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king). Deur gebruik te maak van die sogenaamde rankitsprosedure is bevestig dat die uGGT/uCr-verhouding van hierdie 16 skape normaal verdeel is[10]. Aangesien 95% van 'n normaalverdeling onder die waarde: gemiddeld plus 2 maal standaardafwyking sal lê, is 'n populasieverwysingswaarde vir uGGT/uCr bereken as 43,1[10].

BESPREKING

Die tegniek wat gebruik is om GGT-aktiwiteit in serum te bepaal, kan op gesentrifugeerde skaapurien toegepas word. Ten spyte van verskille in pH en soortlike gewig in die urienmonsters wat ontleed is, het die tegniek herhaalbare resultate gelewer. Dit stem ooreen met navorsing op perde-urien soos gerapporteer deur Adams et al.[1].

Volgens sommige navorsers is dit nodig om skaapurien eers te dialiseer voordat die GGT-aktiwiteit daarin gemeet word[8]. In hierdie ondersoek het bepalings van GGT in skaapurien en serum egter dieselfde resultate gelewer en die teenwoordigheid van nie-spesifieke ensiem inhibeerders kon nie gedemonstreer word nie. Dit blyk dus nie nodig te wees om skaapurien te dialiseer voordat die GGT-aktiwiteit daarin bepaal word nie.

Geen statisties betekenisvolle verandering kon in GGT-vlakke van urien wat teen 4,25 en -20°C gestoor is, vir onderskeidelik 12 en 24 uur, gedemonstreer word nie. Dit verskil van bevindinge ten opsigte van perde en menslike urien waar daar 'n afname in GGT-vlakke was, veral as uriene teen -20°C gestoor is[1]. In die urien van honde wat teen 4°C gestoor is, kon verhoogde vlakke van GGT na 24 uur gemeet word[7]. In skape vergemaklik hierdie bevindings die hanteringsprosedure van urienmonsters voor bepaling van GGT-vlakke.

Die vlakke van urinêre GGT varieër volgens die vloeitempo van urien[1]. Vir die akurate bepaling van die verlies van GGT in urien, is 'n 24-uur urienmonster nodig. In 'n kliniese situasie waar met diere gewerk word, is dit onprakties om 24-uur urienmonsters te versamel[7]. Om hiervoor te kompenseer, kan die urinêre GGT met die uitskeiding van kreatinien in die uriene vergelyk word. In die hond bestaan daar 'n hoë korrelasie tussen die uGGT/uCr en die verlies van GGT oor 24 uur[7]. Soos verwag kan word, het die verlies van GGT in die urien van normale skape ook oor 'n 24-uur periode gevarieër. Indien die verlies van GGT in die urien met dié van kreatinien oor dieselfde periode vergelyk word, blyk dit dat die uGGT/uCr konstant bly. Dit is dus slegs nodig om 'n enkele urienmonster te versamel om die relatiewe verlies van GGT in die urien te bepaal.

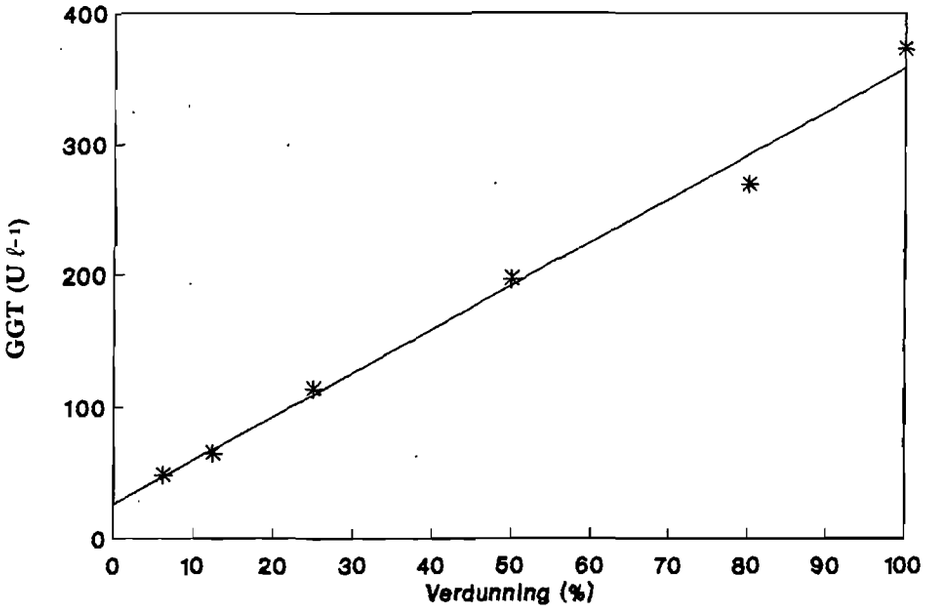

Fig. 2: **GGT-konsentrasies by verskillende verdunnings in skaapurien**

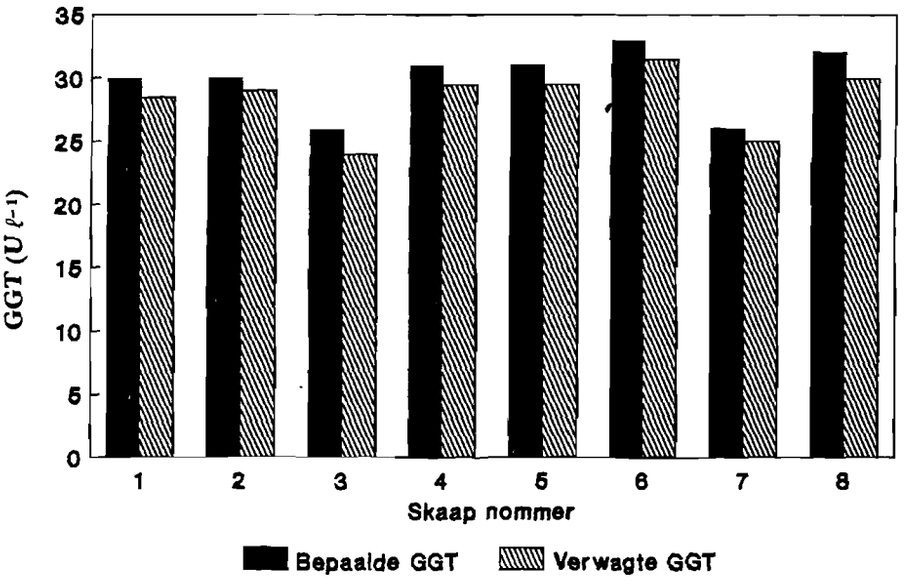

Fig. 3: **Verwagte teenoor bepaalde GGT-konsentrasies in serum**

Tabel 1: **Verandering in GGT-aktiwiteit ($\bar{x}$; ±SD) in urien van skape wat bewaar is teen verskillende temperature vir 12 en 24 uur**

Temperatuur	n	Verandering in GGT (U ℓ⁻¹) na: 12 uur	24 uur
25 °C	8	+ 3,0 ± 9,5	+ 3,5 ± 15,8
4 °C	8	+ 1,1 ± 6,2	- 0,3 ± 3,7
-20 °C	8	+ 0,5 ± 8,8	- 3,4 ± 7,0

Teoreties behoort skape wat relatief meer GGT in die urien verloor 'n verhouding van uGGT/uCr van meer as 43,1 te hê. Die waarde van 43,1 vir die uGGT/uCr-verhouding in skape is heelwat hoër as die verwysingswaarde wat deur Adams et al.[1] in perde bereken is. Die rede hiervoor lê waarskynlik in die feit dat skape ongeveer 64 eenhede GGT per gram nierweefsel bevat teenoor 29 eenhede GGT per gram nierweefsel in die perd[3].

Die gebruik van die uGGT/uCr verhouding om die relatiewe verlies van GGT in urien op 'n gerieflike manier te bepaal, is eers onlangs in die literatuur voorgestel[1,7]. Bayley et al.[2] het serum en

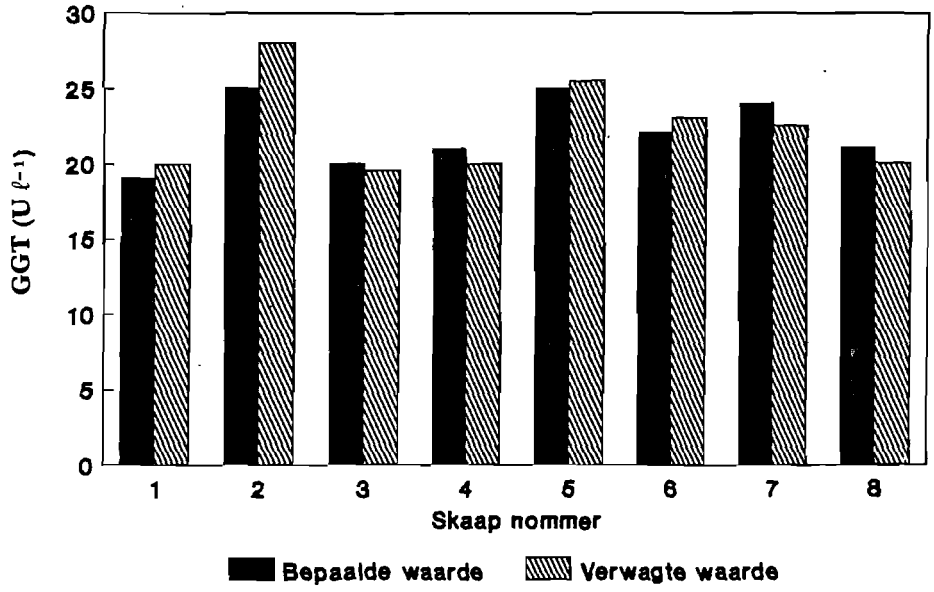

Fig. 4: **Verwagte teenoor bepaalde GGT-konsentrasies in urien**

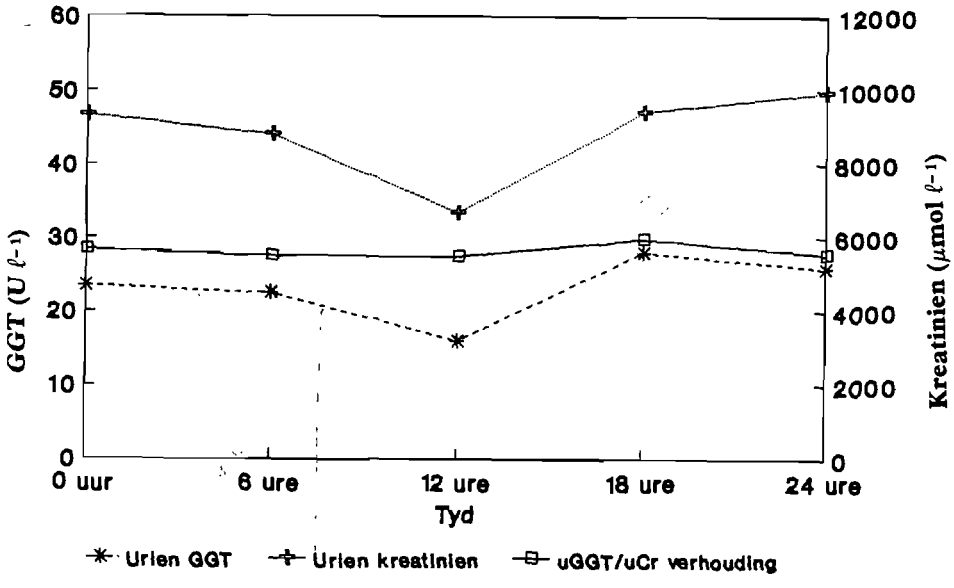

Fig. 5: **GGT- en kreatinienvlakke in urien oor 24 uur**

urinêre biochemiese veranderinge in perde met akute tubulêre nefrose gemonitor. Die uGGT/uCr-verhouding in hierdie perde het toegeneem om die nierskade aan te dui, lank voordat azotemie of verandering in die urien-osmolaliteit ingetree het. Of die uGGT/uCr-verhouding in die urien van skape 'n sensitiewe indikasie van nierbuisskade is soos in die perd, behoort verder ondersoek te word.

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Die tegniese hulp van Mej E Borchard en Mev E Grobler tydens die uitvoering van die proewe word met dank erken. Erkenning word ook aan Prof C F Smit vir die statistiese verwerking van die resultate, gegee.

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INITIAL AND DEVELOPED FREE FATTY ACID CONCENTRATIONS IN MILK FROM PAIRED NORMAL AND SEPTIC SUBCLINICALLY MASTITIC UDDER QUARTERS

L W VAN DEN HEEVER*, Z E KOWALSKI+ and MARIANA OLIVIER*

ABSTRACT

The free fatty acid (FFA) content of milk from the paired normal (N) and septic *(Staphylococcus aureus* or *S. agalactiae)* subclinically mastitic (SSM) quarters of dairy cows was determined by thin layer chromatography. Within-cow comparisons showed the FFA content of milk from the SSM quarters to be consistently significantly higher than that of the opposing N quarters: initially and after warm agitation and both rapid and slow cooling prior to storage at 4°C for 48 h.

No correlation existed between initial and post-treatment FFA levels in milk from N quarters, but a significant positive correlation in the case of SSM milks suggests their greater susceptibility to both spontaneous and induced lipolysis. There was no correlation between the somatic cell content and FFA levels of either freshly-drawn or processed SSM milk. The study emphasises the importance of healthy udders in the production of milk of acceptable flavour and aroma.

Key words: Free fatty acids, hydrolytic lipolysis, bovine mastitis

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INTRODUCTION

Milk and dairy products adversely affected by organoleptically unacceptable defects such as sharp, pungent, bitter, soapy or frankly rancid and related flavour changes are of international[5 9 11 12 37] and national[26 31] concern to the dairy industry. Such defects result from the hydrolytic lipolysis of the milk fat triglycerides (MFTG) contained within the milk fat globules (MFG) of which there are some 15×10^9 ml^{-1} and which have a total surface area of about 80 m$^2\ell^{-1}$. In milk of a good bacterial quality ($< 10^5$ CFU ml^{-1}), such lipolysis results from the action of various intrinsic endogenous or native lipolytic enzymes, especially lipase, normally present in bovine milk. These are present in quantities sufficient to rapidly liberate organoleptically significant quantities of free fatty acids (FFA), particularly C_4-C_{12}, mono- and diglycerides and other products from the MFG lipid core[10 11].

Under normal circumstances, 2 important factors prevent this adverse process: the protective effect of the intact milk fat globule membrane (MFGM) and the remoteness of lipoprotein lipase (m LPL) from its substrate, because of the close bond which exists between the casein micelles and the enzyme in freshly-drawn, uncooled milk[1 21]. In addition, various cofactors and lipolysis inhibitors and activators also play a role in protecting the MFTG from these lipases[8 28].

When lipolysis occurs, the process and resulting rancidity is classified as being either spontaneous or induced. Spontaneous rancidity (SR) develops in the milk of some 3-35% of cows[9] on cooling and during cold storage without prior disruption of the MFGM[11 12]. Conversely, induced rancidity (IR) results from the physical rupture of the MFGM and the consequent exposure of the MFTG to the action of lipase[11 12].

SR is known to be associated with late and prolonged lactation, inadequate nutrition, reduction of milk yield to below 3 kg per milking, blood plasma leaking into the milk within the udder, high somatic cell count and mastitis[8 12]. The extent of SR appears to be determined by the balance of lipase activators and inhibitors in milk and the susceptibility of the MFG[28]. On the other hand, IR is brough about, inter alia, by aeration and turbulence of warm milk, rapid and repeated chilling, freezing and homogenisation, all thermophysical forces which can disrupt the MFGM. SR and IR are however, not necessarily unrelated, as the susceptibility of the MFGM to induced disruption must also be considered[12 31].

Milk lipoprotein lipase (mLPL) is considered to be the most important endogenous lipase in milk, causing the hydrolytic lipolysis of MFTG and the resultant organoleptically unacceptable changes[29]. Factors such as rapid cooling cause dissociation of mLPL from casein (vide supra) and its release into the serum phase of the milk where it attaches to the MFGM and comes close to the MFTG[34]. Other factors which can play a role, are the presence of blood plasma components which can act as cofactors or activators of mLPL[29].

The MFGM is the other important component of milk to be considered in the phenomenon of hydrolytic lipolysis[8 10 11 12 27]. Absolute integrity of the MFGM is essential in its role of separating MFTG from mLPL and all factors affecting this integrity are relevant. The MFGM is partially derived from the lactogenic alveolar epithelial cell wall during exocytosis of the MFTG. The condition of the milk secretory epithelium may therefore have an effect on the structure and composition and behaviour of the MFGM.

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Regression of the secretory epithelium occurs during both physiological (end of lactation) and pathological (mastitis) conditions and the association between SR and prolonged or late lactation is well established[12]. Nutritional and/or physiological parameters may affect the stability of the MFGM while the action of phospholipid or protein-degrading enzymes may also promote disruption of the MFGM[27]. These factors may render MFGM more susceptible to disruption by thermo-physical factors and consequent exposure to lypolytic enzyme.

Downey's review reveals that mastitis can enhance the lipolytic susceptibility of milk[12]. The initial FFA level was found to be 75% higher in mastitic milk and the accumulation of FFA 1,5-2,0 times more rapid in milk positive to the Wisconsin Mastitis Test. The elevated FFA content of mastitic milk may be due to high somatic cell count (SCC) and leucocytic intracellular lipase. Lipolytic defects were more common in farm milk with the SCC ranging from 3,0-5,0 x 10^6 ml^{-1}. The MFGM of mastitic milk has been found to contain fewer phospholipids, less protein and to be of different polypeptide composition[27]. It has been suggested that the greater susceptibility of high SCC milk is due to an alteration in the stability of the MFGM. Other factors may influence lipolysis of milk with a high SCC, eg. breakdown of casein by proteolytic enzymes may lead to release of the lipase bound to casein micelles, while the amount of MFTG available for lipolysis may also increase during mastitis.

A number of milk components including mLPL-activator, cellular lipase, proteolytic enzyme-like substances, anions and MFGM, influence the level of FFA in milk and have an inter-related effect with their role being accentuated in milk with a high SCC[32]. Both bovine serum and high-density lipoprotein activate milk lipase in raw milk and cause an increase in developed unesterified FFA[6].

In investigating the lipolytic changes in milk obtained from cows with aseptic, subclinical mastitis Jurczak & Sciubisz[22] found no correlation between the SCC and initial FFA levels in freshly-drawn milk. However, after storage (4°C/24 h), the FFA level decreased as the SCC rose above $8x10^5$ ml^{-1}. Addition of cells to elevate the SCC of normal milk from $<5x10^5$ to $14x10^5$ ml^{-1}, resulted in a rise in the FFA levels during cold storage. Further elevation of SCC did not, however, lead to a further rise. It was suggested that partial proteolysis of α-lipoprotein and mLPL and consequent inhibition of spontaneous lipolysis may result from the release of intracellular proteases, following somatic cell breakdown. The addition of 1% blood serum caused a 220% elevation of the FFA content of milk, a "spontaneous lipolysis activator" infiltrating from blood into milk possibly playing a role. The most likely factor responsible for spontaneous lypolysis was considered to be α-lipoprotein of blood serum infiltrating through the secretory epithelium into milk during various physiological and pathological conditions.

Vitkov et al. found that the average FFA-content of mastitic (sub-clinical) milk amounted to 7,28 mg 100 ml^{-1} as against 3,96 mg 100 ml^{-1} of normal milk. They suggested that lipolysis within the mammary gland was brought about by serum factors rather than intracellular lipase[37].

The somatic cell response to inflammation of the udder has been shown to result in the appearance of an array of degradation enzymes which do not occur in normal milk[27]. MFGM from mastitic milk had higher levels of acid hydrolases, possibly of PMN-leucocyte lysosomal origin, which may alter the surface charge of the MFGM and therefore its stability[27]. It has been shown that serum mLPL increased in milk obtained from quarters infected with *S.aureus*[1]. The average mLPL-activity of milk derived from quarters with prolonged subclinical septic mastitis has been found to be 27,1% higher than that of milk obtained from normal quarters[2]. Macrophages in udder secretions, secrete lipolytic enzymes[3].

It is apparent from the literature that lipolytic degradation of milk fat in bovine milk is a complex multifactorial problem of world-wide concern. The physiological characteristics of individual cows, oestrus and progressive epithelial involution can play a role in the lipolytic susceptibility of milk. Factors such as disease, mastitis and related elevated SCC and other changes in milk composition, can aggravate the situation by promoting lipolysis. The situation may be further complicated by factors such as mechanical milking, aeration, turbulence and cooling whereby milk is subjected to thermophysical forces which contribute to the occurrence of IR. In combination, these factors may amplify lipolysis to such a degree, that significant organoleptic defects occur.

Irrespective of other factors, it is of practical importance to consider the effect of poor udder health in South African herds in relation to milk for raw consumption or processing into heat-treated milk or dairy products. Continuous monitoring of the SCC of herd milk over several years, indicates that only 25% of herds produced milk with fewer than 250 000 SC ml^{-1}, 38% of herds, milk with 250 000 to 500 000 SC ml^{-1} and 37% with > 500 000 ml^{-1} (21% with 500 000 to 750 00 ml^{-1} and 16% with > 750 000 ml^{-1}). It is evident that in about 75% of herds in South Africa, the health of the cows' udders varies from unsatisfactory to very poor[18]. With reference to the inferred role of mastitis in the cause of rancidity in milk within the Republic of South Africa[26, 31], an investigation into the situation seemed necessary.

Our aim was to examine possible differences in the initial FFA levels of milk freshly drawn from the normal and opposing subclinically mastitic quarters of individual cows, as well as the effect on the FFA content of routine procedures such as rapid and slow cooling prior to storage for 48 h. The influence of turbulence on the FFA content of warm milk from N and SSM quarter pairs, was also compared.

MATERIALS AND METHODS

The experimental design required:

(i) the preliminary identification of lactating cows with opposing pairs of normal and septic subclinically mastitic quarters
(ii) collection within one week of milk samples from such quarter pairs for verification of their health status, follwed by experimental treatment, lipase inactivation and freezing pending FFA determinations.
(iii) FFA determinations and statistical analyses of data from the confirmed opposing pairs of normal and mastitic quarters.

Establishment of health status of udder quarters

Friesland cows (n = 36) in various stages of lactation in commercial dairy herds were used. Foremilk samples from individual quarters were aseptically drawn into sterile stoppered containers after rejection of the first few jets of milk and after swabbing the teat ends with 70% ethanol. The somatic cell content (SCC) of "Somafixed" milk was established by means of a Coulter Counter model ZF, according to standard methods[36]. The individual quarter milk samples were plated out onto Blood Tryptose Agar (BTA) plates and incubated at 37°C for 24 to 48 h. Colonies were identified according to standard methods[7].

Quarters were then classified according to International Dairy Federation (IDF) criteria[23]:
Normal (N): - no mastitis bacteria and a

SCC of less than 500 000 ml^{-1} of milk.

Subclinical septic mastitis (SSM):-
Staphylococcus aureus or streptococci and a SCC of more than 500 000 ml^{-1} milk.

Only quarters of which the initial N or SSM status could be confirmed by re-examination of milk drawn within one week, were included in the study. Each N/SSM quarter pair consisted of either the 2 hind or the 2 fore quarters of one cow.

Quarter sample processing

Immediately after withdrawal of the second (confirmatory) foremilk sample, each quarter was completely milked by hand into a separate sterile container and 10 ml aliquots transferred to 30 ml sterile screw-capped glass test tubes for subjection to one of the following treatments:

IN: lipase inactivation by heating at 80°C for 5 min prior to cooling and freezing (to determine initial FFA content);
SH: horizontal agitation for 10 min at 200 oscillations min^{-1}, prior to lipase inactivation, cooling and freezing;
RC: rapid chilling by immersion into ice water for 3 h prior to storage at 4°C for 48 h, before lipase inactivation and freezing; and
SC: slow chilling by placement for 3 h in a polystyrene container holding freeze packs, prior to storage at 4°C for 48 h and subsequent lipase inactivation and freezing.

FFA determinations

Analytical grade acetic acid (BDH Chemicals, Poole, England), cupric sulphate and phosphoric acid (Merck, Darmstadt, FR Germany), chloroform, methanol, ethanol and ethyl ether of LiChrosolv grade (Merck, Darmstadt, FR Germany), butylated hydroxytoluene (BHT) (Riedel de Haen, Hannover, FR Germany), and commercial lipid standards (Nu Chek Prep, Inc., Elysian, MN, USA), were used. Silica Gel 60 TLC plates (Merck, Darmstadt, FR Germany) (20x20 cm) were scored to provide 20 separate and identical lanes each.

Instruments used were a Jouan (Jouan, Saint-Nazaire, France) 2000 GR 2000SX centrifuge with standard head for a maximum of 4750 rpm, a Linomat III TLC sample applicator from Camag (Camag, Muttenz, Switzerland), and a Shimadzu (Shimadzu, Kyoto, Japan), CS-930 dual wavelength TLC plate scanner with Schimadzu (Shimadzu, Kyoto, Japan) DR-2 data recorder.

After thawing and equilibrating, lipids from the milk samples were extracted, weighed and separated following the method of Folch et al.[16] as modified by Bitman et al.[4]. One ml of milk, 18 ml of chloroform and methanol solvent (mixed 2:1 v/v with 0,01% BHT) and 6 ml of 0,7% NaCl were mixed in a 50 ml centrifuge tube and centrifuged at 10°C for 10 min at 1000 rpm. After centrifugation, the lower (chloroform) phase was collected by syringe, transferred to a reagent tube, dried at ambient temperature under nitrogen for gravimetric determination of total fat by means of an analytical scale and redissolved in 1 ml chloroform for futher TLC processing.

Each TLC plate was spotted by means of the TLC sample applicator set to administer duplicate aliquots of 10 μl each of lipid standard to lanes 1 and 2, and of 50 μl each of the redissolved chloroform phase of milk samples to further lanes. Thereafter the lipid classes were separated by means of the slightly modified two-stage TLC technique of Bitman et al[4].

Stage 1: In a saturated tank with solvent (chloroform, methanol, ethanol, acetic acid mixed 98:2:1:0,1 v/v) kept at 75 ml by regular checking and topping up with new solvent, each inoculated TLC plate was developed at ambient temperature until the solvent front on the plate had risen 17 cm. Thereafter, the plates were transferred to a drying chamber and dried at ambient temperature under nitrogen.

Stage 2: Dried TLC plates were developed at ambient temperature in a saturated tank with solvent (hexane, ethyl ether, acetic acid mixed 94:6:0,2 v/v) kept at 75 ml by regular checking and topping up with new solvent, until the solvent front had risen to the top of the plate. Thereafter, the plate was dried in the drying chamber, immersed in 10% cupric sulphate/8% phosphoric acid and transferred to the drying oven programmed for heating.

In the oven the plates were equilibrated for 3 min at 30°C, gradually warmed up during 10 min to 180°C and kept at that temperature for 3,5 min. The developed plates were transferred for quantitative determinations of the lipid classes by the TLC scanner set for scanning in linear mode at 350 nm and connected to the data recorder. The amount of free fatty acids in each milk sample was calculated from the internal standard graphs prepared from readings obtained from the same TLC plates.

Statistical analyses

To compare the FFA content of the milk of the N and SSM quarter pairs of each of the 36 cows, the Symmetry or Signed Rank Test of Wilcoxon[33] was applied. (Due to sample losses during processing, some comparisons were limited to 32 and 34 quarter pairs.) The data was further analysed by means of Kendall's Rank Correlation Coefficient Method[17] in order to test for a statistically significant positive correlation between the initial FFA content of the N and SSM milk samples respectively and their FFA content after processing (SH, RC and SC). Kendall's test method was also applied for correlations between the SCC and the FFA content of mastitic milk on withdrawal from the udder and after the various methods treatments (SH, RC and SC).

RESULTS

Comparison of milk obtained from the paired N and SSM quarters of the individual cows by means of Wilcoxon's Signed Rank (Two-tail) Test[33] established that:

1. the initial FFA content of the milk of SSM quarters was significantly greater in amount ($p = 0,0214$) than that from opposing N quarters (analysed as $n = 32$ quarter pairs);
2. after SH and RC, milk from SSM quarters contained significantly greater quantities of FFA ($p = 0,0022$ and $0,0024$ resp.) than milk from opposing N quarters (analysed as $n = 36$ and 34 quarter pairs respectively); and
3. after SC, milk from SSM quarters contained more FFA than that from N quarters, the difference being highly significant ($p = 0,0013$) (analysed as $n = 34$ quarter pairs).

In the case of milk from N quarters, there was no significant correlation between the initial FFA content and levels after processing i.e. SH, RC and SC. Conversely, milk from SSM quarters showed highly significant positive correlations between the FFA levels after various treatments (Table 1). The initial quantity of FFA in freshly-drawn N milks varied from 2,64 to 528,47 $\mu g\ ml^{-1}$ and in SSM milk from 11,24 to 1154,60 $\mu g\ ml^{-1}$. The SCC ml^{-1} of the milk from SSM quarters ranged from $556x10^3$ to $19x10^6$. Statistical analysis showed no significant correlation between the SCC and the FFA content of the SSM milk, either initially on withdrawal or after processing (SH, RC, SC).

DISCUSSION

By limiting comparisons to the paired quarters of individual cows, the experimental design of this study elimi-

Table 1: **Correlation between the initial and post-treatment FFA content of normal (N) and mastitic (SSM) milk**

Correlation between initial level and:	Quarter status and Correlation Coefficient	Sample size (n)	Significance level
post agitation (SH)	N: 0,1638 SSM: 0,2985	36	None (p=0,1606) p=0,0325
post RC and 48 h storage	N: 0,1044 SSM: 0,3276	32	None (p=0,3940) p=0,0165
post SC and 48 h storage	N: -0,0133 SSM: 0,4154	34	None (p=0,9136) p=0,0029

nated those factors which are known to affect the FFA content of milk i.e. individual disposition, age, day to day fluctuations, breed, stage of lactation, feed, level of production and variations in susceptibility[12].

The TLC method employed is known not to reflect the presence of FFA with fewer than 8 carbon atoms, a shortcoming shared by some other methods[25]. Short chain FFA are rather volatile and decrease in proportion as the severity of the inflammatory reaction in the quarter increases[19]. Very short chain FA do not play a significant role in rancidity defects, C_{4-12} FFA being responsible for a soapy taste[25]. If our method had been capable of including short chain FFA in the total levels reflected, our results might well have been different.

Many workers have studied the relationship between lipolysis and mastitis[3 14 15 19 22 24 30 32 35 37]. The considerable variation in the degree and nature of inflammation of the mammary gland and its effect on the secretion, may well explain some of their contradictory findings.

This report concerns an investigation into the differences between the milks of paired N and SSM quarters of 32 to 36 individual cows, the status of the quarters being clearly defined in terms of IDF standards[23]. These differences could possibly be ascribed to the higher initial FFA levels in SSM milk as compared to that of N milk and indicates that SSM milk was more susceptible (Table 1) to spontaneous lipolysis due to cooling and to induced lipolysis as a result of agitation and the concommitant turbulence and aeration. The milk was not subjected to organoleptic evaluation. Our findings are in agreement with those of Jurczak & Sciubisz[22] who found no correlation between the SCC and the initial FFA content of freshly-drawn milk. Our study confirmed that SSM milk has a higher FFA content initially as well as after cold storage than N milk.

Jurczak & Sciubisz[22] suggested that when considering only the SCC of milk, milder forms of SSM are more likely to increase lipolysis than the more extensive or severe, albeit subclinical forms of mastitis. In contrast, Hofvendahl[20] reported that only with advanced subclinical mastitis (the milk showing strongly positive reactions to the California Mastitis Test), does the inflammation have the effect of significantly increasing the FFA level of the secretion. Our study has indicated that even with a SCC slightly in excess of 500 000 ml^{-1} was the initial FFA content significantly higher than in the milk of the opposite normal quarter. It has been suggested that in severe or extensive, albeit subclinical forms of mastitis, the presence of inflammatory proteases may inactivate the mLPL and activators of lipolysis[22 28].

Bovine mastitis is a dynamic condition involving the lactogenic or lactiferous units in the gland in various forms of inflammation. In addition, a number of normal physiological parameters such as age, stage of lactation and individual variation can affect the mammary gland and its secretion. These factors may influence the reaction of the mammary gland to bacterial invasion and inflammation. Furthermore the various definitions of mastitis relative to degree, extent, severity, cause and type, make it difficult to explain fully and predict the variable known effects thereof on the nature and composition of the mammary secretion. This also applies to the FFA content of milk, freshly-drawn from the SSM quarters and during subsequent processing. There is, however, sufficient evidence to emphasise the importance of a normal, healthy udder in the production of milk of acceptable initial and final quality.

Bovine mastitis is a significant problem in all countries with a well-developed dairy industry and with high-producing cows. In the Republic of South Africa, about 37% of cows have mastitis in some form or another[13]. It is contended that a considerable proportion of the herds in the RSA have an unsatisfactory to serious mastitis and/or managemental problem[18]. It has been suggested that the prevalence of significant levels of rancidity in milk in the RSA might be associated with mastitis[26 31]. Apart from the initially higher FFA content of freshly-drawn milk obtained from quarters with SSM, our investigation indicates that the milk examined had an increased susceptibility to both spontaneous and induced lipolysis. While the elimination of other factors which promote spontaneous and induced lipolysis is obviously necessary, the findings of this investigation makes it clear that, apart from various other benefits i.e. increased production, successful herd mastitis control programmes would also improve the flavour and aroma quality of milk used for direct consumption or further processing into dairy products.

This study has shown that when comparing milk from quarters of the same cow, milk from a quarter with subclinical mastitis caused by *S. aureus* or *S. agalactiae* showed a higher initial FFA content. Such mastitis milk is more prone to spontaneous lipolysis during cooling and cold storage and is also more susceptible to lipolysis induced by agitation. In dairy herds, where the prevalence of subclinical mastitis is high, organoleptically unacceptable bulk milk may result from both spontaneous and induced lipolysis. This study confirms that mastitis should always be considered among the many factors which may be responsible for rancidity and related taste defects of the milk supply.

It can therefore be concluded that if a milk producer aims to deliver milk with a low initial FFA content and a reduced susceptibility to spontaneous and induced lipolysis, the first step should be to ensure that it is derived from healthy udders. In view of the prevalence of subclinical, septic mastitis in South African dairy herds, efficient mastitis control should enjoy high priority.

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RADIOGRAPHIC PELVIMETRY FOR THE ESTIMATION OF PELVIC DIMENSIONS IN MERINO, DORMER AND S A MUTTON MERINO EWES

S W P CLOETE* and K G HAUGHEY**

ABSTRACT

Investigations into ovine pelvic size and its relationship to repetitive rearing failure require accurate estimation of pelvic dimensions in live animals. Radiographic pelvimetry was used to estimate pelvic dimensions of 84 Merino, 21 Dormer and 20 S A Mutton Merino ewes. Transverse diameter and conjugate diameter were estimated; the area of the pelvic inlet was defined as the product of transverse and conjugate diameters. Dissected measurements obtained from all ewes after slaughter were regressed on estimated dimensions to obtain prediction equations for the correction of radiographic estimates. Prediction equations for the estimation of dissected pelvic dimensions from radiographs were accurate ($r \geqq 0,87$), irrespective of dimension estimated or breed. Derived equations for estimation of dissected pelvic dimensions for the 3 breeds were not statistically different. It was concluded that pelvic dimensions of ewes could accurately be predicted by radiographic pelvimetry.

Key words: Radiographic pelvimetry, pelvic dimensions, adult ewes, regression.

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INTRODUCTION

It was recently established that approximately 60% of a sample of 560 lambs dying in the perinatal period in the western Cape showed lesions indicative of stressful birth, making difficult births the largest single cause of mortality in these lambs (K G Haughey, 1989, research report, Winter Rainfall Region, Private Bag 7607, Elsenburg). High birth masses are commonly associated with birth stress[3], particularly in single lambs[5]. In addition to effect of birth mass, there also appear to be independent contributions of small maternal pelvic size to lamb mortality in some breeds[4]. In this respect, repeated rearing failure was associated with small maternal pelvic size in 2 out of 3 Australian sheep flocks[6], while a similar tendency existed in a local Merino flock[1]. The estimation of pelvic size in ewes younger than 2,5 years is of limited value, owing to the fact that the pelvic centres of ossification only fuse at this approximate age.

In order to study the contribution of pelvic size to rearing failure in South African sheep flocks, it is necessary to obtain reliable estimates of pelvic dimensions in live animals of all breeds. This paper is an assessment of radiographic pelvimetry techniques[7 8] for the estimation of pelvic dimensions in live Merino, Dormer and S A Mutton Merino ewes. In a previous study, pelvic dimensions could accurately be estimated in smaller Merino ewes[1], but it was impossible to obtain reliable estimates for the substantially larger Dormer and S A Mutton Merino sheep with the available equipment at that stage.

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MATERIAL AND METHODS

Ewes > 4 years were used: Merinos (n = 84), Dormers (n = 21) and S A Mutton Merinos (n = 20) with mean live mass ± SD of 54,0 ± 6,3 kg (Merinos), 79,1 ± 11,1 kg (Dormers) and 82,2 ± 9,8 kg (S A Mutton Merinos).

A conventional, mobile x-ray unit was used routinely at a focal spot-film distance of 100 cm. The unit was operated at a fixed milli-amperage of 100, kilo-voltage settings ranging from 63-100, and exposure times of 0,5 - 1,0 s. The ewes were restrained on a specially-designed table[7]. For radiography of the smaller Merino ewes, a bank of 12 x-ray cassettes (305x254 mm) fitted with "Curix" Universal intensifying screens was used. A bank of 8 similar x-ray cassettes, fitted with "Du-Pont Quanta Fast Detail" High-Speed intensifying screens was required for the radiography of the larger Dormer and SA Mutton Merino ewes. All ewes were radiographed on Cronex-4 x-ray film (300 x 240 mm) using a fixed grid (ratio 6:1, 40 lines per cm, focal distance 80 to 100 cm). Radiographic procedure, manpower required and safety procedures followed were similar to those described by Haughey & Gray[7].

Radiographic images were interpreted on a standard 0,3 x 0,8 m viewing box. Vernier calipers were used to measure transverse and conjugate diameters accurately to 0,1 cm; both dimensions were adjusted for the thickness of the subject, using "method C" of Haughey & Gray[7]. The area of the pelvic inlet was defined as the product of the transverse and conjugate diameters.

All ewes were slaughtered after radiography. The pelvises were dissected out after carcases had cooled for 24 h. Transverse and conjugate diameters were measured, using vernier calipers, and the area of the pelvic inlet calculated. These diameters were taken as true measurements, and regressed on values estimated from radiographs, to obtain regression equations for correction of radiographic estimates. The PIR program of the BMDP statistical packet[2] was used in the regression analysis. Overall regression equations over breeds were obtained, together

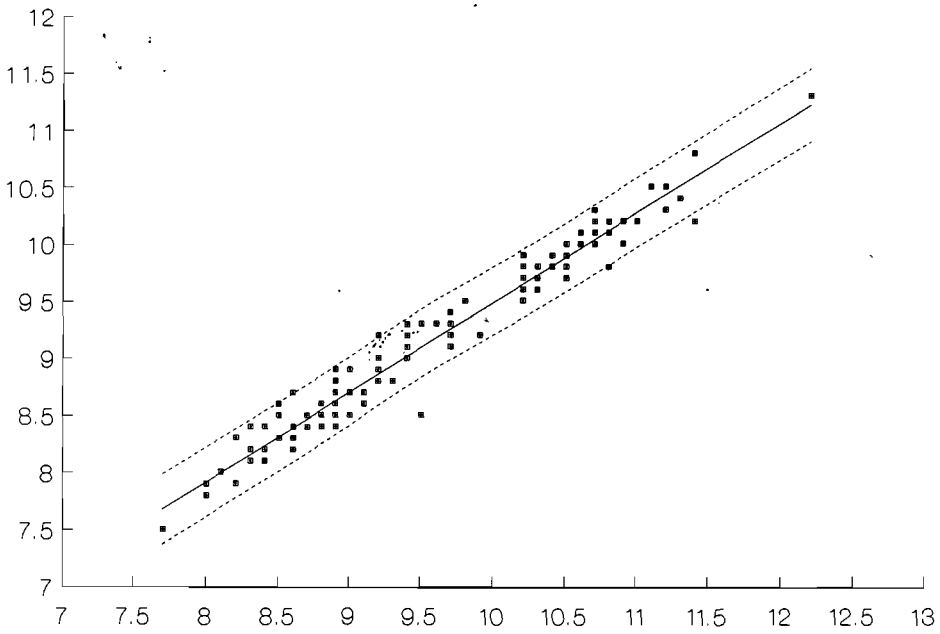

Fig. 1: Scatter-plot of the relationship between dissected transverse diameter (y) and the radiographically estimated transverse diameter (x), with the overall regression line for all ewes (— y = 1,63 + 0,786x; r = 0,98) and 95% confidence limits for individual y-estimates (---).
x-axis: Estimated transverse diameter (cm)
y-axis: Dissected transverse diameter (cm)

Regressions of dissected pelvic dimensions on those estimated by radiographic pelvimetry are given in Table 2. The prediction equations were all significant ($P < 0,0001$), predicting dissected measurements accurately ($r \geqq 0,87$). The error involved in the estimation of dissected dimensions from radiographs amounted to 1,4 - 1,8% of the mean transverse diameter for the respective breeds. Corresponding values for the conjugate diameter and the area of the pelvic inlet ranged from 2,4 to 2,9% and 2,8 to 3,2% of the mean measurements respectively. The comparison of breed-specific regressions revealed that regression equations derived for the transverse diameters of the respective breeds, did not differ significantly ($P = 0,77$). Prediction equations derived for the ewes of the respective breeds were also not significantly different with regard to conjugate diameter ($P = 0,30$) and the area of the pelvic inlet ($P = 0,24$). A scatter-plot, containing the regression line and 95% confidence intervals for transverse diameter from the analysis on all ewes is depicted in Figure 1. The close correspondence between predicted and dissected measurements is evident from the figure.

Table 1: **Mean, standard deviation (SD) and range of pelvic dimensions of Merino, Dormer and SA Mutton Merino ewes, obtained by dissection and by radiographic pelvimetry**

	Method					
	Dissection			Radiography		
Pelvic dimension and Breed	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Transverse diameter (cm)						
Merino (n=84)	8,61	0,40	7,5-9,5	8,88	0,46	7,7-9,8
Dormer (n=21)	10,00	0,29	9,3-10,5	10,60	0,34	9,7-11,3
SA Mutton Merino (n=20)	10,02	0,46	9,2-11,3	10,71	0,56	9,9-12,2
All ewes (n=125)	9,07	0,76	7,5-11,3	9,46	0,95	7,7-12,2
Conjugate diameter (cm)						
Merino	11,69	0,89	10,2-14,4	11,76	0,85	10,3-14,4
Dormer	11,66	0,64	10,6-12,9	11,66	0,69	10,0-12,7
SA Mutton Merino	12,42	0,78	11,3-14,3	12,51	0,77	11,4-13,9
All ewes	11,80	0,87	10,2-14,4	11,86	0,86	10,0-14,4
Area of the pelvic inlet (cm^2)						
Merino	100,8	9,4	78-129	104,5	9,9	80-135
Dormer	116,6	8,3	103-134	123,6	9,4	105-144
SA Mutton Merino	124,4	11,0	111-161	134,0	11,6	120-170
All ewes	107,2	13,4	78-161	112,4	15,5	80-170

with individual regressions for ewes belonging to the respective breeds. The reduction in residual mean squares due to grouping according to breed was used to test whether the slopes and/or y-intercepts of individual breed-specific regressions differed significantly.

RESULTS

Means, standard deviations and ranges of pelvic dimensions are given in Table 1. Radiographic estimates of the transverse diameter and the pelvic area consistently tended to over-estimate dissected measurements.

Corrected pelvic dimensions were calculated from the radiographic estimates, using either breed-specific regression equations for ewes belonging

Table 2: **The regressions of dissected pelvic dimensions of Merino, Dormer and SA Mutton Merino ewes on estimated pelvic dimensions obtained by radiographic pelvimetry**

Pelvic dimension and Breed	Regression equation $y = a + b (\pm SE)x$	SE of estimate Sy.x	Correlation r
Transverse diameter (cm)			
Merino (n=84)	$y=1,52+0,80(\pm 0,04)x$	0,15	0,92
Dormer (n=21)	$y=2,09+0,75(\pm 0,10)x$	0,15	0,87
SA Mutton Merino (n=20)	$y=1,55+0,79(\pm 0,06)x$	0,14	0,95
All ewes (n=125)	$y=1,63+0,79(\pm 0,01)x$	0,15	0,98
Conjugate diameter (cm)			
Merino	$y=0,04+0,99(\pm 0,04)x$	0,28	0,95
Dormer	$y=2,30+0,80(\pm 0,11)x$	0,33	0,87
SA Mutton Merino	$y=1,25+0,89(\pm 0,11)x$	0,37	0,88
All ewes	$y=0,44+0,96(\pm 0,03)x$	0,31	0,94
Area of the pelvic inlet (cm^2)			
Merino	$y=6,7+0,90(\pm 0,03)x$	3,1	0,94
Dormer	$y=19,6+0,78(\pm 0,09)x$	3,9	0,89
SA Mutton Merino	$y=4,8+0,89(\pm 0,07)x$	3,7	0,94
All ewes	$y=12,8+0,84(\pm 0,02)x$	3,4	0,97

Table 3: **Mean (±SD) corrected pelvic dimensions, mean absolute deviations from dissected measurements and the proportion over-estimates: under-estimates of dissected measurements, using breed-specific or overall regressions in the correction procedure for all 125 ewes**

Pelvic dimension	Correction procedure: Breed specific regressions	Correction procedure: Overall regressions
Transverse diameter (cm)		
Mean ± SD	9,07 ± 0,75	9,06 ± 0,75
mean absolute deviation (cm)	0,12	0,12
(%)	1,3	1,3
Over-estimates: under-estimates	0,7:1	1,1:1
Exact estimates* (%)	24,8	30,4
Conjugate diameter (cm)		
Mean ± SD	11,81 ± 0,82	11,80 ± 0,82
Mean absolute deviation (cm)	0,23	0,24
(%)	2,0	2,0
Over-estimates:under-estimates	1,1:1	0,9:1
Exact estimates* (%)	19,2	19,2
Area of the pelvic inlet (cm^2)		
Mean ± SD	107,2 ± 12,9	107,3 ± 12,9
Mean absolute deviation (cm^2)	2,56	2,65
(%)	2,4	2,5
Over-estimates:under-estimates	1:1	0,9:1
Exact estimates* (%)	0,8	1,6

*Corrected dimensions deviating $<0,05$ units from dissected dimensions

to the respective breeds or the overall equations, derived from all ewes (Table 3). Mean corrected pelvic dimensions were similar to dissected means given in Table 1. Mean absolute deviations from dissected measurements were small, irrespective of the correction procedure, generally being $\leq 2,5\%$ of dissected means. Over-estimates and under-estimates of dissected pelvic measurements occurred roughly in a 1:1 ratio, although there appeared to be slightly more under-estimates.

DISCUSSION

The procedure of using true measurements as dependent variables in a regression analysis was considered valid since the calibration sample was a random sample of the intended population of measurements to be predicted[7]. Within breed, coefficients of variation (Table 1) for dissected pelvises were in close agreement with similar results reported by Haughey *et al*[6]. The tendency for radiographic estimates to over-estimate dissected measurements of pelvic area, was also reported previously[1,7]. The obtained y-intercepts, regression coefficients and correlations (Table 2) are well within the range of comparable results in the literature[1,7]. The correlation coefficients for the transverse and conjugate diameters are also in close agreement with a corresponding value (r = 0,97) reported by McSporran & Wyburn[8] for both dimensions. It is evident that dissected pelvic dimensions could be predicted accurately from radiographic estimates, regardless of breed. This finding is emphasised by correlation coefficients of generally $\geq 0,87$ between dissected and estimated dimensions (Table 2), and 95% confidence limits within a narrow margin relative to the prediction equation depicting the regression of dissected on estimated transverse diameter (Fig. 1).

The mean absolute deviations of corrected pelvic dimensions from dissected values were small ($\leq 2,5\%$ of dissected

means), regardless of whether breed-specific regressions or the overall equations over all ewes were used (Table 3). These values compare favourably to those reported by Haughey & Gray[7]. Satisfactory predictions of dissected pelvic dimensions could be obtained from regression equations derived from all ewes. Breed-specific equations evidently had no advantage over these overall equations.

In conclusion, it is evident that actual pelvic measurements of ewes can accurately be predicted from radiographic estimates, regardless of breed and thus mature size. The accuracy level obtained is adequate for our purpose, which is to study the effect of pelvic size on rearing failure in some South African sheep populations. Our results indicate that the use of breed-specific regression equations had no advantage over overall equations for all ewes included in the investigation. The utilisation of overall equations for the correction of pelvic dimensions estimated from radiographs are thus recommended in order to facilitate the correction procedure.

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Book Review/Boekresensie

DIAGNOSTIC CYTOLOGY OF THE DOG AND CAT

R.L. COWELL and R.D. TYLER

1st Edn. American Veterinary Publications, Inc. 5782 Thornwood Drive, Goleta, California, 93117. 1989 pp 259, Numerous colour illustrations, charts and tables, Price US $ 75.45 (ISBN: 0-939674-25-4).

This book is most welcome in that it at last fills what has become a huge gap in the veterinary literature; that is, a comprehensive text covering all aspects of diagnostic cytology. The use of this tecnique has become widespread and has long deserved better documentation. Although it deals specifically with the cytology of the dog and cat, it will nevertheless prove most useful to workers dealing with other animal species.

In general, the text is excellent and covers most aspects in a thorough and up-to-date manner. However, there are some sections, notably the discussions on neoplastic criteria in cytology (Chapter One) and urine sediments (Chapter Nineteen) which are most disappointing.

Much emphasis is placed on the use of algorithms. I find this a most unfortunate attempt to oversimplify what is undeniably a difficult technique requiring much practice. One would prefer a more in-depth discussion on the basic principles of cytology and the recognizable features of different pathophysiological mechanisms affecting cells. In this way the reader could be given a foundation on which to build his experience and to develop his own insights into this fascinating world.

The book boasts a generous number of colour illustrations which are of excellent quality.

All in all, it is an essential and invaluable aid to anyone wishing to become, or who is already, involved in diagnostic cytology irrespective of his/her level of expertise.

CLAIRE MARSHALL

KOLLAGEEN IN BOEREWORS AS MAATSTAF VAN GEHALTE

L W VAN DEN HEEVER*, MARIA C SMIT+ en P H HEINZE+

ABSTRACT

Formalin-fixed samples (n=75) of commercial boerewors (traditional farm style sausage) were examined for total nitrogen (N) and hydroxyproline (Hypro) N to assess their collagen content. Hypro N/total Nx10[3] (unaffected by residual fat) varied from 8,23 to 16,50; 3/75 (5%) of samples had ratios equal to or less than the control (8,38) i.e. sausage home-made from meat without addition of scraps or collagen-rich trimmings. About 95% of samples had ratios greater than 10, and 5% had ratios greater than double the control. In 75% of the surveyed samples, the ratios were more than 1,5 times greater than those of the control.

To ensure boerewors of good compositional quality, it is advocated that formalin-fixed samples of the product be subjected to both histological examination and collagen determinations. Promulgation of legislation to limit the collagen content of boerewors is proposed; a maximum of 12 in the ratio of Hypro N/total Nx10[3] is considered reasonable and attainable in the light of the results of this survey.

Key words: Boerewors, quality, collagen content

Van den Heever L.W.; Smit Maria C.; Heinze P.H. **Collagen in boerewors as a measure of quality.** *Journal of the South African Veterinary Association* (1990) 61 No. 2, 59-61 (Afr.) Department of Veterinary Public Health, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INLEIDING

Regulasie 14(2)(b) van Wet nr 13 van 1929 het vereis dat boerewors gemaak word van die skoon, gesonde en heilsame spiere en vet van die bees, skaap of vark of 'n mengsel van twee of meer hiervan. Dit moes nie minder as 90% totale vleis en 2% proteïenstikstof bevat nie, d.w.s. nie miner nie as 60% maervleis (vleis sonder aangehegte vet). Hierdie regulasie is deur GK R2398 van 25:11:88 herroep en nog nie vervang nie.

Proteïen stikstof (N), chemies bepaal, dek alle proteïen, ongeag die aard en bron daarvan. Die metode onderskei nie tussen dierlike en plantaardige proteïen of tussen spiervleis en ander dierlike weefsels nie. Daarvoor is mikroskopiese ondersoek van gekleurde dun worssky-we nodig, maar sonder spesiale histometriese tegnieke kan die hoeveelheid van 'n weefselsoort nie akkuraat bepaal word nie. Met histologiese ondersoeke word aansienlike hoeveelhede fibreuse bindweefsel (FBW) egter soms waargeneem[5] (L.W. van den Heever, 1972, gemagtigde ontleder).

FBW bind en stut alle weefsels in die liggaam en kom dus ook in 'n mindere of meerdere mate in spiervleis voor. Skeletspiere bevat FBW in 4 histologiese en anatomiese vorms[2]: die diep fascia of spierskede, die septa wat indring om die spierveselbundels te omring en die fyn skede om elke spiervesel. Al hierdie spierbindweefsel kom by die aanhegting van die spier saam en heg direk aan die been of vorm deur middel van 'n massiewe samevoeging van FBW vesels, die pees of sening. Meer as 50% van FBW bestaan uit kollageen[2], 'n familie van fibreuse proteïene waarin hidroksieprolien (Hipro) byna eksklusief gevind word. Die hoeveelheid Hipro in vleisprodukte word dus gebruik om die hoeveelheid kollageen, en dus die hoeveelheid FBW te bepaal[2]. Die hoeveelheid kollageen in vleis wissel van spier tot spier en word ook bepaal volgens ras, ouderdom, soort en geslag van die dier[3 4].

Die hoeveelheid sening of pees wat saam met die spiervleis in die worsmengsel geplaas word, beïnvloed ook die kollageeninhoud van die produk. Anders as elastien, word kollageen na langdurige blootstelling aan temperature bokant 60°C, gepaard met die opname van water, geleidelik tot gelatien omgeskakel en verteerbaar gemaak[2]. Hierdie eienskap word ook deur bogenoemde faktore beïnvloed.

Oormatige hoeveelhede kollageen in boerewors dra by tot die taaiheid van die produk[4], dit lei tot die byvoeging van oormatige hoeveelhede water tydens vervaardiging en die skeletspierweefsel word ten dele vervang met 'n goedkoper bestanddeel van minderwaardige voedingswaarde en 'n ander smaak. Verder is die faktor vir omsetting van Hipro N tot proteïen, slegs 5,55 waar dit 6,25 vir totale vleis N is en gevolglik kan kollageen in wors die totale "vleis"-proteïenvlak hoër as die werklike laat voorkom (Direkteur-generaal 1988 Nasionale Gesondheid en Bevolkingsontwikkeling, persoonlike mededeling).

Daar word soms beweer dat sommige vervaardigers van boerewors dit beskou as 'n aflaaiplek vir allerhande nie-spierweefsels van die slagdier en die afsnysels en los stukke wat andersins nie voordelig bemark kan word nie. In die finale produk, is sulke weefsels immers makroskopies onherkenbaar. Hierdie bewering word soms in 'n mate gestaaf deur histologiese bevinding van onder andere ontoelaatbare weefsels soos lewer, hart, nier, speekselklier, uierweefsel en aansienlike hoeveelhede FBW in sommige handelsboerewors[5]. Laasgenoemde dui daarop dat sommige vervaardigers meer kollageen in die wors laat kom as wat bloot deur die gebruik van suiwer spiervleis verklaar kan word.

Ten einde die juistheid van hierdie indrukke te kan bepaal, is 'n opname van die kollageeninhoud van handelsboerewors uit verskillende dele van die Republiek onderneem.

MATERIAAL EN METODES

Formalien-gefikseerde monsters (n=75) van handelsboerewors wat aan 'n gemagtigde ontleder (L.W. van den Heever) vir amptelike histologiese on-

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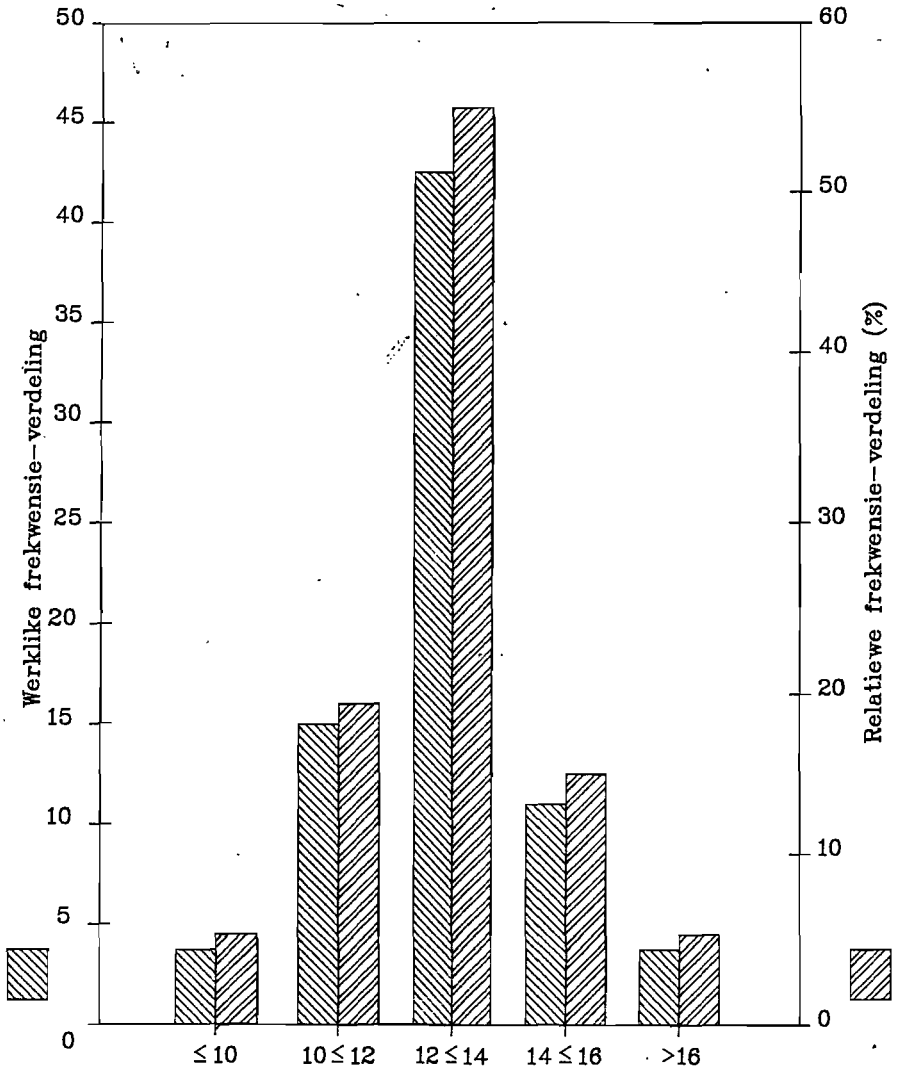

Fig. 1: **Frekwensie-verdeling van die verhouding Hipro N tot totale N in 75 gevriesdroogde boereworsmonsters**

dersoek gestuur is, is vir die opname gebruik. Die monsters is gekoop in Johannesburg, Strand, Knysna, Worcester, Oudtshoorn en verskeie Vrystaatse dorpe. Wors gemaak deur 'n persoon vir eie gebruik van skeletspiervleis en vet, maar sonder byvoeging van afsnysels, het as kontrole gedien, met die veronderstelling dat dié wors van redelike gemiddelde samestelling en gehalte was.

Elke monster het bestaan uit 3 worsstukke van ± 25 mm wat onderskeidelik uit die middel en die 2 punte van 'n 500 mm worslengte gesny is. Na verwydering van die worshuls (-derm) is die worsinhoud deeglik gemeng, deur dit fyn te maal en d.m.v. 'n kaplem te homogeniseer, voordat dit met behulp van petroleumeter ontvet en met 'n infrarooilamp tot konstante massa gedroog is. Die poeier sodoende verkry, is vervolgens in 10 g hoeveelhede in verseëlde glasflessies gevriesdroog.

Die totale stikstof (N) en totale Hipro-N in bogenoemde monsters is in triplikaat bepaal volgens metodes aangeteken deur Cronjé[4]. Die Hipro-bepalings is gegrond op Metode A van Bergman & Loxley[1] soos aangepas vir die Technicon Auto Analyzer Model II. Vir totale N, is 'n metode gevolg wat gegrond is op metode 30-69A (Technicon Auto Analyzer Methodology 1969. Industrial method 30-69A: Total Nitrogen (Kjeldahl) Technicon Corporation, Tarrytown, N.Y. USA) en waarin 'n Technikon Auto Analyzer gebruik is. Die kollageeninhoud van die boerewors is as volg bereken en uitgedruk: mg kollageen per g monster (mg kollageen = mg Hipro Nx7,75); persentasie kollageen proteïen tot totale proteïen (mg Hipro Nx 7,75x100/totale Nx6,25) en die verhouding Hipro N tot totale $Nx10^3$.

RESULTATE

Die kollageeninhoud van ontvette, gevriesdroogde, gehomogeniseerde worshuls (-derm)-vrye handelsboerewors is uitgedruk as:

a) mg kollageen per g worsmateriaal wat gewissel het van 34,10 tot 124,93 met die kontrole op 61,15. Die frekwensieverdeling van die waardes word in Tabel 1 uiteengesit; b) persentasie kollageen tot proteïeninhoud (Nx 6,25) wat gewissel het van 9,55 tot 20,18 met die kontrole op 9,73; Die frekwensieverdeling van hierdie waardes word in Tabel 2 uiteengesit, en c) verhouding Hipro N tot totale $Nx10^3$ wat gewissel het van 8,23 tot 16,50 met 'n kontrolewaarde van 8,38. Die frekwensie verdeling word in Fig. 1 uitgebeeld.

Die verslag oor die amptelike histologiese ondersoek van die opnamemonsters het getoon dat 13/75 (17,3%) daarvan soja-proteïen en 14/75 (18,6%) daarvan dierlike weefsel ander as skeletspier en vet bevat. Die worsmonster met van die hoogste kollageeninhoud (16,5%), het ook groot hoeveelhede FBW met mikroskopiese ondersoek vertoon.

Al 3 wyses van uitdrukking het aangedui dat enkele handelsmonters (5%) ten opsige van kollageeninhoud, selfs van beter gehalte as die kontrolemonster was. Dit dui daarop dat die kontrole 'n redelike basis van vergelyking was. Omdat die kontrolemonster verkry is van wors wat sonder handelsoorwegings, tuis gemaak is deur 'n persoon sonder voorkennis dat dit as kontrole sou dien, word dit beskou as wors van goeie, gewone gehalte soos die verbruiker dit vir eie gebruik sou wou hê.

In gehalte-boerewors skyn 'n maksimum van 15% kollageen wat bykans anderhalf soveel as in die kontrolewors is, 'n redelike en bereikbare standaard te wees.

Vir wors met anderhalf soveel kollageen as wors van "goeie" gehalte skyn 'n maksimum verhouding van 12 (Hipro N tot totale N) sowel redelik as bereikbaar te wees. Toepassing van 'n maksimum verhouding van 14 sou daarin geslaag het om die ± 20% handelsmonsters wat werklik groot hoeveelhede kollageen bevat het, uit die handel te hou.

In Oostenryk, Wes-Duitsland en Switserland word chemiese bepaling van die kollageenvrye vleisproteïeninhoud wetlik vereis ter vasstelling van die samestelling en handelswaarde van vleisprodukte[6]. Vorige en beoogde Suid-Afrikaanse wetgewing wil verseker dat die boerewors in die handel 'n tradisionele produk van hoë gehalte is[6]. Deur neerlegging van minimum hoeveelhede totale proteïen, gegrond op chemiese bepaling van stikstofinhoud alleen, kan die aard van die proteïen nie verseker word nie, omdat plantproteïen en dierlike weefsel wat nie vleis is nie, ook daarby ingesluit sal word. Histologiese ondersoek van boerewors kan en het reeds die byvoeging van bogenoemde ontoelaat-

Tabel 1: **Frekwensie-verdeling van mg kollageen (Hipro N x 7,75) in 75 gevriesdroogde monsters van boerewors**

Klasverdeling	Middelpunt	Frekwensie	Relatiewe frekwensie (Rel. frek.)	Kumulatiewe Rel. frek.
≤40		3	0,0395	0,0395
40< x ≤60	50	2	0,0263	0,0658
60< x ≤80	70	18	0,2368	0,3026
80< x ≤100	90	33	0,4342	0,7368
100< x ≤120	110	17	0,2237	0,9605
120< x		3	0,3095	1,0000

Tabel 2: **Die persentasie kollageen in 75 gevriesdroogde monsters van boerewors ([mg Hipro N x 7,75 x 100]/[mg totale N x 6,25])**

Klasverdeling	Middelpunt	Frekwensie	Relatiewe frekwensie (Rel. frek.)	Kumulatiewe Rel. frek.
≤12,0		6	0,0789	0,0789
12,0< x ≤13,5	12,75	5	0,0658	0,1447
13,5< x ≤15,0	14,25	23	0,3026	0,4474
15,0< x ≤16,5	15,75	27	0,3553	0,8026
16,5< x ≤18,0	17,25	10	0,1316	0,9342
18,0< x		5	0,0658	1,0000

bare weefsel en soja in 'n groot mate beheer. Die kwantitatiewe bepaling van kollageen is vervolgens nodig om te onderskei tussen die hoeveelhede FBW wat in situ in vleis voorkom, en dit wat deur die gebruik van seningryke vleis of kollageenryke afsnysels ("trimmings") in die worsvleismengsel beland. Hiervoor sou die maksimum toelaatbare hoeveelheid kollageen in boerewors neergelê moes word en sou die uitslag van hierdie opname daarvoor as leidraad kon dien. Die maksimum moet egter altyd realisties bereikbaar wees, maar nogtans wors met onaanvaarbare hoeveelhede kollageen uit die handel kan hou.

Die koste van beheer oor die gehalte van 'n produk soos boerewors, deur histologiese en chemiese ontleding, sluit ook in die neem en versending van monsters. Die gebruik van gefikseerde monsters vir beide chemiese en histologiese ondersoek, is 'n logiese manier om kostes te bespaar. Alhoewel geen gepubliseerde verslae oor die bepaling van hidroksiprolien in formalien-gefikseerde vleisprodukte gevind kon word nie, is daar geen bekende rede waarom dit nie gedoen kan word nie. (PA Koolmees, 1985, Rŷksuniversiteit te Utrecht, Nederland, persoonlike mededeling). Die aminosuurgroepe word immers slegs deur die formalien verknoop en hidroksieprolien sou toereties dus normaal gehidroliseer kon word[7]. Om enige onsekerheid uit te skakel, sou dit wenslik wees om hierdie veronderstelling te bevestig.

Ter opsomming toon hierdie opname dat meeste boerewors wat in verskeie dele van Suid-Afrika verkoop word, veel meer kollageen (fibreuse bindweefsel) bevat as wat in boerewors van "goeie" gehalte vir eie gebruik gevind is. Dit dui op die wenslikheid om 'n maksimum kollageeninhoud met hersiening van beheerwetgewing neer te lê. 'n Redelike maksimum, sou 15% kollageen in die totale proteïen, of nog beter, 'n maksimum van 12 in die verhouding van Hipro N tot totale N wees.

ERKENNING

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DIE INVLOED VAN LIGGAAMSMASSA EN KONDISIE OP OVULASIE-TEMPO VAN POLIANDRO-ALBUMIEN GEïMMUNISEERDE S A VLEIS-MERINO-OOIE

S.J. SCHOEMAN* en HELEEN C. ELS*

ABSTRACT

Two groups of 40 S A Mutton merino ewes were used to determine the effect of body mass and condition score on the response of immunisation against androstenedione. The effect of immunisation was significant ($P<0,05$) on ovulation rate (1,64 for non-immunised and 2,08 for immunised ewes) and higher body mass and condition scores acted complimentary to the effect of immunisation. The ovulation rate in control ewes was more closely related to condition score ($r=0,511$) and body mass ($r=0,600$) at the end of the experimental period than in the immunised ewe group ($r=0,302$ and 0,235).

Key words: Sheep, ovulation rate, immunisation, body mass, condition, polyandroalbumin, reproduction

Schoeman S.J.; Els Heleen C. **The influence of body mass and condition on ovulation rate in polyandroalbumin immunised S A Mutton merino ewes.** *Journal of the South African Veterinary Association* (1990) 61 No. 2, 62-64 (Afrik.) Department of Livestock Science, University of Pretoria, 0083 Hillcrest, Republic of South Africa.

INLEIDING

Verhoging in reproduksiedoeltreffendheid in ooie is 'n belangrike meganisme waardeur skaapboerdery meer ekonomies bedryf kan word. Van die komponente van reproduksieprestasie, is ovulasietempo (en dus lampersentasie) waarskynlik dié wat meeste manipuleerbaar is. Aangesien ovulasietempo suksesvol verhoog kan word, kan een van die grootste beperkings in reproduksiedoeltreffendheid, soos by meeste skaaprasse voorkom, oorkom word[5].

Die immunisering van die ooi teen een van haar eie hormone, naamlik androsteendioon, wat vir 'n beperking op ovulasietempo verantwoordelik is, is 'n metode om reproduksieverhoging te bewerkstellig. Sodanige immunisering induseer 'n verandering in die hipotalamus-hipofise-ovaria terugvoersisteem met 'n gevolglike verandering in die funksies van die ovaria en 'n gepaardgaande verhoging in die ovulasietempo van die ooi[3 5 9]. Sedert 1974 het hierdie prosedure homself tot 'n mindere of meerdere mate reeds gevestig[3].

Die resultate verkry met immunisering teen androsteendioon, is onder andere van die voedingspeil afhanklik[4]. Verskeie navorsers toon dan ook dat die effek van immunisering en die voedingspeil additief tot mekaar bydra[1 4 12]. Die verhoging in ovulasietempo as gevolg van die behandeling, is derhalwe addisioneel tot dié deur die peil van voeding bepaal.

Die doel van hierdie studie was om die invloed van liggaamsmassa en kondisie, asook liggaamsmassa- en kondisieveranderings op die ovulasietempo by S.A. Vleismerino-ooie wat met poliandro-albumien geïmmuniseer is, te bepaal. In die algemeen is in vorige studies hoofsaaklik op die effek van liggaamsmassa gekonsentreer[1 4 13]. Dit is egter tot 'n groot mate met kondisie verstrengel[2] en kondisieverskille mag moontlik 'n groter invloed op die sukses van aktiewe immunisering teen androsteendioon uitoefen.

MATERIAAL EN METODE

Tagtig volwasse (4-6 jaar oud) S.A. Vleismerino-ooie is vir 'n tydperk van 4 weke vanaf die veld op kraal, op 'n aanpassingsdieet geplaas. Die ooie is hierna gestratifiseerd ewekansig in 2 groepe verdeel. Een groep is op 'n onderhoudsdieet[7] (8,9% ruproteïen; 9,14 MJ ME/kg) (lae peil) en die ander groep op 'n dieet van ongeveer 1,5 x onderhoud (13,3% ruproteïen; 11,0 MJ ME/kg) (hoë peil) geplaas.

Na 10 weke, is 20 ooie uit elke voedingsgroep ewekansig gekies en met poliandro-albumien (Fecundin^R, Glaxo Animal Health, Coopers) geïmmuniseer deur 'n 2 mℓ (wat 0,6 mg/mℓ poliandro-albumien bevat het) dosis onderhuids toe te dien. Die oorblywende 20 ooie per voedingsgroep het as kontrole gedien, wat buiten die immunisering self, aan dieselfde bestuur en hantering as die behandelingsgroep onderworpe was. Die behandelingsgroep is na 'n verdere 4 weke met 'n onderskragingsdosis geïmmuniseer, en na 'n verdere week is al die ooie met medroksi-progesteroon-asetaat geïmpregneerde sponse (Repromap, Upjohn Pty Ltd) gesinchroniseer. Na 14 dae is die sponse verwyder en bronstige ooie met behulp van koggelramme geïdentifiseer.

Op ongeveer 5 dae na die laaste waarneembare tekens van estrus, is ovulasietempo, met behulp van laparoskopie soos deur Oldham & Lindsay[8] beskryf, bepaal. Ooie is tweeweekliks oor die 18-weke-lange eksperimentele periode geweeg om liggaamsmassaveranderinge te monitor terwyl kondisietelling[10] (skaal 1 tot 5) aan die begin en weer aan die einde van dié tydperk aangeteken is.

Verskille tussen behandelings is met behulp van Student se t-toets vir betekenisvolheid getoets[14].

RESULTATE

Die gemiddelde massas van ooie aan die begin en einde van die proef, gemiddelde massaverandering, kondisietelling en ovulasietempo van die kontrole- en behandelingsgroepe by onderskeidelik die lae en hoë vlakke van voeding word in Tabel 1 aangegee. Die saamgevoegde waardes van die hoë voedingspeil toon 'n aansienlike en betekenisvolle ($P<0,01$) toename (10,45 kg; $Y = 54,50 + 0,47X$) en dié van die lae peil 'n nie-betekenisvolle ($P>0,05$) afname (3,51 kg; $Y = 51,32 - 0,19X$) asook on-

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Tabel 1: **Liggaamsmassa, kondisietelling en ovulasietempo in ooie by 2 vlakke van voeding en immunisering met poliandro-albumien**

	Voedings-peil	n	Gemiddelde massa (±SA)kg Begin	Gemiddelde massa (±SA)kg End[c]	Gemiddelde kondisietelling(±SA)(1-5) Begin	Gemiddelde kondisietelling(±SA)(1-5) End[c]	Gemiddelde ovulasietempo
Behandeling*	Laag	20	53,10 ± 11,63	50,25 ± 12,81	3,05 ± 0,83	2,50 ± 1,05	1,85 ± 0,93
	Hoog	20	54,95 ± 11,67	65,10 ± 11,45	3,10 ± 0,79	4,25 ± 0,72	2,30 ± 0,92
Gemiddeld			54,03 ± 11,54[a]	57,68 ± 14,16[a]	3,07 ± 0,80[a]	3,38 ± 1,25[a]	2,08 ± 0,94[a]
Kontrole**	Laag	19	49,84 ± 9,90	45,63 ± 11,93	2,53 ± 1,07	2,26 ± 0,99	1,32 ± 0,67
	Hoog	20	55,20 ± 12,29	65,95 ± 12,01	3,15 ± 0,59	4,00 ± 0,86	1,95 ± 0,60
Gemiddeld			52,59 ± 11,37[a]	54,77 ± 17,45[a]	2,85 ± 0,90[a]	3,15 ± 1,27[a]	1,64 ± 0,71[b]

*Geïmmuniseer met poliandro-albumien met 4 weke tussenpose
**Geen immunisering
[a,b]P < 0,01 — Waardes in dieselfde kolom met verskillende boskrifte verskil betekenisvol
Geneem na 18 weke tydens laparoskopie

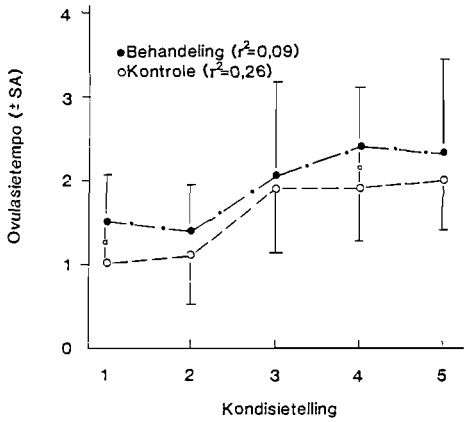

Fig. 1: **Invloed van kondisietelling aan die einde van die behandeling op ovulasietempo by behandelde en kontrole-ooie**

[a]P ≤ 0,05

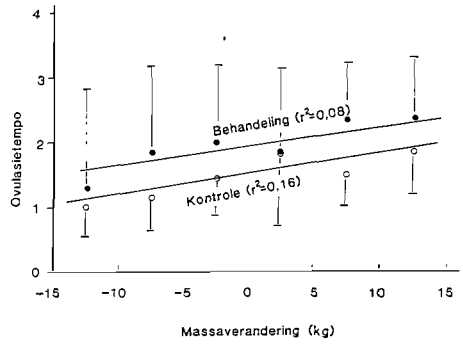

Fig. 2: **Die invloed van massaverandering op ovulasietempo by behandelde en kontrole-ooie**

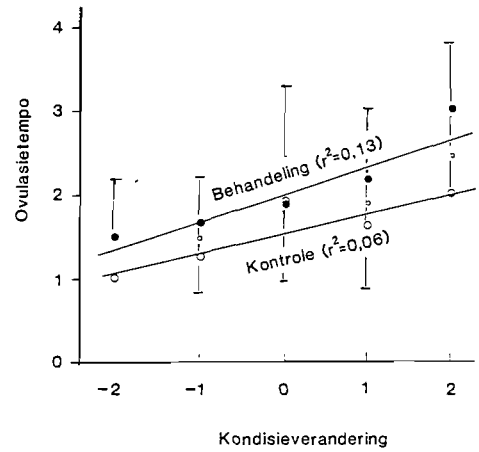

Fig. 3: **Die invloed van kondisieverandering op ovulasie-tempo by behandelde en kontrole-ooie**

[a]P ≤ 0,05; [b]P ≤ 0,01

derskeidelik 'n toename (P < 0,01) en afname (P > 0,05) in kondisietelling.

Immunisering met poliandro-albumien het 'n betekenisvolle (26,8%) (P < 0,01) verhoging van 0,44 ovulasies per ooi in ovulasietempo teweeggebring, terwyl die verhoogde voedingspeil, ongeag die immuniseringseffek, 'n verhoging van 0,54 ovulasies per ooi (34,0%) (P < 0,01) veroorsaak het. Op die lae vlak van voeding het behandeling 'n groter verhoging (P < 0,05) in ovulasietempo tot gevolg gehad (40,2% in die lae voedingsgroep teenoor 18,0% in die hoë voedingsgroep).

Die invloed van kondisietelling aan die einde van die eksperimentele fase op ovulasietempo, word in Fig. 1 aangegee. Die invloed van massa- en kondisieveranderings op ovulasietempo by beide die behandelde en kontrole ooie word in Fig. 2 en 3 onderskeidelik aangedui. In alle gevalle word 'n positiewe verband aangetoon. Die grootste verhoging in ovulasietempo vind plaas vanaf kondisietelling 2 tot 3 (Fig. 1). Hierdie verskil is egter nie betekenisvol (P > 0,05). 'n Verandering van 0,03 vind in ovulasietempo plaas per kilogram massaverandering. In die geval van verandering in kondisie, is die regressiekoëffisiënte 0,23 by die kontrole en 0,33 by die behandelingsgroepe, onderskeidelik.

Enkelvoudige korrelasiekoëffisiënte tussen ovulasietempo en massa, kondisie en massa- en kondisieverandering word vir die behandelings- en kontrolegroepe in Tabel 2 aangegee. In die kontrolegroep is beginmassa, endmassa, massaverandering, begin- en endkondisie betekenisvol (P < 0,01) met ovulasietempo gekorreleer. Alhoewel ook positief, is hierdie korrelasies in die geval van die behandelde ooie, met die uitsondering van kondisieverandering (P < 0,05), nie betekenisvol (P > 0,05) nie.

BESPREKING

Die immunisering van ooie teen androsteendioon, soos in hierdie studie gevind, het 'n aansienlike verhoging in ovulasietempo teweeggebring. Die resultate stem grootliks met dié wat algemeen in die literatuur gevind word, ooreen[3 6 11].

'n Hoër voedingspeil het 'n additiewe invloed tot die behandelingseffek. Hierdie resultaat stem ook ooreen met wat algemeen in die literatuur aangetoon word[1 4 9 12]. Die grootste verhoging in ovulasietempo, wat soos aangetoon, vanaf kondisietelling 2 tot 3 plaasvind, dui waarskynlik op 'n optimum kondisietelling van 3 by beide die kontrole en geïmmuniseerde ooigroepe.

Die verband tussen massa en kondisie aan die een kant en ovulasietempo aan die ander kant, was egter groter in die kontrolegroep as in die behandelingsgroep. Dit wil dus voorkom of ovulasietempo minder massa- en kondisie-

Tabel 2: **Enkelvoudige korrelasiekoëffisiënte tussen massa, kondisie en ovulasietempo by poliandro-albumien geïmmuniseerde (Behandeling) en kontrole-ooie**

Massa/ Kondisie	Ovulasietempo	
	Behandeling	Kontrole
Beginmassa	0,098	0,491**
Endmassa[a]	0,235	0,600**
Massaverandering	0,283	0,396**
Beginkondisie	0,020	0,465**
Endkondisie[a]	0,302	0,511**
Kondisieverandering	0,361*	0,245

*P < 0,05; **P < 0,01
[a]Geneem na 18 weke tydens laparoskopie

afhanklik in geïmmuniseerde ooie as kontrole ooie is, sodat immunisering by skraler ooie 'n beter resultaat as by vetter ooie kan meebring. Dit word ook deur ander navorsers bevestig[4 6 13 15].

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SALMONELLOSIS IN AN ADULT DAIRY COW

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ABSTRACT

An outbreak of diarrhoea occurred in a Jersey herd after the introduction of new stock. One of the cows was examined and treated unsuccessfully. Clinical findings included depression, fever, dehydration, congestion, signs of colic and a severe diarrhoea. The post mortem examination revealed emaciation, pseudomembranous enteritis, mesenteric lymphadenopathy and focal disseminated hepatic necrosis. *Salmonella typhimurium* was isolated from the faeces, mesenteric lymph nodes and liver.

Key words: Salmonellosis, cattle

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Salmonellosis is an important disease both from an economic and a public health point of view. It occurs universally and in all species. More than 2 000 antigenically different serotypes of Salmonella have been identified[2], but only a limited number of these cause salmonellosis in the bovine. The infection rate in dairy cows around the world seems to be between 5 and 15%[2]. The more common species causing disease in the bovine are *S. dublin, S. typhimurium* and *S. newport*[2], but increasing numbers of outbreaks are caused by salmonellae from serogroup E, such as *S. anatum, S. muenster* and *S. senftenberg*[6,7]. The clinical syndromes associated with salmonellosis in the bovine, include acute and chronic enteritis, abortion, septicaemia, polyarthritis, pneumonia, endarteritis and dry gangrene[3].

This paper reports on a fatal case of salmonellosis in a Jersey herd suffering from an outbreak of enteritis.

Diarrhoea first became a problem in a Jersey herd in the eastern Transvaal after the introduction of cows from a herd in the eastern Cape. The first cases of diarrhoea occurred in cows of the permanent population that were subjected to calving stress. Later, calves born on the farm also started to develop diarrhoea from the age of 2 weeks.

The severity varied; severely affected animals showed clinical signs of a fluctuating temperature, anorexia, pronounced drop in milk production and a severe, foul-smelling diarrhoea with mucosal detritus in the faeces, while others showed only a mild diarrhoea.

There was no change in the feeding or management of the herd prior to the start of the problem.

One of the affected cows that had developed severe diarrhoea after calving 2 weeks previously, was referred for examination and treatment.

On admission the cow was depressed and preferred to lie down. The rectal temperature was 40,2°C, the heartrate 72 beats min^{-1} and the respiratory rate 36 min^{-1}. Signs of abdominal pain were present, the mucous membranes were congested and slight dehydration was evident. Ruminal contractions were present as well as a severe, foul-smelling diarrhoea that contained a great deal of mucosal detritus. The fermentation was fairly good, with a methylene blue reduction time of 3 min.

Faecal flotation did not reveal any abnormality. The haematological examination was characterised by a slight leucocytosis, a decrease in mature neutrophils, a left shift and a monocytosis.

Based on the history and the clinical findings, a preliminary diagnosis of salmonellosis was made. Faecal as well as blood cultures were performed in an attempt to confirm the diagnosis. Treatment was immediately instituted using trimethoprim plus sulphadimethylpyrimidine (Amphoprim, Krüger-Med), procaine benzylpenicillin (Depocillin, Coopers Animal Health) and flunixin meglumine (Finadyne, Centaur). The condition of the animal deteriorated despite the treatment, and blood appeared in the faeces. The animal died 4 d after admission and a post mortem examination was conducted.

The blood cultures did not yield any bacterial growth, but a bacterium was isolated from the faeces which was identified as *Salmonella typhimurium* 1,4,5,12: i : 1,2. The results of an antibiogram that was performed, indicated that the organism was fairly resistant with the highest sensitivity to colistin and polymixin B.

The carcase revealed a moderate degree of emaciation and dehydration. Significant lesions were restricted to the alimentary tract, mesenteric lymph nodes, liver and spleen. The forestomach content was reduced in amount, dark greenish-gray in colour and foul-smelling. The intestinal lumen contained a thin, gray, flocculent material rich in mucus. A severe pseudomembranous enteritis which extended from the distal part of the duodenum to the ileo-caecal valve was present in the small intestine. The entire mucosa was transformed into a dull, yellowish-gray and slightly friable pseudomembrane that was firmly attached to the underlying tissue. On physical removal, a raw, haemorrhagic area of intestinal wall remained. In addition to the enteric lesion, marked mucosal and submucosal congestion of the fundic portion of the abomasum together with mild mucosal congestion and severe mucosal and submucosal oedema of the caecum and colon were present. The mesenteric lymph nodes were enlarged (up to 10 times their normal size), oedematous and showed an increase in the cortical component with associated loss of corticomedullary distinction.

The liver was enlarged, congested and evinced a bronze discolouration together

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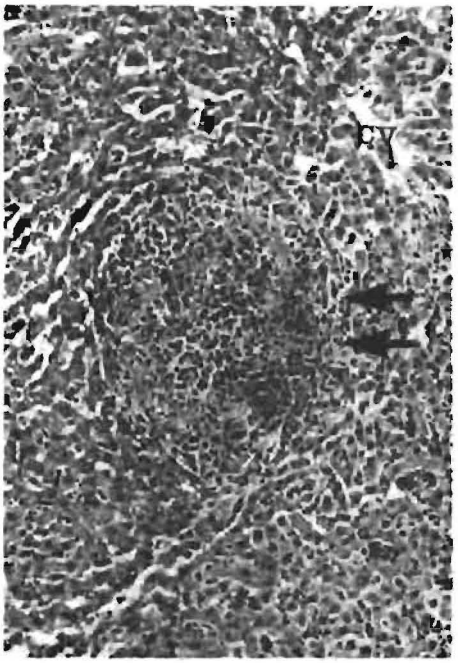

Fig. 1: **Focus of coagulative necrosis (arrowed) in the midzonal region of a hepatic lobule. (CV denotes the central vein). HE X100**

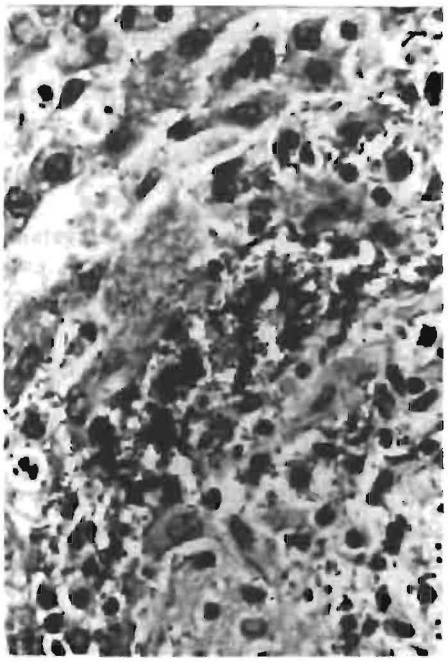

Fig. 2: **Bacteria at the periphery of the lesion. Gram X400**

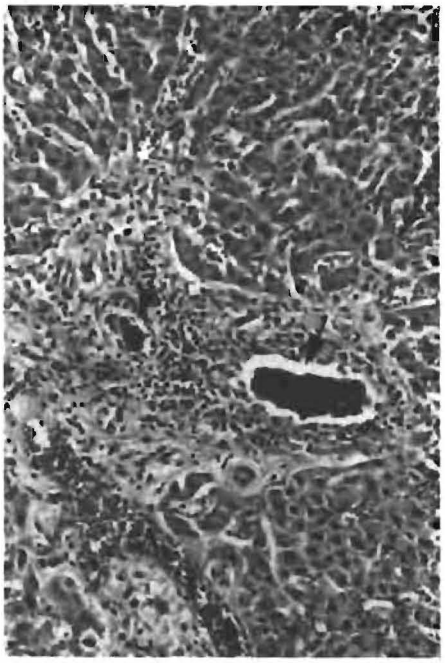

Fig. 3: **Accumulates of bile (arrowed) within the bile ducts in a portal triad. HE X100**

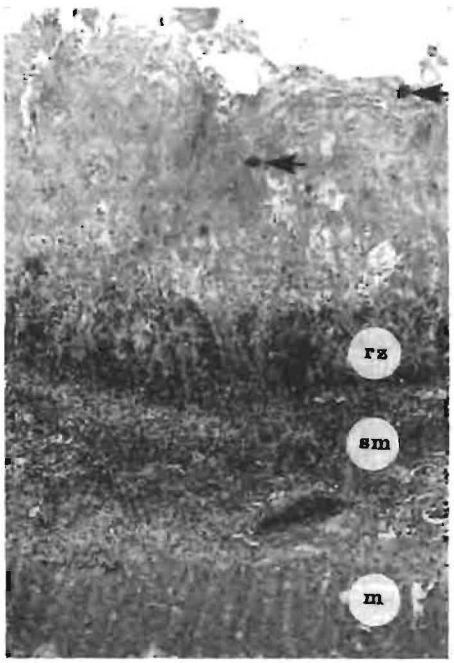

Fig. 4: **Diphtheritic enteritis. Note (1) the necrotic pseudomembrane (top) containing colonies of bacteria (arrowed) separated from the viable tissue by a reaction zone (RZ) and (2) the congestion, oedema and leukocytic infiltration of the submucosa (SM). (M denotes the circular muscle layer). HE X40**

with focal disseminated necrosis. The foci of necrosis were minute (pinpoint in size) and although present throughout the liver, were most readily seen from the parietal surface. Moderate splenic atrophy due to a reduction in both white and red pulp was an added feature.

Specimens of liver, spleen, mesenteric lymph node and ileum were submitted for the isolation of potential pathogens. *Salmonella typhimurium* 1,4,5,12, : i: 1,2 was isolated from the mesenteric lymphnodes and liver.

Specimens of liver, spleen, selected portions of the intestinal tract, kidneys, lungs and heart were collected and preserved in 10% buffered formalin for histopathological evaluation. Sections were routinely prepared and stained with haematoxylin and eosin (HE) and by the Gram (Brown-Hopps modification) method.

Examination of the sections served largely to confirm the macroscopic findings. Foci of coagulative necrosis, usually with a midzonal distribution, were present in the liver (Fig. 1). Gram-negative, rod-shaped bacteria, free and in clumps, were invariably encountered at the periphery of these foci (Fig. 2). Sinusoidal leukocytosis, mild in extent, and cholestatis of the biliary system (Fig. 3) were associated findings in the liver. Lymphocytic depletion and a mild reactive hyperplasia of the mononuclear phagocytic elements characterised the splenic lesion. The pseudomembranous enteritis was classified as diphtheritic with extensive caseous necrosis of the mucosa, leaving only crypt remnants (Fig. 4). Bacteria, free and in colonies, with similar morphological and staining characteristics to those encountered in the liver were scattered throughout the necrotic tissue. The necrotic and viable components were separated by a reaction zone consisting of haemorrhage, thrombosis and leukocytic (predominantly neutrophilic) infiltration. The underlying submucosal and subserosal layers were congested, oedematous and infiltrated by leukocytes. Reactive hyperplasia and oedema dominated the reaction in the lymph nodes and was accompanied by a variable degree of focal disseminated (coagulative) necrosis. No significant findings were evident on examination of the kidneys, lungs and heart.

DISCUSSION

The clinical diagnosis of salmonellosis was underpinned by the hepatic, lymphonodular and, to a lesser extent, by the enteric lesions. Focal disseminated necrosis of the liver and mesenteric lymph nodes (as well as other organs such as the spleen and kidneys) are characteristic findings in bovine salmonellosis[1,5]. However, an unusual feature, in our experience, was the presence of numerous bacteria (presumably *Salmonella* spp. in view of their morphology and staining characteristics) in association with the necrotic foci in the liver. The bronce discolouration of the liver may be ascribed to the associated cholestasis. Although the nature of the enteritis was perhaps not all that unusual, the severity and extent of the lesion was unexpectedly marked. The

diagnosis was confirmed by the positive culture results.

Salmonella typhimurium is an ubiquitous organism with a wide host range. This is in contrast to *S. dublin* which is considered host adapted to the bovine. The spread of these organisms is facilitated by intensive housing techniques which is often applied in dairy herds[4]. Diarrhoea only became a problem in this herd after the introduction of new stock and it only affected the cows that had been on the farm prior to the introduction as well as the newborn calves. It is therefore possible that the specific serotype was introduced into the herd by the cows that were brought in. Adult cattle infected with *S. typhimurium* shed the organism for a limited period only and do not become permanent carriers as with *S. dublin* infection[8 9]. Despite this, and the fact that *S. typhimurium* is an ubiquitous organism, most infections in cattle are derived from other cattle[9]. Other sources of infection that may play a role include environmental contamination, humans, wildlife and animal feedstuffs[9].

Although the infection affects all age groups, it occurs more commonly in calves[2]. Due to the fact that the disease caused by *S. typhimurium* in adult cattle is mainly of the acute type, treatment is in general less successful and the mortality rate higher than in the case of *S. dublin*[9]. The resistance of the serotype isolated in this case, to many of the commonly used antimicrobials, highlighted the importance of this resistance as a contributing factor to unsuccessful treatment.

This case indicates the importance of salmonellosis as a differential diagnosis for diarrhoea in adult cattle.

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Book Review/Boekresensie

PRACTICAL CANINE DERMATOLOGY 3ND EDN
PRACTICAL FELINE DERMATOLOGY 2ND EDN
PRACTICAL EQUINE DERMATOLOGY 2ND EDN

L.J. ACKERMAN

American Veterinary Publications, inc. 5782 Thornwood Drive, Goleta, California 93117. 1989 pp 380, 276, 268, Colour illustrations 48, 24, 18, Price US $ 34.95 each (ISBN 0-939674-23-8, 21-1, 22-x).

These three manuals certainly have a place in the veterinary literature. They should also find a place on the shelf of any general practitioner working with these species. However, one doubts that they will spend much time on the shelf. In the ever-expanding jungle of veterinary dermatology, the practitioner needs a survival manual such as this one.

Here is a highly readable, quick reference, clinically-orientated text which will most effectively serve the undergraduate student and the non-specialist practitioner. Each manual begins with an introduction on basic anatomy and physiology and diagnostic methods. Thereafter each disease is dealt with in a similar manner i.e. a brief discussion on its pathophysiology, a description of clinical signs, diagnostic methods and criteria and of the treatment. The most important up-to date information is supplied. Finally, there is a short list of references listing the most informative articles available in the most accessible veterinary journals for each specific disease.

A chapter each on systemic and topical therapy, gives the most pertinent information on these subjects. Unfortunately, many of the products mentioned, are not available in this country. Appendices listing names and addresses of pharmaceutical companies, diagnostic laboratories and board-certified veterinary dermatologists, will also be of limited use to the local reader. However, the glossary of dermatological terms and the conversion tables will probably be helpful.

A few colour plates at the end of each manual is, to my mind, less than sufficient when dealing with the clinical aspects of a subject such as this. On the other hand, the lack of illustrations makes these books more reasonably priced and therefore more accessible to us.

In order for these books to remain as useful as they are at present, they will have to be frequently updated as the discipline grows.

CLAIRE MARSHALL

CONGENITAL SUPERNUMERARY ECTOPIC LIMBS IN A BRAHMAN-CROSS CALF

SHERYL L FOURIE*

ABSTRACT

Supernumerary ectopic limbs growing from the withers of a calf, were successfully removed by surgical excision. The anatomy of the limbs is described and a possible embryological basis for the abnormal development is suggested.

Key-words: Congenital limb deformity, ectopic limbs, calf.

Fourie S.L. **Congenital supernumerary ectopic limbs in a Brahman-cross calf.** *Journal of the South African Veterinary Association* (1990) 61 No. 2, 68-70 (En.) Department of Surgery, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

Congenital limb abnormalities are commonly encountered in domestic animals[6]. However, there have been relatively few reports[1-4] in the literature of cases of supernumerary limbs or polymelia in cattle.

As 3-week-old, female, Brahman-cross calf was presented with 2 supernumerary thoracic limbs growing from the area between the 2 normal scapulas. There had been no similar limb abnormalities in any of the other cattle in the herd from which the calf originated.

Apart from the extra appendage, the calf was clinically normal. The extra appendage protruded from the dorsal midline between the 2 normal scapulas and hung down on the left side of the calf. From observation and palpation, the appendage appeared to consist of a single, deformed scapula and humerus and it divided, more distally, into 2 complete limbs. The calf was unable to move the extra limbs voluntarily and sensation was absent. The limbs could be manually lifted dorsally or swung cranially or caudally, pivoting at the dorsal attachment of the body (Fig. 1).

Radiographic examination of the area where the limbs were attached to the body, revealed a deformed scapula which cranially consisted of 2 parts and caudally ended in a long cylindrical portion which appeared to be lying next to the dorsal spines of thoracic vertebrae T2-T4. Two dorsal spines were present on the second thoracic vertebra (T2), one of which was paddle-shaped. The second spine was forked, consisting of a short cranial branch and a longer caudal branch which ran parallel to the dorsal spine of the adjacent vertebra (T3) (Fig. 2).

Fig. 1: **The calf with 2 supernumerary limbs**

The supernumerary limbs were removed surgically. The extra limbs originated from the tissue between the *Mm. rhomboidei thoracis* and were not functionally attached to any structure.

The deformed scapula was diamond-shaped. The split distal end formed 2 articulation facets for the 2 humeri. The humero-scapular joint was stabilised by ligamentous structures. The 2 humeri were attached to each other with thick fascia at their proximal and distal ends. The distal end of the humeri were fused to the abnormal olecranon, fixing the elbows in a flexed position. Thick ligamentous structures ran from the distal humeri to the proximal radii in the cranial angles of the elbow joints. The radio-carpal and intercarpal joints were ankylosed. More distally, the limbs were normal. A large artery and vein, equivalent to the A. and V. brachialis in the normal bovine limb were present, supplying the limbs. The artery and the vein divided into 2 branches — each supplying a limb. These vessels ran medially along the radius and then on the palmar surface of the metacarpus where they divided into 2

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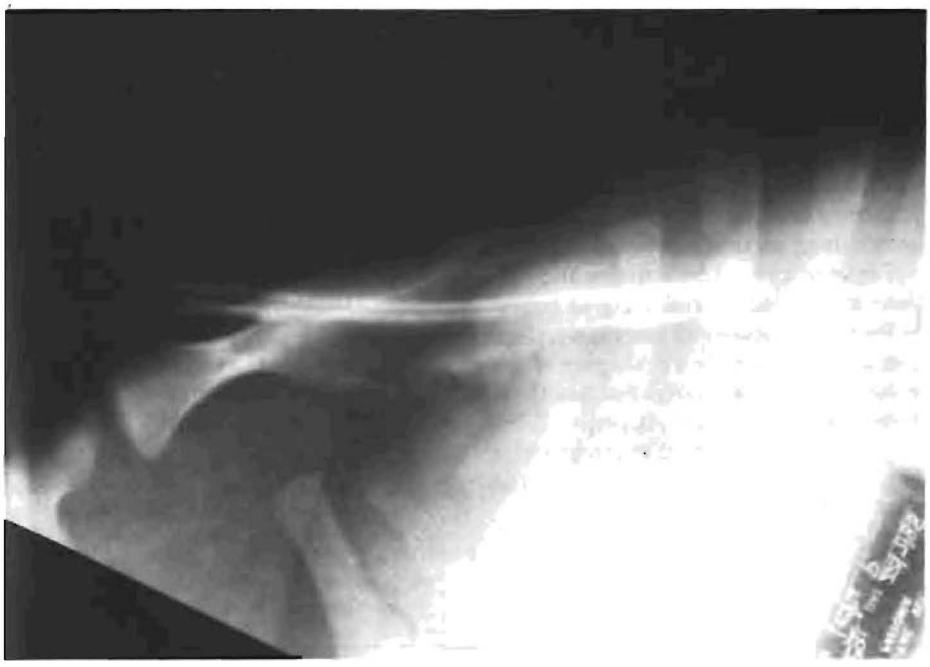

Fig. 2: **Lateral radiograph showing the deformed scapula, the proximal ends of the humeri (far left) and the split dorsal spine of T2**

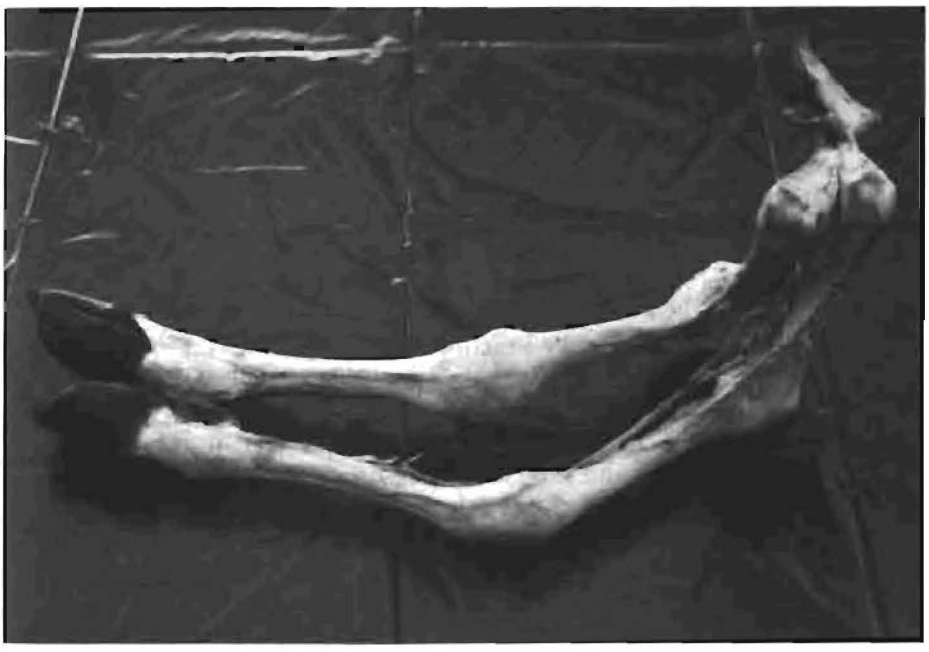

Fig. 3: **The dissected limbs showing blood vessels and bony structures**

branches; hereby supplying each digit. No muscles were present on the limbs except the remnants of the muscles which attached the limbs to the calf's body proximally. The bones were covered in thick fascia. There were very small nerves running parallel with the blood vessels but these appeared to terminate in the fascia. This suggested that the fascia was the remnant of the muscles.

From the carpus, distally, tendons were present on both limbs. The flexor and interosseus tendons were present on the palmar surface. Two extensor tendons were present on the dorsal surface.

The shoulder, elbow and carpal joints had all undergone arthrogryposis. The angles of all joints were filled with large amounts of fat, giving the legs a well-rounded appearance (Fig. 3).

The normal embryological development of the limbs has been described by Latshaw[5], Nodon & De Lahunta[6], and Tuchmann-Du Plessis & Haegal[7]. The critical stage of limb development in the bovine foetus is from Day 24 to 40 of gestation[6]. At this stage, the limb tissues are undergoing early differentiation and are most susceptible to disruption[6]. Any insult at this time of development, results in a structural malformation due to abnormal differentiation[5].

In the present case, there was abnormal embryological development, resulting in the formation of the ectopic limbs. It is suggested that a limb field formed in an atypical site.

In the Wolffian Crest, a lateral ectodermal fold on the embryo, cells caudal to the normal limb bud position continued to develop into an extra limb bud. Possible reasons for this are:

a. The inductive stimulus for growth separated into 2 components and the limb was duplicated or an inductive stimulus arose in an aberrant position and resulted in the formation of an ectopic limb[5].
b. Morphogenic substances may have reached levels above the threshold value needed to stimulate growth in the area of the atypical limb field and stimulated the continued growth of cells in this area of the Wolffian Crest forming an extra limb[5].
c. The original limb-forming area of tissue, may have divided into 2 separate cranial and caudal halves. Owing to embryonic regulation, the ability to form a complete and normal limb was then re-established in both halves and 2 complete limbs were formed[6].
d. The limb bud mesenchyme has the ability to initiate the formation of an apical ectodermal ridge (AER) or thickening of the surface ectoderm in any position. This AER organises limb development as a whole. An extra AER may have been formed, caudal to the normal AER, resulting in the formation of an extra limb[6].

The ectopic limb had one scapula which divided into 2 parts near its distal end. Further distally, the appendage consisted of 2 separate limbs, each with all the bony components. The limb must have been partially developed with the scapula partially formed when an extra AER appeared on the extra limb bud. This extra AER had the ability to reorganize the underlying limb mesenchyme and, as a result of this, the scapula divided into 2 at its distal end and a second set of distal skeletal elements were formed, thus duplicating the distal tissues of the ectopic limb[6].

Nervous innervation of the limbs was very poorly developed. The limbs were also devoid of distinct skeletal muscles and their appropriate nerve supply. This was either due to an early absence of myotome-derived cells or secondary muscle degeneration[6]. If an abnormal

AER was formed in the dorsal midline away from the myotomes, this AER would not have been able to induce muscle and nerve development. The blood vessels were supplied with nerves but these grow in with the vessels. It is therefore suggested that the most likely reason for the development of these supernumerary limbs was the formation of an extra AER caudal to the normal AER.

Secondary muscle degeneration could have been caused by the lack of innervation and movement of the limbs[6]. If nerves innervating the limb muscles fail to develop completely, the muscle cells undergo 'denervation atrophy' or progressive shrinkage[6]. As tendons develop separately to muscles[2], these were present on the limbs.

The joints had undergone arthrogryposis and ankylosis. Arthrogryposis could have been due to the lack of muscle contraction or secondary abnormal joint formation[6]. Owing to sustained immobility of these limbs in utero, modification of the joint capsules, ligaments and articular surfaces occurred, resulting in permanent fixation or ankylosis of some of the joints[6].

Tensions exerted by muscles and tendons are needed for reshaping of bones[3,6]. Because of abnormal muscle development, stress-dependent reshaping of the proximal bones did not occur and these bones had an abnormal shape.

It is unlikely that the condition is hereditary and the initial trigger for the abnormal development is unknown.

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Book Review/Boekresensie

METABOLIC AND NUTRITIONAL DISEASES OF CATTLE

J M PAYNE

1ST Edn. Blackwell Scientific Publications, Oxford OX 20 EL. 1989 149 Pages (ISBN 0-632-01969-7).

This book forms part of the Library of Veterinary Practice series, a series that is designed "to supply up-to-date information relevant to the subject in a concise format so that specific facts can be located quickly and easily."

An introductory chapter is followed by chapters on disorders associated with mineral, water, electrolyte, energy, nitrogen and protein metabolism. There are also specific chapters on urolithiasis, deficiencies of trace elements and vitamins, congenital disorders affecting the metabolic system and disorders associated with toxic factors such as fluorosis and copper poisoning. Finally there is also a short chapter on the various interactions that can affect infertility, the skeletal system and the functioning of the alimentary tract. No references are given.

I found the text easy to read and the contents are presented in a concise and lucid manner. Only the important and relevant facts are discussed which makes this book a good buy for the busy practitioner who wants to keep up-to-date on the subject of metabolic conditions. The book is not an in-depth study of the subject, but it was not the intention of the author that it should be so. It is obvious that the author was an authority in this field. Professor Payne tragically, died suddenly when the manuscript was almost complete. Fortunately his co-worker and wife dr. Sylvia Payne was able to complete the final chapters.

The book is recommended for practising veterinarians, veterinary students and agriculturists as a tool for continuing education in the field of metabolic and nutritional diseases.

G H RAUTENBACH

SYSTEMIC CRYPTOCOCCOSIS IN A CAT

W L BERRY*, I B J VAN RENSBURG** and MARIJKE M HENTON***

ABSTRACT

A three-year-old, castrated, male, domestic cat presented with an antibiotic-resistant rhinitis, generalised lymphadenopathy, and skin nodules distributed over the neck, thorax and abdomen. *Cryptococcus neoformans* was identified on cytology and histopathology specimens, and cultured from all the specimens submitted. The cat died without antimicrobial therapy being instituted. Systemic cryptococcosis was confirmed on necropsy. Lesions were found in the upper and lower respiratory tracts, skin, subcutis, skeletal musculature, lymph nodes, kidney, eye and brain.

This report details a case of systemic cryptococcosis in a cat and gives a review of feline cryptococcosis.

Key words: Cat, *Cryptococcus neoformans*, systemic mycosis

Berry W.L.; Van Rensburg I.B.J.; Henton M.M. **Systemic cryptococcosis in a cat.** *Journal of the South African Veterinary Association* (1990) 61 No. 2, 71-75 (En.) Department of Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INTRODUCTION

Cryptococcosis is an acute, subacute or chronic respiratory, meningeal or systemic mycosis, caused by the saprophytic encapsulated yeast, *Cryptococcus neoformans*[2 9]. Although there are many members of the genus, *C. neoformans* is the only regularly pathogenic species in humans[7 9] and animals[1 4]. Although isolated from many natural sources, it is most frequently found in soils enriched with pigeon excreta[2 9].

The organism has a worldwide distribution[9] and is cited as the most common systemic mycosis of cats[1 13]. Despite this, feline cryptococcosis has not previously been reported in South Africa. Only 2 cases of cryptococcosis have been reported in the Republic of South Africa[5 6].

HISTORY, CLINICAL AND LABORATORY FINDINGS

A three-year-old, neutered, male domestic cat was originally presented with a complaint of submandibular lymph node enlargement of 10 d duration. A lymphadenitis was diagnosed and the cat treated with amoxycillin and clavulanic acid (Synulox, Beecham Pharmaceuticals) for 4 d. Subsequent histopathological examination of a biopsy of a lymph node revealed a granulomatous lymphadenitis, suggestive of an infectious agent. One month later, the cat was returned with respiratory distress and coughing. Thoracic radiographs were normal. The cat was discharged with amoxycillin and clavulanic acid, in addition to bromhexine (Bisolvon, Boehringer Ingelheim). Two and a half months later the cat was returned, suffering from inspiratory dyspnoea, a sero-purulent nasal discharge, generalised lymphadenopathy, and multiple intradermal nodules distributed over the neck, thorax and abdomen. The cat was then referred to the Department of Medicine, Faculty of Veterinary Science, University of Pretoria, for further investigation.

On clinical examination the cat was alert, had a rectal temperature of 39°C, and pulse and respiratory rates of 156 and 40 per min, respectively. Multiple, well-circumscribed, erythematous nodules ranging from 3 to 10 mm in diameter, were present in the skin of the neck, thorax and abdomen. All peripheral lymph nodes as well as the tonsils were enlarged. Enlargement of the retropharyngeal lymph nodes resulted in bulging of the pharyngeal mucosa. A bilateral sero-purulent nasal discharge gave rise to an inspiratory dyspnoea, with increased breath sounds audible over the lung fields. The kidneys were easily palpated and both had an irregular surface.

Cytological examinations of stained preparations (Romanowski stain, Cam's Quick-Stain, C.A. Milsch; supra-vital staining, New Methylene Blue) of fine needle aspirates of the lymph nodes, skin nodules, lungs, trans-tracheal aspirate and the nasal flush, revealed the presence of yeast-like organisms. An India ink preparation revealed the presence of a thick halo, similar to that of *Cryptococcus sp.*

A significant urinalysis finding was a specific gravity of 1,021. The sediment was acellular and contained no yeast cells.

Biopsies from the mandibular and cranial cervical lymph nodes, and skin nodules were submitted for histopathology and fungal culture.

The results of blood chemical and haematological investigations are presented in Table 1. An ELISA test (Leukassay, Pittmann Moore) for FeLV was negative. Concomitant corona virus (feline infectious peritonitis) infection was excluded on the basis of a low antibody titre (<80) using an immunofluorescence test (Department of Infectious Diseases, Faculty of Veterinary Science, University of Pretoria). Interstitial and peribronchial infiltration, especially of the ventral parts of the lung lobes, was identified on examination of the thoracic radiographs.

The anterior lobes showed evidence of consolidation, and the presence of air bronchograms.

The cat became depressed and severely dyspnoeic on Day 5 after admittance, and died 12 h later.

BIOPSY FINDINGS

The biopsy specimens (skin and lymph node) were routinely prepared and stained with haematoxylin and eosin (HE). Examination of the skin sections re-

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Table 1; **Pertinent laboratory results obtained on examination of blood, serum and urine from a cat with systemic cryptococcosis**

Laboratory test	Salient Features
Haematology	White cell count $16,5x10^9$ ℓ^{-1} (7-20) Neutrophils (mature) 9,07 (2,5-12,5) Neutrophils (immature) 2,64 (0-0,3) Lymphocytes 2,97 (1,5-7,0) Monocytes 0,33 (0,085) Eosinophils 1,32 (0-1,5) Basophils 0,16 (rare)
Chemical pathology	Total serum proteins 90,1 g ℓ^{-1} (54-72) Globulin 59,8 g ℓ^{-1} (17-48) Urea 12,6 mmol ℓ^{-1} (7,1-10,7) Creatinine 171,1 μmol ℓ^{-1} (141)
Protein electrophoresis	Gamma globulins 30 g ℓ^{-1} (7-24)
Urinalysis	Specific gravity 1,021

*Normal parameters in brackets

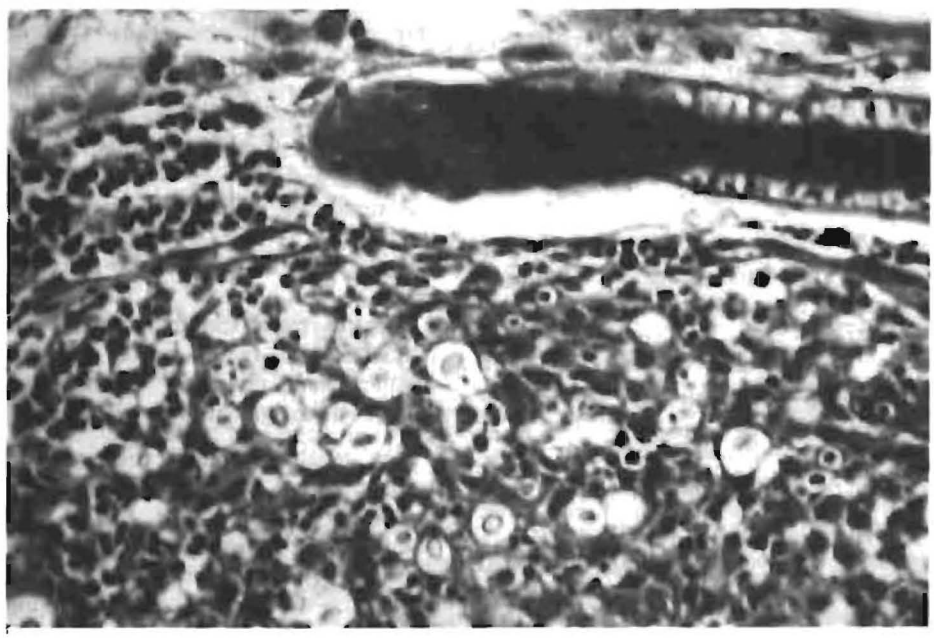

Fig. 1: **Cryptococcal granuloma in the dermis. HE x 400**

vealed masses of cryptococcal organisms in dense clusters, mostly at the level of the hair follicles and extending into the dermis and subcutis. A moderate cell infiltration of neutrophils, eosinophils, lymphocytes, plasma cells and macrophages accompanied the dense clusters of organisms (Fig. 1). The organisms showed typical morphological features of *Cryptococcus sp.*, particularly when stained with periodic acid schiff (PAS), Gomori's methenamine silver (GMS) and a superimposed GMS-HE combination.

Examination of lymph node sections revealed sub-capsular and medullary sinuses filled with cryptococcal yeasts, and lymphoid tissues reduced to small strands of round cells comprising mostly plasma cells. No lymphoid follicles or paracortical areas were discernible (Fig. 2 & 3).

Hyphae were not detected in any of the sections examined.

A diagnosis of systemic cryptoccccosis with extensive involvement of the skin and lymph nodes, was confirmed.

MICROBIOLOGY

Skin, lymph node biopsies, and a sample of the trans-tracheal aspirate fluid, were cultured on bovine blood tryptose agar (Biolab), Sabourauds agar, Sabourauds agar containing chloramphenicol and cycloheximide, potato agar, and trypan blue agar. All were incubated at 25°C, except the blood tryptose agar which was incubated at 37°C. After 48 h a pure heavy growth of a yeast occurred on all culture media, except the Sabourauds agar containing chloramphenicol and cycloheximide. The colonies were white and dry initially, and the colour deepened through cream to a tan colour when aged. The growth appeared dark blue on the trypan blue medium. India ink preparations revealed a yeast resembling *Cryptococcus,* but with thin capsules.

Culture tests on niger seed agar *(Guizotia abyssinica),* for urease production, sugar fermentation and assimilation (API 20c, Auxanogramme strip, Ayerst Lab.) confirmed the identity of the isolate to be *Cryptococcus neoformans.* This was supported by an independent laboratory (The South African Institute for Medical Research, Hospital Street, Johannesburg, Republic of South Africa.)

The isolate was sensitive, in vitro, to nystatin and miconazole, with partial sensitivity to ketoconazole, clotrimizole, and amphotericin B. It was resistant to flucytosine.

NECROPSY FINDINGS

The carcass was in a good condition. Multiple granulomas were present in the skin and subcutaneous tissue, and were cream coloured and waxy in appearance. The size ranged from 2 to 10 mm in diameter. Many larger round to oval granulomas (up to 20 mm in diameter), of similiar appearance to those in the skin and subcutis, were present in the skeletal muscles (Fig. 4). On cut surface, the granulomas were solid and without signs of necrosis, liquefaction or pus formation. All superficial lymph nodes were markedly enlarged and waxy in appearance. Some mesenteric lymph nodes were similarly affected, but the bronchial and mediastinal lymph nodes were normal.

The kidneys were extensively affected with large, pale, granulomatous, proliferative tissue replacing most of the cortices, similar to extensive renal lymphosarcoma. A mucopurulent rhinitis, sinusitis, and tracheitis were observed. The lungs showed fairly diffuse alveolar emphysema, areas of atelectasis and small granulomatous lesions. Keratitis and a moderate periocular myositis were present in the left eye. The liver showed moderate centrilobular degeneration..

MICROSCOPIC FINDINGS

Microscopy of skin and lymph node sec-

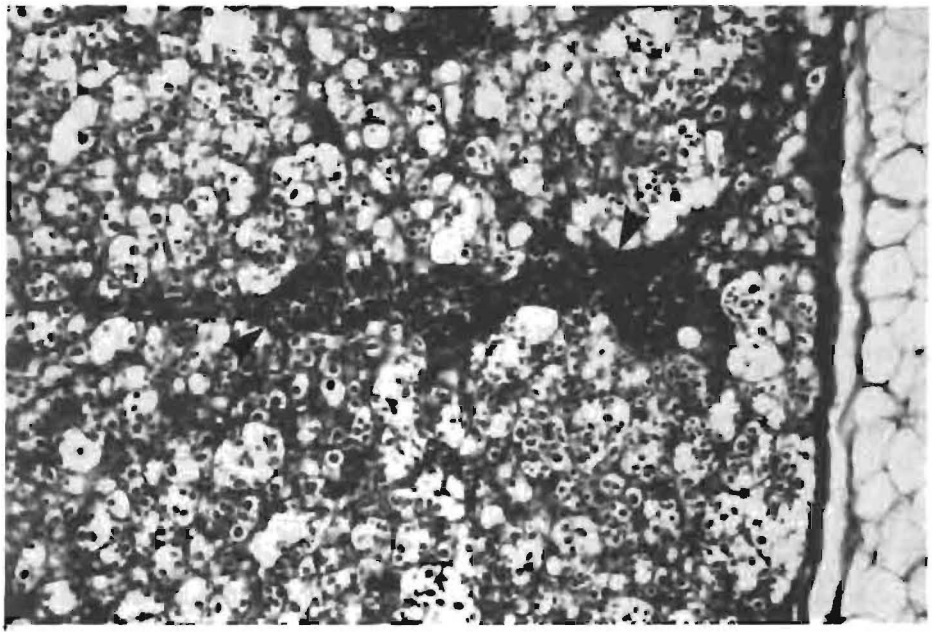

Fig. 2: **Peripheral lymph node, showing massive infiltration of cryptococcal yeasts with almost total obliteration of the lymphoid elements. (arrows) HE & GMS x 100**

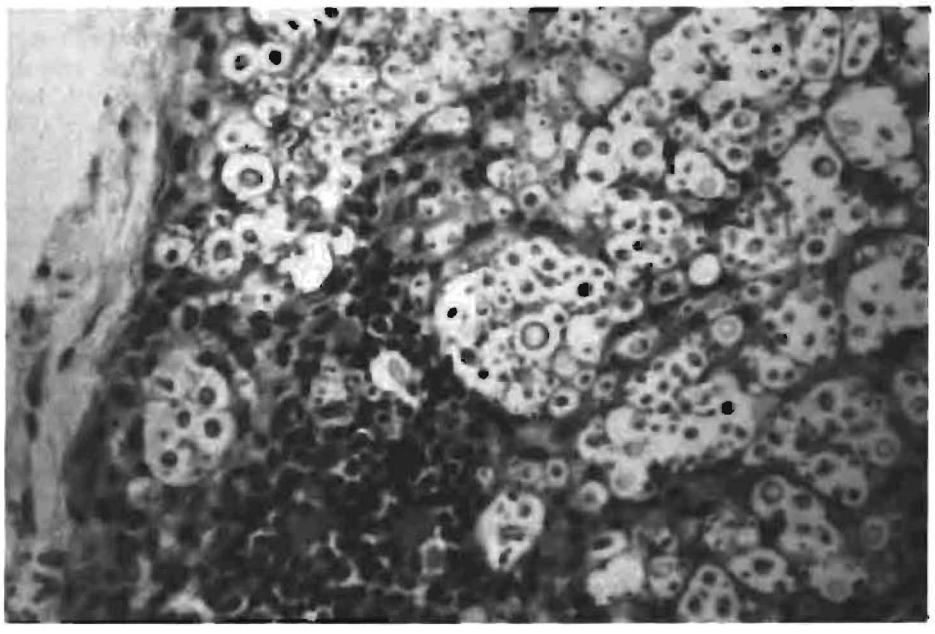

Fig. 3: ***Cryptococcus neoformans* in lymph node sinuses, mixed with a few remaining lymphocytes. HE x 400**

tions was the same as that described in the biopsy sections. In the skeletal muscles, the cryptococcal colonies were divided into compartments by delicate connective tissue stroma, with scant inflammatory cell infiltration (Fig. 5).. The meninges were similarly affected, the mass of organisms following the normal contours of the cerebrum (Fig. 6). No invasion of nervous tissue had taken place. A small focus of mycotic retinitis and periocular myositis was present in the left eye.

The kidneys were extensively infiltrated by masses of yeasts involving the perirenal tissue, renal capsule and renal cortices. Large mycotic granulomas were present with conspicuous infiltrations of lymphocytes, plasma cells, and few neutrophils and macrophages (Fig. 7). The tubular epithelium showed granular degeneration and fatty change.

A purulent bronchitis with lymphocytic peribronchial inflammatory infiltrate was present. Partial atelectasis of the lungs was present and many alveoli were filled with macrophages. Crytococcal organisms appeared to be scarce in the lung sections. The cryptococcal organisms seen in the lung sections were not related to areas of intense cell reaction.

DISCUSSION

Cryptococcus neoformans infection may involve many organ systems, the clinical signs being determined by the organs involved. Most commonly the upper respiratory tract, central nervous system (CNS), skin and eyes are affected[1 2 8]. This cat had involvement of all of these systems, including the lower respiratory tract, kidneys, lymph nodes and skeletal muscles.

Although the route of infection is still unknown, some authors believe that the respiratory tract is the main portal of entry, with haematogenous or lymphogenous spread[1 9]. Others have argued that the diameter of cryptococcal organisms (4-20 μm) prevents deep lung deposition, as only particles smaller than 2 μm in diameter can reach the terminal airways and alveoli[24]. Neilson et al.[17] demonstrated that viable *C. neoformans* smaller than 2 μm in diameter can exist in soil, which, if inhaled, would then be capable of deep lung deposition. Although the case presented here did have lung involvement, few organisms were found in the alveolar tissue during microscopic examination, even though organisms were found with relative ease in fine needle lung aspirates. When the history, clinical signs at initial presentation, and the necropsy findings are considered, it is conceivable that the primary site of infection was the upper respiratory tract.

Dissemination from the primary site of infection to the central nervous system (CNS), skin and eyes occurs frequently[1 13 24]. Although this cat did not display overt signs of CNS involvement, a meningitis was found on histopathological examination. Cutaneous lesions occur most frequently on the head[13 24]. A non-painful peripheral lymphadenosis is usually associated with skin lesions. Ocular lesions may exist without the animal displaying a visual deficit[13]. Although an opthalmoscopic examination was not performed in this case, histopathological examination of the left eye revealed a focal retinitis.

Despite extensive involvement of the skeletal muscles, lameness and pain on palpation were not clinically detectable. Cryptococcal myositis appears to be a less common manifestation of the disease[1 24].

A diagnosis of cryptococcosis may be made relatively easily by identification of the organism on cytological examination of fine needle aspirates or nasal swabs. An India ink preparation is very useful to demonstrate the round to oval cells, each surrounded by a typical, clear

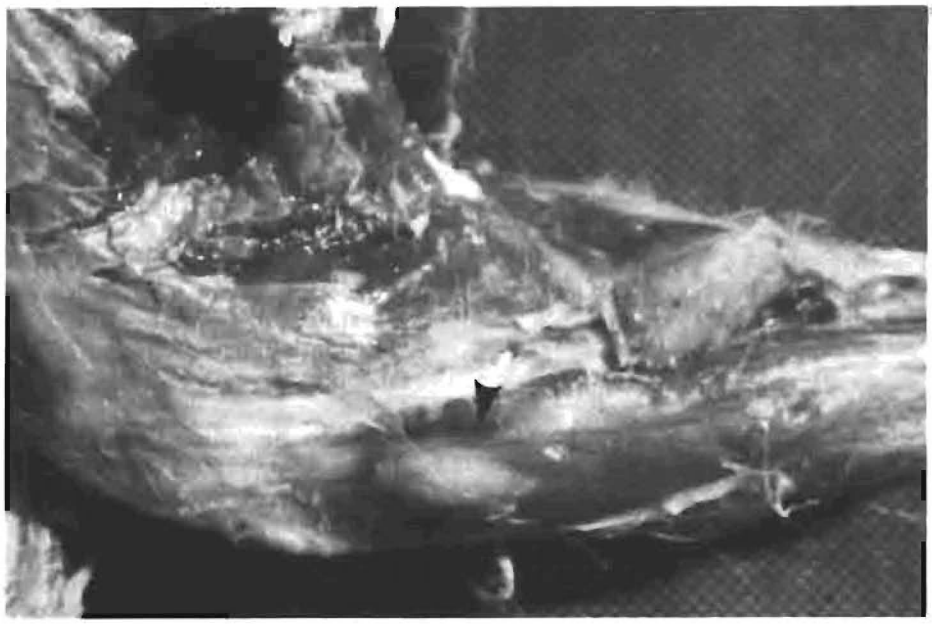

Fig. 4: **Cryptococcal granuloma (arrow) in the muscles of the forelimb**

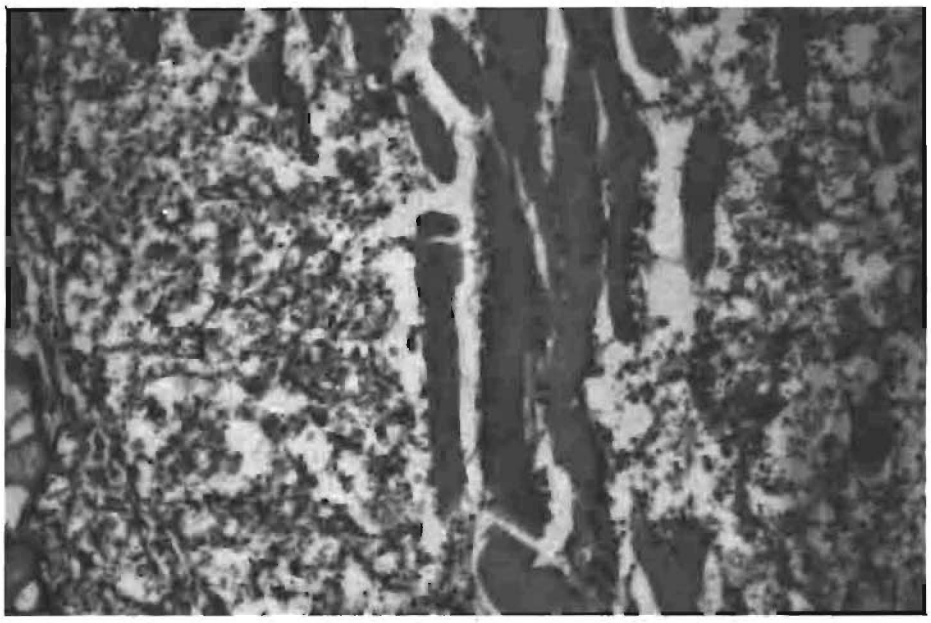

Fig. 5: **Cryptococcal myositis, showing extensive muscle involvement, the presence of many yeasts and a small number of round cells. HE x 150**

halo corresponding to the thick mucinous capsule[9 14]. Serologic tests are available as a diagnostic aid and have proved valuable in monitoring the response to therapy[12 13 18 19 22]. The agglutination test for antibodies does not allow for differentiation between active and previous subclinical disease, and as antibodies may be bound to cryptococcal capsular polysaccharide, a negative antibody titre may result[13 19]. The latex cryptococcal agglutination test (LCAT), which detect soluble cryptococcal polysaccharide antigen, is reported to be more reliable and sensitive. As the titre is proportional to the antigenic load, an index of the severity of infection may be ascertained[13].

Definitive diagnosis of cryptococcosis rests with culture and positive identification of the organism. An interesting feature in this case, was the typical appearance of the thick capsule in tissue specimens, yet after culture, the capsule appeared thinner and smaller in diameter. Variations in capsule diameter and thickness may occur[9], and when grown on artificial media, the capsule may be thin or absent[2]. However, when in tissues, the organism always produces a capsule[2], which may be thin in some strains of *C. neoformans*[2].

Immuno-suppression is believed to be an important predisposing factor, particularly in man[9 10]. Although feline leukaemia virus (FeLV)-positive cats have been reported with cryptococcosis[1 15 26], 2 of which were successfully treated[1 15], it is difficult to draw valid conclusions on the relationship between FeLV and cryptococcosis[13]. Cell-mediated immunity is of greater importance than antibody production in preventing cryptococcosis[11]. It is therefore conceivable that FeLV may make cats more susceptible to cryptococcosis[13], and the same may be true for feline immunodeficiency virus (FIV). However, in a review of immunity in cryptococcosis, Fromtling & Shadomy[10] suggest that immunity to the organism may depend upon a complex interaction of both humoral and cellular immune factors. This cat was ELISA negative for FeLV, had a low FIP titre, and exhibited a polyclonal gammopathy which was interpreted as an indication of immune-stimulation. Polyclonal gammopathies are reported in other cases of feline cryptococcosis[18 19], and in this case is consistent with the lymph nodes populated almost entirely by plasma cells (Fig. 2 & 3).

The tissue reaction to *C. neoformans* is very variable, and may range from little or no reaction to a purely granulomatous reaction[4]. The paucity of both cell-mediated and humoral reactions evident in tissues, is thought to be due to the relative non-antigenicity of the capsular polysaccharide[14]. It is reported that poorly-encapsulated intracellular cryptococcal organisms produce a marked granulomatous inflammatory reaction, unlike well-encapsulated yeasts where little or no reaction occurs[4 10]. In the case under discussion, the tissue reaction was variable. Minimum reaction in the lymph nodes and muscles extended to a marked granulomatous reaction in the dermis, renal and perirenal tissues. Where little response occurs, the yeast cells multiply profusely, displacing normal tissue components and distorting the architecture[4 13]. This was clearly evident in the lymph node and muscle sections.

Feline cryptococcosis is usually associated with a poor prognosis, particularly in debilitated animals with widespread disease, or those with CNS involvement[13]. Untreated cases of clinical infection are fatal, as borne out by this case. Although an immuno-compromised patient is at greatest risk, a cat positive for FeLV does not necessarily imply a hopeless prognosis. FeLV-positive cats have been successfully treated while suffering from localised nasal cryptococcosis[1 15].

As treatment for cryptococcosis is protracted and not always successful, it is advisable to test the sensitivity to various antifungal agents. Amphotericin B has in the past been the treatment of

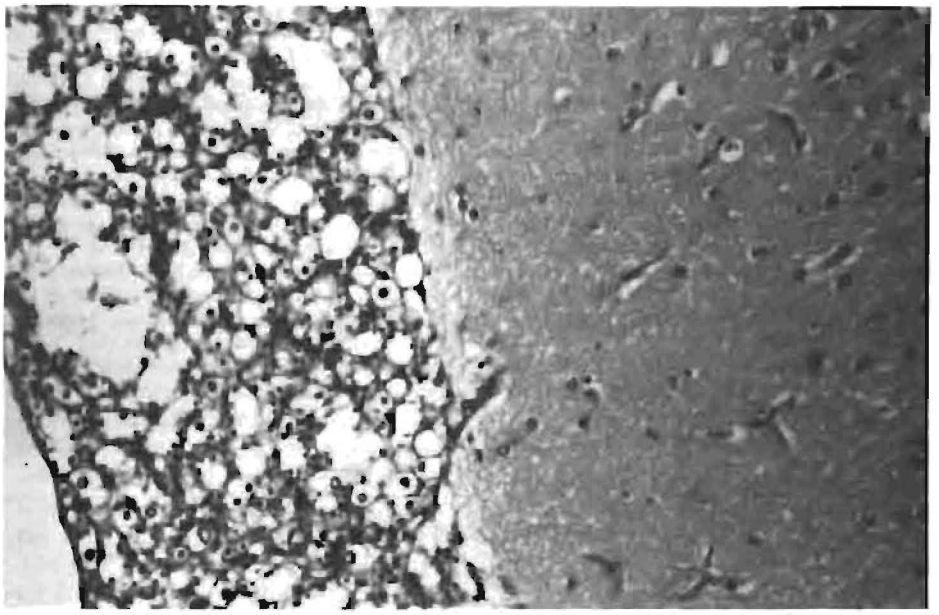

Fig. 6: **Cryptococcal meningitis. HE x 200**

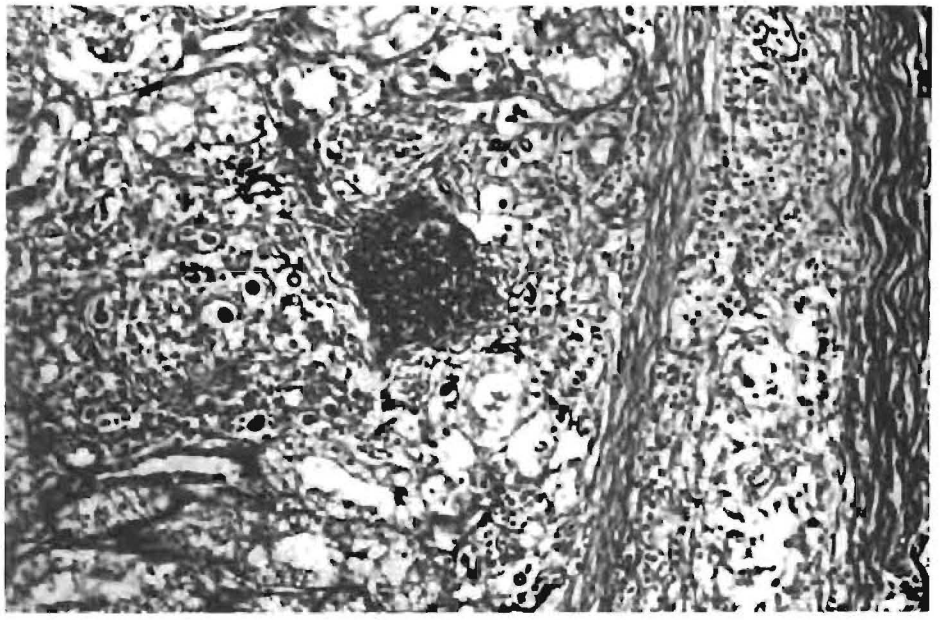

Fig. 7: **Kidney, showing intracapsular and interstitial proliferation of yeasts and round cell infiltration. HE & GMS x 400**

choice[2]. Alternative treatments have been investigated in an attempt to avoid the nephrotoxic side-effects of amphotericin B, and its failure to adequately penetrate the cerebrospinal fluid[13]. In humans, the treatment of choice is a combined amphotericin B and 5-fluorocytosine (5-FC) regimen[3]. The reported benefits are: a reduced dose and shorter duration of therapy than with amphotericin B alone, the prevention of fungal resistance to 5-FC, more and faster cure rates, a more rapid sterilisation of cerebrospinal fluid, and fewer unsuccessful treatments and relapses than with the use of amphotericin B alone[3]. This combination therapy has been reported as successful in 3 cases of feline nasal cryptococcosis[1 20 23].

The successful use of ketoconazole has been reported in the treatment of localised nasal and dermal cryptococcosis[12 13 18 19 21].

5-Fluorocytosine has also been used alone, but with variable success. The major disadvantage is the development of resistance by initially susceptible organisms[1 13]. Although Holzworth et al[13] reported limited success with 5-FC alone, there are reports of successful 5-FC therapy in cases of feline cryptococcosis involving the nasal passages and skin[16 25].

Recently, Shaw[22] reported the successful treatment of 10 cats with combined 5-FC and ketoconazole therapy, the combination allowing reduction in the dosage of both drugs, with minor side effects.

Autogenous vaccines have been utilised as adjunctive therapy in some cases of feline cryptococcosis[25]. Owing to the weak immunogenicity and antiphagocytic characteristics of *Cryptococcus neoformans*[10], some doubt exist as to the efficacy of autogenous vaccines.

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A REVIEW OF ENERGY METABOLISM IN PRODUCING RUMINANTS

2. Control of nutrient partitioning

J G VAN DER WALT* and MARGARET J LININGTON+

ABSTRACT:

Insulin is the primary short-term hormonal regulator of metabolism in the resting ruminant. The concentration of plasma insulin is positively correlated with energy intake. Diets producing hyperinsulinaemia, direct the balance towards body gain (anabolic). However, in lactating animals, the postprandial rise in insulin is reduced, thereby favouring movement of nutrients to the mammary gland and promoting gluconeogenesis. Similar mechanisms balance the demands of foetal and maternal growth. Glucagon, on the other hand, stimulates both glycogenolysis and gluconeogenesis in the liver from glucogenic amino acids, thereby indirectly diminishing protein synthesis in muscle.

Homeorhetic hormones from both the pituitary and reproductive glands, play a major role in the long-term control of nutrient partitioning. Oestrogens appear to affect feed intake, promote RNA and protein synthesis and inhibit gluconeogenesis in the liver, thereby promoting the metabolic adaptations necessary for pregnancy. Progesterone, on the other hand, appears to block the action of the oestrogens at cellular level, and may actually increase feed intake. The pituitary hormones, prolactin and somatotropin, bring about significant improvements in production, especially in milk yield. The action of the somatomedins appears to be responsible for the paradoxical spectrum of effects attributed to somatotropin.

Key words: Ruminant, hormone, homeostasis, homeorhesis, energy, metabolism

Van der Walt, J.G.; Linington, M.J. **A review of energy metabolism in producing ruminants. 2. Control of nutrient partitioning.** *Journal of the South African Veterinary Association* (1990) 61 No. 2, 77-79 Department of Physiology, Faculty of Veterinary Science, University of Pretoria, 0110 Onderstepoort, Republic of South Africa.

INTRODUCTION

The influx of nutrients into the metabolism of the ruminant is partitioned between catabolic and anabolic processes. This partitioning may be influenced by dietary composition[53] and by hormones, acting both in the short- or long-term, i.e. homeostatic or homeorhetic. In this review, the effects of both types of hormone on the metabolism of the productive ruminant are examined.

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HOMEOSTATIC CONTROL

The endocrine glands having major effects on minute-to-minute control of energy metabolism, are the pancreas and the adrenal glands which produce, inter alia, insulin, glucagon, somatostatin, glucocorticoids and catecholamines[8 10 15]. Since glucose occupies a central role in energy metabolism, the control of gluconeogenesis, glycogenesis and glycolysis may be used to illustrate the interaction of these hormones.

Pancreatic hormones: The regulation of insulin and glucagon secretion in ruminants, differs from that in monogastric mammals. Supraphysiological concentrations of volatile fatty acids (VFA) are more potent stimulators of insulin and glucagon release than glucose[34 42]. Normal physiological concentrations of propionate and butyrate may also influence insulin and glucagon release[2 44], as may the autonomic nervous system[3 52]. Vagal stimulation increases, while adrenalin and sympathetic nervous system stimulation depresses insulin and glucagon release[6 13]. Thus insulin secretion appears to be regulated by nutrient intake and is influenced by innervation and adrenal catecholamine secretion, while no clear correlation exists between glucagon secretion and nutrient intake[5].

Ruminants are generally believed to be more resistant to insulin than non-ruminants, since a plasma concentration of 50-60 mU ℓ^{-1} of insulin is required to suppress the hepatic output of glucose in sheep[7 55 56] (due to the inhibition of both glycogenolysis and gluconeogenesis[18 21]) compared to 30 mU ℓ^{-1} in humans[48]. Insulin enhances uptake of glucose by muscle and adipose tissue[31 38], but not by the mammary gland or uteroplacental complex[31 39], in both of which uptake is dependent on the glucose concentration gradient. In ruminants, insulin seems to increase protein synthesis and decrease proteolysis in muscle tissue[12 35], and to stimulate fat synthesis[58]. Although the mechanism is not clear, it probably involves increasing the uptake of glucose by adipose tissue[38]. The inhibition of lipolysis by insulin (and glucose) is diminished during late pregnancy[31] and lactation[39], thereby ensuring an adequate supply of acetate and FFA for milk fat synthesis. The exact mechanism by which the effect of insulin on adipose is reduced, is not known. Insulin also suppresses hepatic production of ketone bodies[14], independently of FFA concentration in the blood, while facilitating peripheral utilisation of ketone bodies.

The overall effect of insulin is, therefore, anabolic, and it achieves this, by directing the movement of nutrients

into muscle and adipose tissue, by promoting the entry of glucose into tissues where it can be oxidised or stored and by partitioning glucogenic nutrients to muscle instead of liver, thereby reducing gluconeogenesis[15].

Glucagon stimulates both glycogenolysis and gluconeogenesis by increasing the hepatic extraction of alanine, glutamine, serine, threonine and lactate[17 25].

However, propionate conversion to glucose is not affected by glucagon[19], which acts on pyruvate carboxylase, an enzyme not involved in propionate metabolism. The effect of glucagon on protein metabolism appears to be indirect, by promoting the uptake of amino acids by the liver for gluconeogenesis and thereby diverting them from muscle protein synthesis[17]. Although glucagon has been implicated in the control of lipid metabolism and ketogenesis[4 9 14], the actual role played is not certain at this stage, and awaits further investigation.

Adrenal hormones: While glucocorticoids and catecholamines are not essential components of the hormonal control of the metabolism of resting, well-fed ruminants, lack of these hormones may impair the ability of ruminants to respond to stress[52]. Increased glucocorticoid concentration in blood causes an initial reduction in hepatic glucose production and a decrease in peripheral uptake[46], followed by an increase in gluconeogenesis about 24 h later[47]. Protein synthesis is reduced and catabolism increased by elevated cortisol levels[47]. Thus, the glucocorticoids seem to reduce the overall response of metabolism to insulin[16]. The importance of the catecholamines is to provide for immediate supplies of glucose and lipid fuels in times of stress, hypoglycaemia, and during exercise[16]. While adrenalin is a potent hyperglycaemic agent, noradrenalin is only 20% as effective[3 58]. Both lipolysis and glycogenolysis (in both liver and muscle) are increased[52], thereby providing extra suppliers of glycerol and lactate for hepatic gluconeogenesis[3].

HOMEORHETIC CONTROL

The hormones that are implicated in the long-term control of metabolism may be placed into 2 categories, those produced by the reproductive organs, and those produced by the pituitary gland in response to large-scale changes in the life cycle. While not much is known about the mechanism of action of any of these hormones at the cellular level, considerable information exists about their integrated effect on feed intake, growth, pregnancy and lactation.

Reproductive hormones: Oestrogens have a biphasic effect on feed intake[22], exogenous dosages below 40 ug d^{-1} cause feed intake to increase, while higher dosages lead to a decline in intake[26 27]. At the tissue level, oestrogen promotes RNA and protein synthesis in the liver, which lead to a promotion of glycogen synthesis and an inhibition of gluconeogenesis[43]. Furthermore, insulin secretion by the pancreas is enhanced, while lipoprotein lipase in adipose tissue is inhibited[29]. The net effect of these changes is to increase the level of circulating triglycerides[50]. Although progesterone may stimulate feed intake in cycling rats, the effect in ruminants is not as clear. Progesterone appears to modify the effect of the oestrogens in ruminants, by directly blocking the action at cellular level[27].

Pituitary hormones: Two hormones that probably play the central role in regulating the overall long-term control of metabolism are somatotropin (or growth hormone) and prolactin[37]. Both are large polypeptide hormones, produced and released in irregular, episodic bursts by the adenohypophysis[6 30 36]. Due to their effect on nutrient partitioning, both hormones may bring about a considerable improvement in production[40 41]. For example, direct administration of exogenous somatotropin to growing calves may increase growth by about 10%[20], although removal of the inhibitory factor somatostatin by autoimmunisation, may increase growth rate by up to 15%[51].

The exact mechanism by which somatotropin causes these effects on production, is not known[11]. However, some of the intermediate steps have been elucidated. Lipid reserves are preferentially mobilised when nutrients are in short supply[54]. In the case of growth rate, the major component of the increase in growth is an increase in nitrogen retention[28], which is due to an increased uptake of protein and not due to a decrease in breakdown[24 25].

Comparatively little data has been obtained on the effect of prolactin on the partitioning of nutrients. The effect of prolactin in ruminants appears to differ considerably from that in all other mammals. Although lactation may be totally inhibited in non-ruminants by blocking the action of prolactin, the course of lactation in cows is unaffected by such a blockade[1 33]. While exogenous ovine prolactin in non-ruminants promotes growth and nitrogen retention, its effect on ruminants remains uncertain[23].

Treatment with exogenous prolactin causes gut hypertrophy, affects lipid metabolism by inhibiting lipogenesis and promoting lipolysis[57], releases somatomedin C from the liver[32] and assists in the absorption of calcium from the gut lumen[49]. Nothing is known about the mechanism of action at cellular level.

While it is self-evident that the metabolism of the animal is considerably influenced by these, and, possibly, other similar hormones, the mechanisms by which these hormones exert their effects, are still largely unknown. However, considerable progress is currently being made in the application of these hormones and their synthetic analogues in promoting animal production.

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THE VETERINARY PROFESSION IN SOUTH AFRICA: 5. — THE COAT OF ARMS AND MOTTO OF THE S.A.V.M.A.†

By Dr. H.H. CURSON, Onderstepoort

The design appearing below was approved as the Coat-of-Arms of the South African Veterinary Medical Association at the General Meeting held at Onderstepoort on the 26th of September 1932. It was drawn up as a result of the labours of a sub-committee (consisting of Mr. C.G. Walker, Artist; Dr. P J. du Toit, Dr. A.D. Thomas, Mr. C. Jackson, B.Sc. BVSc; and Dr. H.H. Curson, F.R.C.V.S.), appointed 24th October 1931. It was agreed to leave the choice of a motto to the members of the Association.

It was sought to indicate the following features: (a) The triple sources of the veterinary profession: (b) the fact that the Association is South African; (c) the relationship of the local Faculty of Veterinary Science to the University of Pretoria; (d) the incorporation as charges of the crests of the Royal College of Veterinary Surgeons and the University of Pretoria; and (e) the colour associated with the veterinary profession, namely maroon (dark cherry).

On examining the design it will be seen that the shield, similar to that of the University of Pretoria, has been divided into three fields**. These fields are gold red and green as occuring in the arms of the Union of South Africa, and the area of division is maroon, the veterinary colour adopted in South Africa as far back as 1899 when the Natal Volunteer Veterinary Corps was created.

As typical of South Africa is the head of an Afrikander bull, the colour being dark cherry. The crests of the University of Pretoria (established 1930) and the Royal College of Veterinary Surgeons (instituted 1844) are shown in silver and black respectively. These three charges occupy the three fields of the shield. The serpent and arrow occuring on the upper limb of the partition indicate the medical nature of the profession

The letters on the upper scroll are S.A.V.M.A. and stand for the name of the Association with its Afrikaans equivalent.

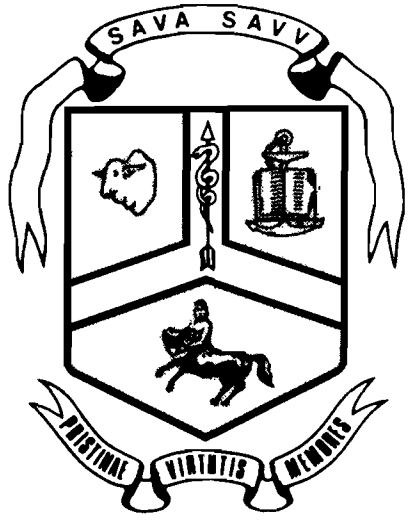
MOTTO(⁰)

The lower scroll contains the motto *Pristinae virtutis memores,* which was chosen by ballot and had the approval of 88 members out of a total of 96 who voted.

The motto is that of the 8th (King's Royal Irish) Hussars which was the first unit in this country to have a veterinarian. This veterinarian, Thomas Burrows (or Burrowes) was indeed the first veterinary surgeon in South Africa. (Burrowes graduated (30/3/1799) at the Royal Veterinary College Londen).

By adopting this motto (to which heraldically there is no objection) not only is this historical association maintained, but at the same time the memory of pioneers such as Wiltshire, Hutcheon, Lambert, Duck, Rickmann, Watkins-Pitchford, Theiler and others is honoured. The motto will recall pride for the past, faith in the present, and hope that our successors may prove worthy of the traditions handed on to them.

As a profession we have not asserted ourselves sufficiently and it is clear that apart from that of the mining engineers, no other profession has done so much for South Africa.



†Journal of the South African Veterinary Medical Association (1933) Vol. 4: 107-110

**Notice of the registration of the colours as is used today appeared in Government Notice 1665, Government Gazette 1268, 29 October 1965

⁰It is interesting to note that the motto is a variation of the well-known saying of Paul Kruger, viz — "Zoekt in't verlede al't goede en schone...vormt daarna uw toekomst" (Search the past for all that is good and beautiful...then build your future)

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