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

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ABSTRACT

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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, Escherichia coli, Bacillus,* yeast and fungus contamination were determined. The extremes and reasonably attainable quality standards were deduced from the highest frequency and mean values of these figures. The total bacterial count was not statistically predictable from variables such as pH, moisture, protein and fat, but was found to be related to the combined effect of all 4 independent variables.

Résumé

DÉTERMINATION DE LA QUALITÉ PHYSICO-CHIMIQUE ET MICROBIOLOGIQUE D'ALIMENTS DE CARCASSE, D'OS ET DE SANG

La valeur pH et l'humidité, la teneur en graisse et en protéine des sousproduits d'abattoir qui sont commercialement disponibles en République d'Afrique du Sud ont été l'objet d'un examen et le compte bactérien total et l'importance de la contamination par Salmonella, Escherichia coli, *les levure et les fungus a été déterminée. Les standards de qualité extrèmes et raisonnablement procurables furent déduits des fréquence les plus élevées et les valeurs moyennes de ces chiffres. Le compte bactérien total ne fut pas statistiquement en mesure d'être prédit à partir des variables comme le pH, l'humidité, la protéine et la graisse mais fut trouvé être en relation avec l'effet combiné de toutes les 4 variables indépendantes.*

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 consitute a health hazard and may also affect the quality of the feeds. Keil & Keller (1961) reported that the total bacterial contents of 9 specimens of carcass meal ranged from 9×10^3 to 2×10^{10} organisms per gram, while Morehouse & Wedman (1961) and Keil & Keller (1961) reported that these products contained pathogenic bacteria. According to Gumanson, Hurvell, Nardblom, Rutquist & Thale (1974), meat meal and abattoir by-products were responsible for the largest number of *Salmonella* isolates in Sweden during the period 1968–1972. These workers regarded the isolation and reporting of such isolates as the first step towards the prevention of the spread of salmonellosis to man. *Salmonella* spp. have also been isolated from carcass meal in the Republic of South Africa (RSA) by Van den Heever & Van der Made (1977).

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 carcassess 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 15 365 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 fungi 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 anlysed.

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,

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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 according 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* group I (faecal), the Eijckman test was performed on 1 g of meal, i.e. the formation of gas in MacConkey's broth (1 g of meal in 10 mℓ), as well as the production of indole in peptone water (1 g of meal in 10 mℓ) after 24 h incubation at 44 °C. This is an adaptation of the method described by Cruickshank (1965).

The isolation of *Salmonella* spp. was performed by enriching 1 g of material in 10 mℓ of brilliant green broth* and incubating the material for 18 h at 37 °C. Sub-cultures were made onto SS agar* and incubated for 18–24 h at 37 °C for selection of lactose negative colonies. These were further typified on T.S.I. agar* and carbohydrate fermentation tests, with final identification by slide agglutination tests, using non-specific antisera**.

The presence of *Clostridium* organisms was determined by suspending 1 g of by-product meal in 10 mℓ of saline, filtering it through a double layer of cheese cloth and centrifuging at 4 220×g for 10 min. The sediment was plated onto sulphite iron agar (Mossel, De Bruin, Van Diepen, Vendrig & Zoutewelle, 1956), and incubated at 37 °C for 24 h in anaerobic jars*** in a hydrogen atmosphere.

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 calculated 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 pH**** was measured on 10 g of meal which had been stabilized for 1 h in 50 mℓ of 0,15 M NaCl solution.

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, cumulative frequency polygons, arithmetic mean values, partial and highest correlations, variation inflation 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, moisture, pH and fat, either singly or in combination.

Results

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

TABLE 1 pH, moisture, fat, protein and total aerobic bacterial counts of by-products meals

	Characteristics of by-products meals											
	Carcass meal				Blood meal				Bone meal			
	Range		Mean	Highest frequency	Range		Mean	Highest frequency	Range		Mean	Highest frequency
	Min.	Max.			Min.	Max.			Min.	Max.		
pH.....	5,1	7,0	6,7	6,7	5,5	8,5	7,3	7,5	5,7	7,4	6,8	6,9
Moisture %...	3	13,6	6,4	5	3,6	57	11,6	7,5	2,5	10,4	5,6	4,5
Fat %.....	5,2	33,8	15,5	10	0,5	5,2	2,2	3	1	12	7,8	7
Protein %.....	31,9	68,3	50,7	50	43,1	90	78,5	80	19,8	62,1	29,3	20
Total aerobic bacterial count/g.....	2×10^2	3×10^8	1×10^5	1×10^5	0	1×10^9	1×10^5	1×10^6	8×10^2	6×10^8	1×10^8	1×10^5

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TABLE 2 Percentage contamination of by-product meals

Contaminants	Percentage of specimens contaminated		
	Carcass meal	Blood meal	Bone meal
Yeasts	25	23	25
Fungi	25	20	46
E. coli	59	68	54
Bacillus spp.	20	61	64
Clostridium spp.	44	28	37
Salmonella spp.	17	3	8

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.

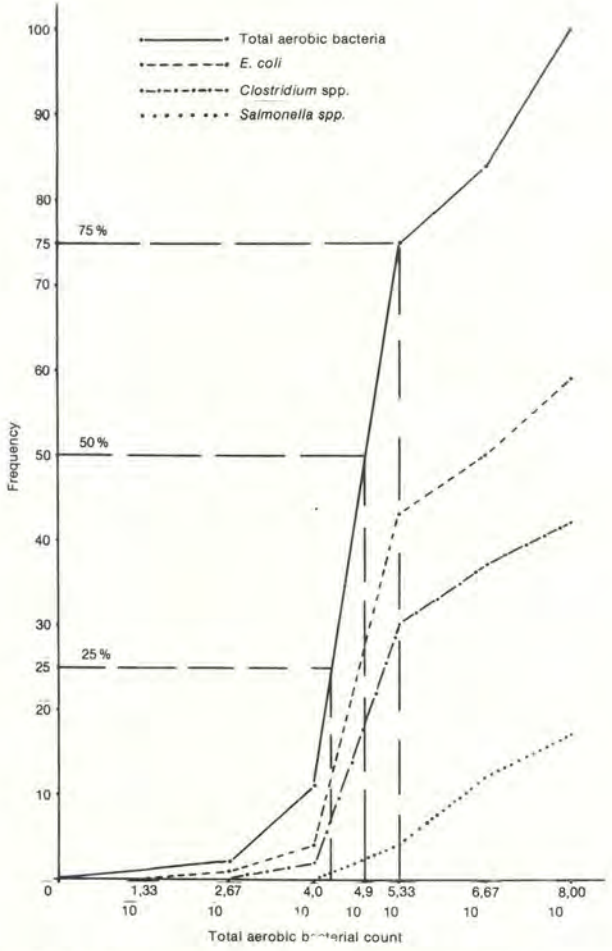

FIG. 1 Carcass meal: Cumulative frequency polygons of total aerobic bacterial count and total aerobic bacterial count contaminated with *E. coli, Clostridium* spp. and *Salmonella* spp.

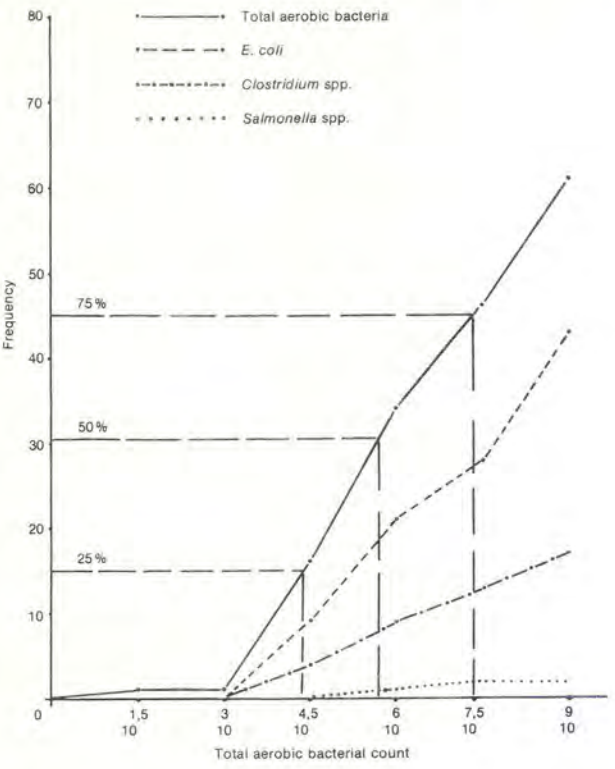

FIG. 2 Blood meal: Cumulative frequency polygons of total aerobic bacterial count and total aerobic bacterial count contaminated with *E. coli, Clostridium* spp. and *Salmonella* spp.

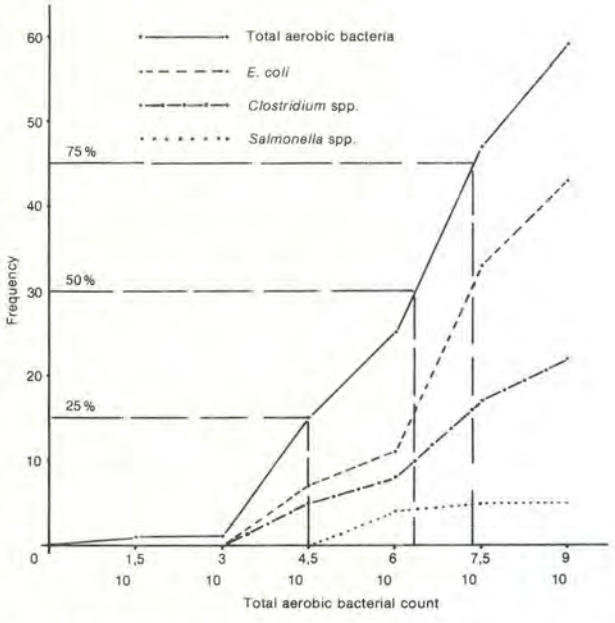

FIG. 3 Bone meal: Cumulative frequency polygons of total aerobic bacterial count and total aerobic bacterial count contaminated with *E. coli, Clostridium* spp. and *Salmonella* spp.

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.

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

Variables	Products and correlations					
	Carcass meal		Blood meal		Bone meal	
	Simple	Partial	Simple	Partial	Simple	Partial
pH: moisture	−0,0524	0,0099	0,1485	0,2073	−0,1333	0,1230
pH: fat	−0,3556*	−0,2936**	0,0384	0,0184	0,0543	0,0083
pH: protein	0,1774	0,1468	−0,0298	0,1543	−0,0127	0,0257
pH: bact. count	−0,2404*	−0,2137*	0,0589	−0,0684	−0,3429**	−0,3426**
Moisture: fat	−0,0066	−0,0430	0,1997	0,0484	−0,3409	−0,3036
Moisture: protein	0,1542	0,0895	−0,7902**	−0,7516**	0,1566	0,1574
Moisture: bact. count	0,3900**	0,3711**	0,6180**	0,5246**	0,6559**	0,6178**
Fat: protein	−0,2205*	−0,1863	−0,1826	−0,0508	0,1088	0,2816*
Fat: bact. count	0,1530	0,1199	0,1543	0,0486	−0,2118	0,0037
Protein: bact. count	0,1457	0,1637	−0,4092	0,1743	0,1442	0,0587

* Significant
** Highly significant

The F-values from the regression analysis (Table 4) indicate a highly significant relationship between total aerobic bacterial count as 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 R^2 values which were less than the minimum of 0,75 (Table 6).

The percentage of fit ($R^2 \times 100\%$) 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

Variables	Carcass meal	Blood meal	Bone meal
1. pH, moisture, fat, protein	7,00**	9,10**	13,20**
2. pH	4,50*	0,25	7,18**
Moisture	15,02**	20,51**	38,93**
Fat	1,37	0,13	No contribution
Protein	2,59	1,69	0,2102

* Significant
** Highly significant

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

Variables	Carcass meal	Blood meal	Bone meal
pH	1,2201	1,0495	1,1536
Moisture	1,1959	3,8630	1,9782
Fat	1,1970	1,0460	1,2296
Protein	1,1223	2,8103	1,1178
Bact. count	1,2965	1,6744	1,9945

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

	Model	Carcass meal	Blood meal	Bone meal
R	1	0,4782	0,6347	0,7061
	2	0,4479	0,6180	0,7048
R^2	1	0,2287	0,4028	0,4986
	2	0,2025	0,3844	0,4900
$R^2 \times 100(\%)$	1	22,87	40,28	49,86
	2	20,25	38,44	49,00

Model 1. $Y = +_1X_1 +_2X_2 +_3X_3 +_4X_4$ (carcass, blood, bone)
Model 2. $Y = +_1X_1 +_2X_2$ (carcass, bone)
$Y = +_1X_2$ (blood)
Where Y = bacterial count
X = pH
X_2 = moisture
X_3 = fat
X_4 = protein
A = intercept, B_1, B_2, B_3 & B_4 = 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 grequency 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 1×10^4/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 statistical 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 culd 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 2,5% 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 1×10^3 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.

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