# A COMPARATIVE HISTOLOGICAL STUDY OF THE NUMBER AND SIZE OF THE MYENTERIC GANGLIA AND NEURONES IN THE FORE-STOMACH AND ABOMASUM OF GREY, WHITE AND BLACK KARAKUL LAMBS

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## **ABSTRACT**

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Homozygous grey Karakul lambs are born with a lethal genetic factor responsible for death at weaning age. When put on a high roughage diet under field conditions they develop distended, thinwalled rumens and sand impacted abomasa. Homozygous white Karakul lambs have a similar factor but survive for a longer period. Black Karakul lambs are not affected. The present study was undertaken to compare by image analysis the number and size of the myenteric ganglia, and the number of myenteric neurones in the walls of the fore-stomach and abomasum of 24-h-old grey, white and black Karakul lambs. One square centimetre samples were taken from analogous areas of the rumen, reticulum, omasum and abomasum of 38 embalmed Karakul lambs. Haematoxylin and eosin stained histological sections of each sample were studied with a Vids 2 Image Analyzer. One way analysis of variance indicated a significant difference between the groups regarding the number and size of the myenteric ganglia and in the number of myenteric neurones in the reticulum, rumeri and abomasum. The number and size of the ganglia and the number of neurones was greatest in the black lambs and decreased progressively in the white and grey lambs. The omasum was not affected. It is suggested that the paucity of myenteric ganglia and neurones in the regions examined is instrumental in causing the lethal condition described above.

### INTRODUCTION

Homozygous grey Karakul lambs are born with a lethal genetic factor responsible for death at weaning age (Nel & Louw, 1953). When put on a high roughage diet under field conditions they develop distended thin-walled rumens and sand impacted abomasa (Nel, 1965). These lambs have a potbelly appearance, become emaciated and die. Homozygous white Karakul lambs are born with the same genetic factor and show the same clinical signs as the homozygous grey lambs, but they survive for a longer period. Black Karakul lambs are unaffected. Homozygous grey and white Karakul lambs can be identified at birth by the lack of pigmentation of the tongue, palate and ears (Nel & Louw, 1953).

Previous studies in 24-h-old grey, white and black Karakul lambs showed a significant difference (P<0,05) in the length and width of the rumen, and in the volume of the rumino-reticulum, between the three groups (Groenewald & Booth, 1989). The grey lambs displayed the largest rumen and the black lambs the smallest. In the homozygous grey and white lambs the distended rumens were filled with milk (Groenewald & Booth, 1989). Studies on the dimensions of the tunica muscularis showed a significant decrease in thickness in the rumen, reticulum and abomasum in homozygous grey and white lambs when compared to normal black lambs (Groenewald & Booth, 1990).

The vagus nerve with its myenteric ganglia and neurones is responsible for the movement of the stomachs in ruminants (Duncan & Phillipson, 1951; Cook, 1986). Studies by Newhook & Titchen (1974) showed that the normal passage of liquids to the

abomasum of lambs was interrupted after cervical or abdominal vagotomy, resulting in the liquids being channelled to the reticulum and rumen. The same effects were achieved when the nerve endings were blocked by hexamethonium (Newhook & Titchen, 1974). Habel (1956) performed dorsal, ventral and total vagotomies on sheep and found a distended rumen and reticulum resulted from dorsal and total vagotomies. The clinical signs (distended rumen and impacted abomasum), milk filled rumens, and the decrease in the thickness of the tunica muscularis, thus indicated a decrease in nerve supply to the fore-stomachs and abomasa of the affected lambs.

As macroscopic dissections of the vagus nerve revealed no differences in its pattern between the grey, white and black lambs (Groenewald & Booth 1989), it was decided to compare by image analysis the number and size of the myenteric ganglia and the number of myenteric neurones in the walls of the fore-stomachs and the abomasum of 24-h-old grey, white and black Karakul lambs. The study indicated a significant decrease in the number and size of the myenteric ganglia and number of myenteric neurones in the grey and white lambs when compared to black lambs. The decrease of myenteric ganglia and neurones could be responsible for the clinical manifestations of this condition.

### MATERIALS AND METHODS

Thirteen grey, fifteen white, and ten black 24-h-old Karakul lambs were embalmed with 10 % formalin via the left common carotid artery immediately after they had been slaughtered and skinned. Grey and white lambs with unpigmented tongues, palates, and ears were specifically selected. Black lambs were randomly selected and served as the control group.

A one square centimetre sample was taken from an analogous area of the rumen, reticulum, omasum and obomasum in each of the 38 embalmed lambs.

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The samples of the rumen were taken from the middle of the left dorsal, left ventral, right dorsal and right ventral sacs. The other samples were taken from the right surface of the reticulum, the parietal surface of the omasum, and the greater curvature of the abomasum. Routine histological sections were prepared from each sample and stained with haematoxylin and eosin for microscopic evaluation. All counting and measuring of the myenteric ganglia and myenteric neurones was performed with a Vids 2 Image Analyzer.

The number of myenteric ganglia and myenteric neurones, as well as the surface area (size) of the myenteric ganglia, was determined in each section and the mean for each group was calculated. The number of myenteric ganglia and neurones was counted and measured in the left dorsal, left ventral, right dorsal and right ventral ruminal sacs and the mean for the rumen was calculated.

One way analysis of variance was implemented to determine whether significant differences existed between the three groups of lambs regarding the number and size of the myenteric ganglia and the number of neurones.

## RESULTS

The myenteric ganglia were found between the inner circular and outer longitudinal layers of the tunica muscularis in all the lambs. The number (Fig. 1) and size (Fig. 2) of the myenteric ganglia and the number of myenteric neurones (Fig. 3) in the rumen, reticulum and abomasum differed significantly between the three groups of lambs. The number and size of the ganglia, and the number of neurones,

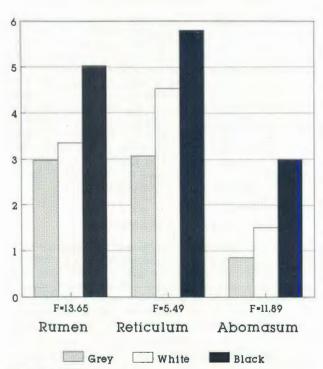


FIG. 1 Mean number of ganglia per cm

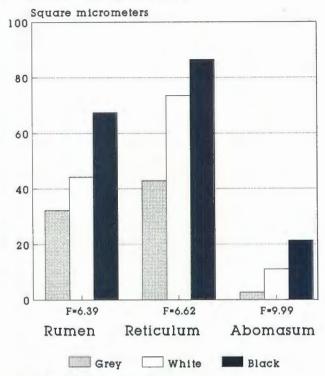


FIG. 2 Mean surface area of ganglia

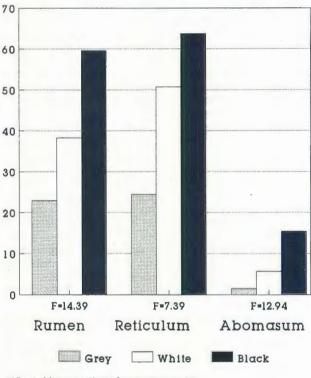


FIG. 3 Mean number of neurones per cm

showed a progressive decrease between the black, white and grey lambs. The omasum was not affected.

## DISCUSSION

The results of the present study clearly indicate a paucity in the number of myenteric ganglia and myenteric neurones in the homozygous grey Karakul lambs, and to a lesser extend in the homozygous white Karakul lambs, when compared to the black control lambs.

Since nerve supply can play a role in the normal conservation of muscle, the reduction in the number of myenteric ganglia and neurones could be the cause of the reduction in thickness of the tunica muscularis (Groenewald & Booth, 1990). However, this phenomenon could also be a developmental defect as the reduction in thickness of the muscle layers is present 24 h after birth.

Habel (1956) demonstrated a distended rumen and reticulum by doing a total vagotomy on sheep. The same result was achieved when he performed a dorsal vagotomy. The clinical signs demonstrated by the lambs with the lethal factor correspond to the features found by Habel (1956).

In suckling lambs the suckling reflex causes the milk to bypass the rumen and go to the abomasum by means of the oesophageal groove (Habel, 1956; Swensen, 1984). Studies by Newhook & Titchen (1974) revealed that milk passes into the rumen and reticulum instead of the abomasum after cervical or abdominal vagotomy because of a oesophageal groove disfunction. By blocking the nerve endpoints with hexamethonium they achieved the same results. Therefore, the presence of milk in the rumen of the affected lambs in this study could be the result of malfunctioning of the oesophageal groove. The volume of milk in the rumen could then cause a distention and subsequent additional thinning of the ruminal wall.

The myenteric ganglia and neurones are responsible for movement of the fore-stomachs (Habel, 1956; Duncan & Phillipson, 1951) which is a prerequisite for digestion of roughage in the ruminant. Cooke (1986) found neural connections between the myenteric and submucosal ganglia and suggested that they work as a single integrative system. She also found that the submucosal ganglia project into the mucosa and suggested that they function to control and coordinate absorptive and secretory function, blood flow, and contractility of the muscularis mucosa. As the myenteric and submucosal ganglia work as a single integrative system (Cooke, 1986), a decrease in the number of myenteric ganglia and neurones may lead to impaired absorption of nutrients, decreased secretion of enzymes and decreased movement of villi. Reduced movement of the fore-stomachs, coupled with the thin tunica muscularis and decreased blood flow, would lead to impaired digestion and absorption of nutrients, leading eventually to emaciation and death.

The impaction of the abomasum is most probably caused by reduced peristalsis resulting from the reduction of nerve supply. Further distention of the rumen is caused by accumulation of water and roughage in the rumen because of reduced ruminal movement.

White Karakul lambs, when compared to grey lambs have a greater number of myenteric plexuses and ganglion cells and a thicker tunica muscularis in the reticulum, rumen and abomasum which would result in better movement of the stomachs and better digestion of roughage, thus prolonging survival.

The omasum develops from the lesser curvature of the foetal stomach, the rumen and reticulum from the greater curvature, and the abomasum from both (Bryden, Evans & Binns, 1972). The structures that develop from the greater curvature of the stomach are mainly supplied by the dorsal vagal trunk and the structures that develop from the lesser curvature are mainly supplied by the ventral vagal trunk (McGeady & Sack, 1968). The omasum also receive a branch from the dorsal vagal trunk (McGeady & Sack, 1968) and that would explain why the number of myenteric ganglia and neurones were unaffected in this organ.

In the developing autonomic nervous system the preganglionic fibres of the nerves are formed by neurones that originate in the ventrolateral plate, and the ganglionic cells and postganglionic fibres are neural crest derivatives (Latshaw, 1987). Melanoblasts responsible for pigmentation also develop from the neural crest (Latshaw, 1987). The lack of pigmentation of the tongue, palate and ears, and the reduction of myenteric plexuses and ganglion cells in the rumen, reticulum and abomasum of the affected lambs, therefore strongly suggest neural crest involvement in the condition described.

It is concluded that the paucity of myenteric ganglia and neurones in the rumen, reticulum and abomasum is instrumental in causing this lethal condition in grey and to a lesser extend in white Karakul lambs.

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