
For the total aerobic bacterial count, 10 g of by-
product meal was suspended in 50 ml of sterile
physiological saline containing 0.01% Tween 80.
Ten fold dilutions of this suspension were made ac­
cording to the method of Miles & Misra (1938), plated
on blood tryptose agar* and incubated for 18 h at
37 °C. The colony forming units of bacteria (CFU)
were counted at the appropriate dilution and the
degree of contamination was expressed as the number
of organisms/g.

To determine the presence of Escherichia coli
Bacteriological examination
For the determination of the numbers of yeasts and
fungi present, 1 g of material was suspended in 10 ml
of saline and further tenfold dilutions were made for
inoculation of Wickerham agar (Wickerham, 1951). The
number of organisms/g of material was cal­
culated after 7 days incubation at 30 °C.

Physicochemical determinations
The protein content was determined by the method
of Horwitz (1970), using a conversion factor of
0.875.

The moisture content was determined on the
Brabender*** apparatus, by heating the meal for
4 h at 105 °C.

The fat content of the meal was determined on 1 g
material by the method of Lea, Parr & Carpenter
(1960).

Statistical analysis
The bacteriological and physicochemical results
were analysed statistically (Snedecor & Cochran, 1967;
Ostle, 1954), rendering frequency distributions, cumu­
lative frequency polygons, arithmetic mean values,
partial and highest correlations, variation infla­
tion factors and multiple linear and polynomial regression
analyses. The regression analyses were performed to
determine the predictability of total aerobic bacterial
counts from the independent variables of protein,
mucrose, pH and fat, either singly or in combination.

RESULTS
Irrespective of the origin of the by-product meals,
the values of the physicochemical and microbiological
parameters showed wide variations (Tables 1 & 2).

<table>
<thead>
<tr>
<th>Characteristics of by-products meals</th>
<th>Carcass meal</th>
<th>Blood meal</th>
<th>Bone meal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Highest</strong></td>
<td><strong>Range</strong></td>
</tr>
<tr>
<td>pH..................................</td>
<td>5.1</td>
<td>7.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Moisture %....</td>
<td>3</td>
<td>13.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Fat %........</td>
<td>5.2</td>
<td>33.8</td>
<td>15.5</td>
</tr>
<tr>
<td>Protein %.....</td>
<td>31.9</td>
<td>68.3</td>
<td>50.7</td>
</tr>
<tr>
<td>Total aerobic bacterial count/g.....</td>
<td>2×10³</td>
<td>5×10³</td>
<td>1×10⁴</td>
</tr>
</tbody>
</table>

* Difco, Michigan, USA
** Section of Bacteriology, Onderstepoort
*** Gallenkamp & Co., London, England
**** E488—Metrorho, Hrvisan
TABLE 2 Percentage contamination of by-product meals

<table>
<thead>
<tr>
<th>Contaminants</th>
<th>Percentage of specimens contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carcass meal</td>
</tr>
<tr>
<td>Yeasts</td>
<td>25</td>
</tr>
<tr>
<td>Fungi</td>
<td>25</td>
</tr>
<tr>
<td>E. coli</td>
<td>59</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>20</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>44</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>17</td>
</tr>
</tbody>
</table>

The relationship between the frequency of contamination of by-product meals with Salmonella, E. coli and Clostridium, and the frequency of total aerobic bacterial counts are presented in the cumulative frequency polygons (Fig. 1, 2 & 3).

The calculation of simple and partial correlations (Table 3) indicates highly significant correlations between moisture and total aerobic bacterial count in all 3 types of by-product meals.

The latter findings are supported by the results of the multiple linear regression analysis (Table 4). This analysis was justified because the values of the variation inflation factors of pH, moisture, fat, protein and total aerobic bacterial count ranged between the statistically essential values of 1,0 and 5,0.
THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF CARCASS, BONE AND BLOOD MEAL

TABLE 3 Simple and partial correlations between variables in carcass, blood and bone meal

<table>
<thead>
<tr>
<th>Variables</th>
<th>Carcass meal</th>
<th>Blood meal</th>
<th>Bone meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple</td>
<td>Partial</td>
<td>Simple</td>
</tr>
<tr>
<td>pH: moisture</td>
<td>-0.0524</td>
<td>0.0099</td>
<td>0.1485</td>
</tr>
<tr>
<td>pH: fat</td>
<td>-0.3556*</td>
<td>-0.2936**</td>
<td>0.0384</td>
</tr>
<tr>
<td>pH: protein</td>
<td>0.1774</td>
<td>0.1468</td>
<td>0.1543</td>
</tr>
<tr>
<td>pH: bact. count</td>
<td>-0.2404*</td>
<td>-0.2137*</td>
<td>0.0589</td>
</tr>
<tr>
<td>Moisture: fat</td>
<td>-0.0066</td>
<td>-0.0430</td>
<td>0.1997</td>
</tr>
<tr>
<td>Moisture: protein</td>
<td>0.1542</td>
<td>0.0995</td>
<td>-0.7902**</td>
</tr>
<tr>
<td>Moisture: bact. count</td>
<td>0.3900**</td>
<td>0.3711**</td>
<td>0.6180**</td>
</tr>
<tr>
<td>Fat: protein</td>
<td>-0.2205*</td>
<td>-0.1863</td>
<td>-0.1826</td>
</tr>
<tr>
<td>Fat: bact. count</td>
<td>0.1530</td>
<td>0.1199</td>
<td>0.1543</td>
</tr>
<tr>
<td>Protein: bact. count</td>
<td>0.1457</td>
<td>0.1637</td>
<td>-0.4092</td>
</tr>
</tbody>
</table>

* Significant
** Highly significant

The F-values from the regression analysis (Table 4) indicate a highly significant relationship between bacterial count and a dependent variable and the combined effect of 4 independent variables, and only a limited relationship with pH alone. The feasibility of predicting total aerobic bacterial counts from the highly significant F-values (Table 4) was disproved by multiple correlation R values and particularly by the R² values which were less than the minimum of 0.75 (Table 6).

The percentage of fit (R² X 100%) in Table 6 indicates that 22.87%, 40.28% and 49.86% of the fluctuations in the total aerobic bacterial counts in carcass blood and bone meal respectively can be ascribed to the combined effect of the 4 independent variables.

TABLE 4 F-values from multiple linear regression analysis for the independent variables of pH, moisture, fat and protein, and the dependent variable of total bacterial count in carcass, bone and blood meal

<table>
<thead>
<tr>
<th>Variables</th>
<th>Carcass meal</th>
<th>Blood meal</th>
<th>Bone meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple</td>
<td>Partial</td>
<td>Simple</td>
</tr>
<tr>
<td>pH, moisture</td>
<td>7.00**</td>
<td>9.10**</td>
<td>13.20**</td>
</tr>
<tr>
<td>Fat, protein</td>
<td>4.50*</td>
<td>0.25</td>
<td>7.18**</td>
</tr>
<tr>
<td>Moisture</td>
<td>15.02**</td>
<td>20.51**</td>
<td>38.93**</td>
</tr>
<tr>
<td>Fat</td>
<td>1.37</td>
<td>0.13</td>
<td>No contrib-</td>
</tr>
<tr>
<td>Protein</td>
<td>2.59</td>
<td>1.69</td>
<td></td>
</tr>
</tbody>
</table>

* Significant
** Highly significant

TABLE 5 Variation inflation factors calculated for pH, moisture, fat, protein and total aerobic bacterial count in carcass, blood and bone meal

<table>
<thead>
<tr>
<th>Variables</th>
<th>Carcass meal</th>
<th>Blood meal</th>
<th>Bone meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1.2201</td>
<td>1.0495</td>
<td>1.1536</td>
</tr>
<tr>
<td>Moisture</td>
<td>1.1959</td>
<td>3.8630</td>
<td>1.9782</td>
</tr>
<tr>
<td>Fat</td>
<td>1.1970</td>
<td>1.0460</td>
<td>1.2296</td>
</tr>
<tr>
<td>Protein</td>
<td>1.1223</td>
<td>2.8103</td>
<td>1.1178</td>
</tr>
<tr>
<td>Bact. count</td>
<td>1.2965</td>
<td>1.6744</td>
<td>1.9945</td>
</tr>
</tbody>
</table>

TABLE 6 Multiple correlation coefficients between the fitted and the experimental data of carcass, blood and bone meal

<table>
<thead>
<tr>
<th>Model</th>
<th>Carcass meal</th>
<th>Blood meal</th>
<th>Bone meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>0.4782</td>
<td>0.6347</td>
<td>0.7061</td>
</tr>
<tr>
<td>R²</td>
<td>0.4799</td>
<td>0.6180</td>
<td>0.7048</td>
</tr>
<tr>
<td>R X 100 (%)</td>
<td>22.87</td>
<td>40.28</td>
<td>49.86</td>
</tr>
<tr>
<td>Model 1. Y = X1 + X2 + X3 + X4 (carcass, blood, bone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2. Y = X1 + X2 (carcass, bone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y = X1 (blood)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Where Y = bacterial count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X = pH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X1 = moisture
X2 = fat
X3 = protein
A = intercept, B1, B2, B3 & B4 = regression coefficients

Significant non-linear relationships were determined by polynomial regression analysis for moisture in carcass and blood meal and for fat in blood meal. When these terms were included in the statistical models, no additional improvement for the predictability of bacterial count was obtained.

DISCUSSION

A wide variation in the quality of the different by-product meals is evident from the results of the analyses.

pH

The highest frequency of pH occurring in carcass, blood and bone meal is acceptable as the standard pH for these products.

Moisture content

The highest frequency value of 5% moisture in carcass meal and the mean value of 6.4% indicate that the present 10% legal maximum value (Anon., 1947) is too high. In blood meal most specimens
comply with the maximum legal limit, although the arithmetic mean of 11.6% indicates a considerable number of values in excess of this limit. No legal limit has been set for the moisture content of bone meal, but a mean value of 5.6% and the highest frequency 4.5% indicate a reasonable legal maximum of 7%.

**Protein content**

The control regulations require that “carcass meal” and “carcass and bone meal” contain a minimum protein content of 50% and 40% respectively. In the specimens analysed, the lowest protein value of 31.9% was well below both the above minimum values, even though it was obtained by the mean and highest frequency values.

In blood meal the mean protein value of 78.5% and the highest frequency value of 80% were well above the minimum legal requirement of 75%.

**Microbiological counts**

*Salmonella* spp. were found in 17% of the specimens of carcass meal, *Clostridium* spp. in 44% and *E. coli* in 59%. Blood meal contained *Salmonella* spp. in 3% of the specimens, *E. coli* in 68% and *Bacillus* spp. in 61%. The bone meal was contaminated by *Salmonella* spp. in 8% of the specimens, *E. coli* in 54% and fungi in 46%. The maximum total and mean aerobic bacterial counts in all by-product meals can be regarded as extremely high.

The cumulative frequency polygons (Fig. 1, 2 & 3) indicate that the cumulative increase in total bacterial count is accompanied by an increased frequency of contamination by potentially pathogenic and pathogenic organisms. In each case *Salmonella* as a contaminant emerged at total bacterial counts in excess of $1 \times 10^4$/g. In all the by-product meals this point is found between the first and second quartiles ($Q_1$ & $Q_2$) and supports the finding of extremely high rates of bacterial contamination in by-products.

The statistic analysis proved that no statistical model exists by which bacterial count could be predicted from any of the independent variables of pH, moisture protein and fat, and that the bacterial count is largely dependent on the combined effect of these variables.

**Conclusions**

In general, the proposed changes in the existing specifications promulgated under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act 36 of 1947) (Anon., 1947) are as follows: The moisture content in carcass, blood and bone meal could be reduced to 7%, 10% and 7% respectively. The fat content of carcass meal is at a realistic level at 10%, but that of blood meal could be raised to 12.5% and that of bone meal be limited to 8%. The protein level of carcass meal is at a realistic level, but that of blood meal could be raised to 80%, as most specimens comply with this specification.

From the analysis of the microbiological contents of specimens it is clear that good quality products can be regarded as those containing less than $1 \times 10^4$ aerobic organisms/g. Contaminants which occur in particularly high numbers in carcass meal are *E. coli*, *Salmonella* spp. and *Clostridium* spp.; in bone meal, fungi, *E. coli*, *Bacillus* spp., *Clostridium* spp. and *Salmonella* spp.; and in blood meal, *E. coli*, *Bacillus* spp. and *Clostridium* spp.

The percentage of contaminated specimens, and particularly the high incidence of *Salmonella* spp. in all by-product meals, is a cause for concern. To prevent the spread of this infection to food animals and so also to man it is imperative that such contaminated by-products be excluded from animal feed mixes.

From the results of the statistical analysis of the 4 dependent variables and 1 independent variable, it is clear that no single criterion of measurement is indicative of the quality of these by-products and, consequently, all parameters such as fat, protein, moisture and microbiological counts would have to be established individually.

**References**


