Influence of breed and age on collagen content and solubility of some ovine and goat muscles

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Introduction

Tenderness is one of the most important meat quality characteristics. The intrinsic tenderness of meat is supposed to be dependent on two main factors, namely the contractile and sarcoplasmic proteins, and the connective tissue proteins. The properties of the contractile and sarcoplasmic proteins could easily be significantly altered through the use of tenderizing agents and by aging the meat. The properties of the connective tissue could, however, not be changed that easily. Even the aseptic aging for 6 months at 37°C had no tenderizing influence on connective tissue (Sharp, 1963). It is thus important to investigate the characteristics of connective tissue in meat.

As collagen is the most abundant protein in connective tissue, it should also have a major influence on meat tenderness. Two important factors in this regard are the collagen content and the solubility of the collagen. In general, the higher the collagen content and the lower the solbility thereof, the tougher the meat should be.

Much research has been done on collagen content and solubility, but mainly on cattle (Herring, Cassens & Briskey, 1967; Hunsley, Vetter, Kline & Burroughs, 1972; Cross, Carpenter & Smith, 1973; Prost, Pełczynska & Kotula, 1975), although some research has already been done on sheep (Smith & Carpenter, 1970), pigs (Boccard, 1968) and certain goat breeds (Gonzalez, Owen & Cereceres, 1983). In cattle it has been shown that breed and age have a marked influence on muscle collagen content and solubility (Boccard, Naudé, Cronje, Smit Venter & Rossouw, 1979). It was therefore decided to study the effect of breed and age of four sheep breeds and one goat breed on collagen content and solubility. The breeds studied were chosen because of their existence in South Africa and also because of certain other intrinsic factors in these breeds. The breeds chosen, and the reasons for being chosen, are as follows :

Pedi	- an indigenous, unimproved fat-tailed sheep breed (Hugo, 1968)
Merino	- very important for it's wool production (Hugo, 1968)
Boer Goat	- excellent meat producer (Naudé & Hofmeyr, 1981)
Dorper	- excellent meat producer, especially at a young age (Hugo, 1968)
South African Mutton Merino	- known as a mutton-wool breed; and the lambs possess a good lamb conformation (Hugo, 1968)

The data was analysed statistically on an IBM personal computer with the NWA STATPAK (1984) programs.

Results and Discussion

The results of a three-way analysis of variance (breed, muscle and age) on collagen content, collagen solubility and index value are given in Table 1.

1. Collagen content

1.1. Collagen content as influenced by breed

Breed had a highly significant ($p < 0,01$) influence on collagen content (Table 1). In order of decreasing collagen content the breeds were the Boer Goat, Pedi, Merino, S.A. Mutton Merino and Dorper. These breeds could be grouped into two groups according to their collagen contents : group A consisting of the breeds Pedi, Merino and Boer Goat, and group B consisting of the breeds Dorper and S.A. Mutton Merino. The collagen contents of the breeds in group A were significantly higher ($p < 0,01$) than those of group B and a definite pattern was noted in that the collagen contents of breeds between groups differed significantly ($p < 0,01$), but not significantly ($p < 0,05$) within groups (Table 2).

Table 1 : Three-way analysis of variance showing the effect of breed, muscle and age on collagen content, collagen solubility and index value

		F value						
	CV%	Breed (A)	Muscle (B)	Age (C)	AxB	AxC	BxC	AxBxC
Content	57	** 57,17	** 322,17	** 36,85	** 3,29	** 8,08	** 2,21	0,88
Solubility	41	** 26,62	** 367,66	** 1350,37	** 4,96	** 3,83	** 3,47	** 1,83
Index	43	** 72,91	** 90,15	** 12,04	** 5,42	** 8,11	** 3,21	0,82

* = $p < 0,05$
** = $p < 0,01$

Materials and Methods

Fifteen lambs or kids were used from each breed. The animals were weaned at an age of six weeks and five animals from each breed were slaughtered at 1, 4 and 18 months of age. After starvation overnight, the slaughter groups were transported from the Animal and Dairy Science Research Institute at Irene to the abbatoir of the Veterinary Research Institute at Onderstepoort, about 40 km from Irene. The animals were slaughtered according to normal slaughtering procedures. The carcases were chilled overnight at 4°C and 95 % RH after which the carcases were transported back to Irene. The following muscles were dissected from the right side 24 hours post mortem : *M. longissimus dorsi* (LD), *M. semimembranosus* (SM), *M. supraspinatus* (SS), *M. biceps fermoris* (BF), *M. semitendinosus* (ST), *M. psoas major* (PM) and *M. infraspinatus* (IS). The muscles were frozen at -20°C until required for analysis.

As required, the frozen muscles were allowed to thaw overnight. All external fat and connective tissue was removed. Slices were cut from an area in the middle of the longest axis of the muscle. The samples were homogenized and subsequently used for the determination of the collagen content and solubility.

Of the homogenized sample, 150 g was freeze dried and pulverized in a mill at 20 000 rpm (Ultra-Turrax, Janke & Kunkel). Total nitrogen was determined with a Technicon Auto Analyzer according to the method of Weber (1973) after the sample (0,1 g) was digested in a micro-Kjeldahl system. The hydroxyproline was determined on a hydrolysed sample (0,5 g) of pulverized freeze-dried muscle according to Method A of Bergman and Loxley (1963) as adapted for use with a Technicon Auto Analyzer. All samples were performed in triplicate. The ratio of hydroxyproline to total nitrogen was determined, and the result expressed as a ratio to total nitrogen.

The solubility of the intramuscular collagen was determined according to the method of Hill (1966). Fresh muscle was homogenized, and 4 g of this added to a flask containing 50 ml of a 1 % NaCl solution. The flask was capped with a rubber cap fitted with a reflux tube. The suspension was heated in a glycerol bath at 90°C for 4 hours. The suspension was then filtered, the filtrate dried in a rotary evaporator and both the dried filtrate and residue digested with 6N HCl for 9 hours. The hydroxyproline content of the residue and filtrate was determined according to Method A as described by Bergman and Loxley (1963). Determinations were done in triplicate. Collagen solubility was expressed as the percentage hydroxyproline in the filtrate as compared to the total hydroxyproline (filtrate plus residue).

The combined effect of collagen content and solubility were taken into account by using the following formula to calculate an index :

$(X_i/X_n) \times (100 - Y)/100$

X_i = collagen content of muscle

X_n = collagen content of breed with highest mean collagen content at 1 month of age = Boer Goat (6,5540)

Y = collagen solubility of the muscle

Table 2 : Mean values for collagen content (Hypro N/Total N x 10^3), solubility (%) and index value of the different breeds

	Pedi	Merino	Boer Goat	Dorper	S.A. Mutton Merino
Content	5,5065[a]	5,4596[a]	5,5309[a]	3,7888[b]	4,0116[b]
Solubility	35,6394[ac]	31,9793[b]	32,9276[b]	35,5206[a]	37,1644[c]
Index value	50,0401[a]	53,2233[a]	52,2480[a]	34,7388[b]	34,6053[b]

abc Within each row, values with no common superscript differ highly significantly ($p < 0,01$).
The following differed significantly ($p < 0,05$) from each other :
Solubility : Pedi and S.A. Mutton Merino
Index value : Pedi and Merino

It seems that the type of sheep breed (bred primarily for meat or wool production) has a marked influence on the collagen content of the muscles, with the meat-type breeds having the lower average collagen content. It would thus seem that the meat-type of breeds would produce the better quality meat regarding tenderness if only the collagen content had an influence on the tenderness of the meat. The Boer Goat, although a meat producing goat, fell into the category of non-meat type sheep. Boccard, Naudé, Cronje, Smit, Venter and Rossouw (1979) also indicated that significant differences in collagen content exist between the cattle breeds Afrikaner and Friesland. The same results were also found between the pig breeds Large White and Piëtrain (Boccard, 1968). The results of this experiment are thus generally in accordance with the results obtained in the above-mentioned experiments, although it was conducted with other species. Breed may thus have an influence on the collagen content of muscles, but the results from this experiment also indicate that breeds could be divided into different groups, depending on their general application, i.e. divided into groups related to primarily meat or wool production (except for the Boer Goat which is a meat type goat, but of a different species).

1.2. Collagen content in the different muscles

Highly significant differences ($p < 0,01$) were found in collagen content between various muscles (Table 1). The only non-significant differences were found between SS and BF, SS and PM, and BF and PM (Table 3). The IS had the highest collagen content of all the muscles investigated, while the LD had the lowest, approximately 28,37 % of the IS value. The muscles in descending order of collagen content were IS-(BF-PM-SS)-ST-SM-LD. The PM is generally regarded as a very tender muscle, whereas these results indicate a high collagen content which would indicate a tougher type of muscle, the conclusion must be that there are other factors also influencing tenderness and therefore not collagen content alone. The connective tissue content in the different muscles of bovine from the highest to the lowest content was found to be IS-BF-ST-PM (Prost et al., 1975). Nottingham (1956) also found the PM to have the lowest content of connective tissue in sheep (mainly Southdown-Romney crosses), while the muscles in decreasing order were SM-LD-PM. Although the order of the SM and LD is in accordance with obtained results reported in this experiment, the position of the PM there disagrees with the results found in this experiment. Nottingham (1956) also found that, although the PM is generally regarded as a tender muscle, the shear force of the PM was higher than that of the LD when cut across the muscle fibre direction, but lower when cut parallel to the fibre direction. This again indicates that other factors, in this case muscle fibre, may also influence tenderness.

1.3. Collagen content as influenced by age

Age had a highly significant influence ($p < 0,01$) on collagen content (Table 1). The general tendency in all the breeds was a decrease in collagen content with age (Table 4). In bovine a highly significant difference ($p < 0,01$) was found in the different muscles as influenced by age (Boccard et al., 1979). A decrease in collagen content was found from birth to the older ages, although a slight increase was found after 8 months, whereas a constant level was reached and maintained from the age of 16 months. This was not found in the sheep and goat breeds in this experiment, as the collagen content generally tended to decrease with age. No general change was observed in the connective tissue content with age in bovine, was found, although calves had a higher content than older animals (Prost et al., 1975). No significant differences with age could be shown by Kim, Ho and Ritchey (1967), although the collagen content in the LD between veal (ca 3 months) and mature beef (older than 5 years) differed significantly ($p < 0,05$). Their findings of a general increase in collagen content in the muscles LD and BF disagree with the results from this experiment and with those of Boccard, Naudé, Cronje, Smit, Venter and Rossouw (1979).

Table 3 : Mean values for collagen content (Hypro N/Total N x 10^3), solubility (%) and index value for the different muscles.

	LD	SM	SS	BF	ST	PM	IS
Content	2,8799^a	3,3161^a	4,5597^b	4,6396^b	3,8125^c	4,5655^b	10,1503^d
Solubility	33,5102^{ac}	29,2789^b	31,8232^a	32,0225^c	25,2925^d	35,6509^e	54,9456^f
Index value	28,8882^a	35,9408^b	47,1255^c	47,7090^c	43,1446^c	43,2430^c	68,8969^d

abcdef Within each row, values with no common superscript differ highly significantly ($p < 0,01$)

The following differed significantly ($p < 0,05$) from each other :

Content : LD and SM

Solubility : LD and SS, LD and BF

Index value : SS and ST, SS and PM, BF and ST, BF and PM.

Table 4 : Mean values for collagen content (Hypro N/Total N x 10^3), solubility (%) and index value between the different slaughter ages

	1 Month	4 Months	18 Months
Content	5,3771^a	4,9323^b	4,2690^c
Solubility	45,9880^a	35,8009^b	22,1498^c
Index value	41,6843^a	45,7244^b	47,5046^c

abc Within each row, values with no common superscript differ highly significantly ($p < 0,01$)

The general conclusion is that the collagen content is a poor indicator of tenderness (Herring et al., 1967; Hunsley et al., 1972; Cross et al., 1973) and that other factors must, therefore also have an influence on observed tenderness.

2. Collagen solubility

2.1. Collagen solubility as influenced by breed

Breed had a highly significant influence ($p < 0,01$) on collagen solubility (Table 1). Significant differences ($p < 0,05$) were found between all breeds except between the breeds Pedi and Dorper, and between Merino and Boer Goat (Table 2). This means that in the groups A and B as described previously, breeds differed significantly in collagen solubility within groups, as well as between groups. Breeds could therefore not be grouped into the same solubility-based groups as in the case with collagen content. With reference to collagen solubility, the breeds could be divided into three groups, group X consisting of the fat tailed breeds, namely Pedi and Dorper; group Y consisting of the breeds Merino and Boer Goat; and group Z consisting of the breed S.A. Mutton Merino. This grouping tends to divide the sheep breeds into groups consisting of fat-tailed breeds (group X), woolled breeds (group Y) and woolled-mutton breeds (group Z), with the Boer Goat falling in the same category as the Merino. Significant differences exist in collagen solubility between the cattle breeds Afrikaner (meat producing) and Friesland (milk producing) (Boccard et al., 1979).

2.2. Collagen solubility of the different muscles

Significant differences ($p < 0,01$) were found in collagen solubility between muscles (Table 1). The muscles in descending order of collagen solubility were IS-PM-LD-BF-SS-SM-ST, with all of them differing significantly ($p < 0,05$) from each other (Table 3). Cross, Carpenter and Smith (1972) found this order for the mean solubility across age to be BF-ST-SM in sheep which disagrees slightly with the results obtained in this experiment. In cattle, Herring, Cassens and Briskey (1967) found the solubility of the SM to be less than that of the LD, while Cross, Carpenter and Smith (1973) found the order of adjusted solubility to be LD-(SM-BF)-ST in bovine muscles. This again agrees with the results found with this experiment using sheep and goat breeds. Although the collagen content of the LD was low with a high solubility (an expected combination for high tenderness), this was not observed by the taste panel or objective shear force measurements resulting in the conclusion that connective tissue contributes little to observed toughness (Cross et al., 1973). It would thus seem there must be other factors that also influence tenderness of meat aside from collagen content and solubility.

2.3. Collagen solubility as influenced by age

Age had a highly significant influence ($p < 0,01$) on collagen solubility (Table 1). The general trend was a decrease in solubility with age (Table 4). These results support the findings of previous researchers (Hill, 1966; Cross et al., 1972; Boccard et al., 1979). The general theory in this regard is that with an increase in age, more cross linkages form between the collagen fibres resulting in a reduction in solubility, and a reduction in the tenderness of the meat.

In general, it seems that the solubility of collagen was a better indicator of observed tenderness (Herring et al., 1967; Cross et al., 1973) although Smith and Carpenter (1970) found that percent soluble collagen was not consistently related to tenderness. It may thus be possible for other factors besides collagen solubility to influence observed tenderness.

3. Index comprised of both the collagen content and solubility

3.1. Index as influenced by breed

Breed had a highly significant influence ($p < 0,01$) on the index value (Table 1). Again, the breeds could be divided into the same two general groups as before with the collagen content, with group A consisting of the breeds not directly bred for meat production, i.e. the Pedi, Merino and Boer Goat, and group B consisting of the breeds bred for meat production, i.e. Dorper and Mutton Merino. The breeds differed significantly ($p < 0,01$) between groups, but not within groups ($p > 0,05$), except between the Pedi and Merino in group A which differed significantly ($p < 0,05$) from each other (Table 4). The breeds in group B had the lowest index values which should indicate more tender meat.

3.2. Index values of the different muscles

The index values between the muscles differed significantly ($p < 0,05$) except between the muscles SS and BF, and between the muscles ST and PM (Table 3). The muscles, in order of decreasing index value (increasing toughness according to the assumption that a high index value should indicate tender meat), were IS-(BF-SS)-(PM-ST)-SM-LD.

3.3. Index as influenced by age

Age had a highly significant influence ($p < 0,01$) on the index value (Table 4). The general trend was a higher index value with increasing age, thus indicating a decrease in tenderness as would be generally expected.

Conclusion

It may thus safely be concluded that factors other than the collagen content and solubility must also have an influence on the tenderness of meat. It has already been indicated by Veis (1966) that the nature of the collagen fibre weave and the system of intermolecular cross linkages are involved in structural stabilization. Hence it should also play a part in the whole concept of meat tenderness. Nottingham (1956) has already indicated that muscle fibre influences the tenderness of meat, and Herring, Cassens and Briskey (1967) have shown that the state of contraction of the muscle fibres has a marked influence on tenderness. This has also been shown when muscles contracted (cold shortening) due to the over-effective chilling of hot carcases (Lawrie, 1979).

The results of this experiment indicate that the collagen content of breeds bred primarily for wool production and meat production differ substantially. The breeds bred for meat production should have the best meat quality regarding the tenderness of meat. In general, the collagen content seems to decrease with age, but this phenomenon could be explained by the increase of muscle fibre volume during the growth of the animal, and not directly by a decline in the absolute content in collagen. As expected, the collagen solubility declined with age. Other factors must also play a major role in the tenderness of meat, specially the type of matrix (weave) formed by the collagen, the muscle fibres and the state of muscle contraction. It should thus be worthwhile to repeat this experiment in which also the tenderness of the muscles is determined by Warner-Bratzler type of shear force measurements and also to determine, histologically, the type of matrix the collagen has formed as well as the state of contraction of the muscle fibres. It is suggested that only effectively electrical stimulated carcases be used to try to minimise the effect of muscle contraction (cold shortening) in an effort to determine the real overall influence of collagen on meat tenderness. It is further suggested that a combination of these factors related to objective tenderness would give a better result in evaluating the effect of age, breed and different muscles on overall tenderness.

References

Bergman, I. & R. Loxley, 1963. Two improved and simplified methods for the spectrophotometric determinations of hydroxyproline. Analyt. Chem. 35, 1967.

Boccard, R., 1968. Variation de la teneur en hydroxyproline de muscles de porcs Large White et Piétrain. Ann. Zootech. 17, 71-75.

Boccard, R.L., R.T. Naudé, D.E. Cronje, Maria C. Smit, H.J. Venter & Ellenor J. Rossouw, 1979. The influence of age, sex and breed of cattle on their muscle characteristics. Meat Science 3, 261-280.

Cross, H.R., Z.L. Carpenter & G.C. Smith, 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. J. Food Sci. 38, 998-1003.

Gonzalez, F.A.N., J.E. Owen & M.T.A. Cereceres, 1983. Studies on the Criollo Goat of Northern Mexico : Part 2 - Physical and chemical characteristics of the musculature. Meat Science 9, 305-314.

Herring, H.K., R.G. Cassens & E.J. Briskey, 1967. Factors affecting collagen solubility in bovine muscles. J. Food Sci. 32, 534-538.

Hill, F., 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31, 161-166.

Hugo, W.J., 1968. The small stock industry in South Africa. Government Press, Pretoria.

Hunsley, R.E., R.L. Vetter, E.A. Kline & W. Burroughs, 1972. Effects of age and sex on quality, tenderness and collagen content of bovine longissimus muscle. J. Anim. Sci. 33, 933-938.

Kim, C.W., G.P. Ho & S.J. Richey, 1967. Collagen content and subjective scores for tenderness of connective tissue in animals of different ages. J. Food Sci. 32, 586-588.

Lawrie, R.A. 1979. Meat Science 3d Edition. Pergamon Press, Oxford. p 203.

Naudé, R.T. & H.S. Hofmeyr, 1981. Meat production. In : Goat production. Ed. C. Gall. Academic Press, London. p 285.

Nottingham, P.M., 1956. The connective-tissue content and toughness of sheep muscles. J.Sci. Food Agric. 7, 51-56.

NWA STATPAK, (1984). NWA STATPAK : Multi-function statistics library. Version 3.1. Northwest Analytical, Inc. Portland, Oregon.

Prost, E., E. Pełczynska & A.W. Kotula, 1975. Quality characteristics of bovine meat. I. Content of connective tissue in relation to individual muscles, age and sex of animals and carcass quality grade. J. Anim. Sci. 41, 534-540.

Sharp, J.G., 1963. Aseptic autolysis in rabbit and bovine muscle during storage at 37°. J. Sci. Food Agric. 14, 468-479.

Smith, G.C. & Z.L. Carpenter, 1970. Lamb carcass quality. III. Chemical, physical and histological measurements. J. Anim. Sci. 31, 697-706.

Weber, R., 1973. The determination of hydroxyproline and chloride in meat and meat products. Simultaneous operation with nitrogen and phosphorus determinations. Technicon International Division S.A., Technical Report no. 7. Technicon International Division, Geneva.

References

Bergman, I. & R. Loxley, 1963. Two improved and simplified methods for the spectrophotometric determinations of hydroxyproline. Anal. Chem. 35, 1961-1965.

Boccard, R., 1966. Variation de la tendreté et hydroxyproline des muscles de bovins.

Boccard, R.L., R.T. Naudé, D.E. Cronje, M.C. Smit, H.J. Venter & E.J. Rossouw, 1979. The influence of age, sex and breed of cattle on their muscle characteristics. Meat Science 3, 261-280.

Cross, H.R., Z.L. Carpenter & G.C. Smith, 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. J. Food Sci. 38, 998-1003.

Gonzalez, F.A.N., J.E. Owen & M.T.A. Cereceres, 1983. Studies on the Criollo Goat of Northern Mexico : Part 2 - Physical and chemical characteristics of the musculature. Meat Science 9, 305-314.

Herring, H.K., R.G. Cassens & E.J. Briskey, 1967. Factors affecting collagen solubility in bovine muscles. J. Food Sci. 32, 534-538.

Hill, F., 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31, 161-166.

Hobbs, J., 1986. The small stock industry in South Africa. Government Printer, Pretoria.

Hunsley, R.E., R.L. Vetter, E.A. Kline & W. Burroughs, 1971. Effects of age and sex on quality, tenderness and collagen content of bovine longissimus muscle. J. Anim. Sci. 33, 933-938.

Kim, C.W., G.P. Ho & S.J. Richey, 1967. Collagen content and subjective scores for tenderness of connective tissue in animals of different ages. J. Food Sci. 32, 586-588.

Lawrie, R.A. 1979. Meat Science 3d Edition. Pergamon Press, Oxford. p.303.

Naudé, R.T. & H.S. Hofmeyr, 1981. Meat production. In : Goat production. Ed. C. Gall. Academic Press, London.

Nottingham, P.M., 1956. The connective tissue content and toughness of sheep muscle. J. Sci. Food Agric. 7, 85.

NWA STATPAK, (1984). NWA STATPAK : Multi-function statistics library. Second Ed. Northwest Analytical, Inc. Portland, Oregon.

Prost, E., E. Pelczynska & A.W. Kotula, 1975. Quality characteristics of bovine meat. II. Collagen of connective tissue in relation to individual muscles, age and sex of animals and carcass quality grade. J. Anim. Sci. 41, 534-540.

Sharp, J.G., 1963. Aseptic autolysis in rabbit and bovine muscle during storage at 37°. J. Sci. Food Agric. 14, 468-479.

Smith, G.C. & Z.L. Carpenter, 1970. Lamb carcass quality. III. Chemical, physical and histological measurements. J. Anim. Sci. 31, 697-706.

Weber, R., 1973. The determination of hydroxyproline and chloride in meat and meat products. Simultaneous determination of nitrogen and phosphorus in meat products. Technicon International Division S.A., Geneva.