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Is collagenolytic activity present in the blood of ruminants?

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Abstract In the present study, collagenolytic activity was investigated in two separate experiments using blood plasma or serum. Two separate experiments were conducted at different times. The first experiment involved the analysis of blood plasma from 16 rams, 6 goat bucks, and 3 lactating cows. The rams and goat bucks were randomly divided into two equal heads groups (control and treated), and they did not exhibit any sexual problems. In the second experiment, twelve calves (5 males and 7 females) with a mean age 152 days, and weight of 128 kg, were selected and randomly divided into two groups. One group received a total mixed ration (TMR) plus a feed block (22 kg weight), while the other group received only TMR. Blood serum from all calves was utilized for collagenolytic activity. Bovine Achilles' tendon collagen served as the substrate for measuring collagenolytic activity, while hydroxyproline (HYP) was employed as a product for activity measurement. The results revealed the presence of the enzyme(s) in the blood samples of all the experimental animals, albeit at varying levels of activity. It was observed that the activity was significantly inhibited by cupric sulfate as a metal ion. Furthermore, notable positive and significant correlations were found between the daily gain of sheep and collagenolytic activity in blood samples, while negative correlations were observed between the age of the calves and collagenolytic activity. These results supported the hypothesis that enzyme activity is associated with growth.

Keywords: collagenolytic activity, hydroxyproline, enzyme inhibition

Introduction

The term "collagen" originates from the Greek word "kolla," meaning glue (Pachence, 1996). Collagen serves as the primary protein in animal connective tissue and is the most abundant protein found in mammals, making up about 25% to 35 % of the whole-body protein content. It constitutes 1% to 2% of muscle tissue and contributes to 6 % of the weight of robust, tendinous muscles. Collagens are widely distributed throughout the tissues of living organisms (Canty and Kadler, 2005).

Collagen is known to be degraded to HYP containing peptides and free HYP (Prockop and Kivirikko, 1967). Plasma free HYP is commonly used as an index of collagen turnover. It increases during bone growth (Klein and Teree, 1966), acromegaly (Lee and Lloyd, 1964), and the postpartum period when the uterus undergoes involution (Klein, 1964). The process by which vertebrates degrade collagen in their connective tissue during growth or repair is referred to as collagenolytic activity. Collagenase has been suggested to play a role in bone resorption and remodeling (Lenaers-Claeys and Vaes, 1979).

Starcher et al. (1980) demonstrated that zinc deficiency in chicks significantly reduces the activity of bone collagenase, which may disrupt the delicate balance between bone growth and remodeling by impairing collagen breakdown. Collagenolytic enzymes have been identified in human serum by Gries et al. (1970). It is believed that these human serum enzymes differ in

their specificity from collagenases found in other vertebrates. However, research on collagenase activity and its relationship to growth and weight gain in ruminants is limited. Therefore, we aimed to investigate whether a similar class of enzymes exists in the blood of ruminants and, if so, whether their activity is associated with growth.

Materials and methods

Management and experimental design

Two separate experiments were conducted at different times. Both experiments were carried out in accordance with ethical guidelines and regulations reported by Izmirli et al. (2010). Animal care and handling protocols were designed to minimize stress and discomfort, and all procedures adhered to the ethical guidelines for animal experimentation. The details of each experiment and the experimental design are described below:

First experiment (Sheep, Goats, and Lactating Cows)

Sixteen mature and sexually inexperienced rams (7 Baluchi and 9 Kurdi Iranian native breed), along with six native goat bucks, were selected for this study. The average body weight for Baluchi and Kurdi rams was 53.0 ± 8.7 kg and 58.2 ± 9.2 kg, respectively. The average body weight for goat bucks was 70.9 ± 5.8 kg. The mean ages for rams and goat bucks were 13.1 ± 2.2 months and 53.3 ± 2.5 months, respectively. Before the start of the experiment, all rams and goat bucks were isolated from animals of the opposite sex as much as possible for more than 3 months.

The rams and goat bucks were randomly divided into two equal heads groups (control and treated), and they did not exhibit any sexual problems. All control rams and three of the goat bucks were fed a ration containing 15.5% crude protein (CP). The ration for the treated rams and the remaining goat bucks contained 77% rumen-degradable protein (RDP), using 1.5% feed-grade urea (on a dry matter basis), while the control group received a ration with 65% RDP (Table 1). Clean, fresh water, salt rock, and mineral blocks were available ad libitum. The treatment ration was offered to all animals for approximately 70 days.

Three lactating cows, at the middle of lactation period (180 ± 20 days in milk), aged 62 ± 2 months, were selected for blood sampling. These cows were fed with a balanced total mixed ration (TMR) consisting of 22.28% alfalfa hay, 19.8% corn silage, and 57.92% dairy cow concentrate (dry matter basis). They had unrestricted access to water and TMR throughout the day. All animals were kept under natural daylight conditions (latitude 51°27'N) and ambient temperature.

Second experiment (calves)

Immediately after birth, all calves were separated from their mothers and transferred to individual pens. In the first 6 hours after birth, colostrum was fed to the calves using a bottle, at a rate of 10% of their body weight, divided into three consecutive feedings. Subsequently, fresh cow's colostrum was fed to all calves for three consecutive days. Fresh cow's milk was then fed according to the farm's schedule and was the same for both groups. The calves received 6 kg of milk daily until 8 weeks of age, after which the amount was reduced to 4 kg until 11 weeks of age, and further reduced to 2 kg until 12 weeks of age. Milk was offered twice a day at 6:00 and 15:00 hours. All calves were weaned at 90 days of age. Starter mixture was introduced to all calves from the third day of birth until weaning. High-quality alfalfa hay was provided in a separate bucket from the age of two weeks. Clean, fresh water was available ad libitum in a bucket. After weaning, twelve calves (5 males and 7 females) aged 152 ± 40 days, with a weight of 127.9 ± 27.3 kg, were selected and randomly divided into two groups. One group received a TMR plus a feed block (22 kg weight), while the other group received only TMR (Table 1). The calves were fed their rations twice a day at 07:00 and 16:00 hours, with half of the ration given at each feeding. A new feed block was provided after approximately 90% of the previous one was consumed. Clean, fresh water, salt rock, and mineral blocks were offered ad libitum. Daily feed intake was recorded, and any remaining feed was collected before each morning feeding (usually no remnants were left). The treatment ration was offered to all calves for about 70 days.

Blood collection

For rams and goat bucks, on day 71, and for cows, on day 180 of lactation, blood samples were collected from the jugular vein using vacutainers containing heparin as an anticoagulant, 4 hours after the morning feeding. Plasma was obtained by centrifuging the blood samples at 2500 RPM for 10 minutes. For calves, blood samples were collected from the jugular vein at 71 days after birth. Each sample was allowed to clot and stand at room temperature for 2-3 hours, after which serum was harvested using a Pasteur pipette.

Collagenolytic activity assay

The collagenolytic activity assay employed in this study was a modification of the method described by Gries et al. (1970). Tris-HCl buffer adjusted to pH 7.2 was used for all enzyme incubations with the media. Bovine Achilles' tendon collagen (Sigma, C4387) was utilized as the substrate for measuring collagenase activity. The incubation was carried out at 30°C for five hours, which was found to be more convenient than the original method of 25°C for 1 hour (Bannister and Burns,1972). The reaction was stopped by adding 10% (w/v) citric acid, and the hydroxyproline (HYP) content in the media was measured spectrophotometrically at 550 nm against a blank, using the method described by Stegmann and

Stalder (1967). The assay determined collagenolytic activity based on the release of HYP from Achilles' tendon collagen as the substrate. One unit of collagenolytic activity was defined as the amount of enzyme that released 1.0 µmol of HYP per hour at 30°C and pH 7.2. The HYP equivalent in the background was determined, and the enzyme activity was reported as µmol per deciliter (100 mL) of the sample. A calibration curve using known concentrations of HYP was prepared with each series of determinations. A blank, containing substrate but no serum (or plasma), was also prepared. All samples were assayed in duplicate.

Effect of cupric sulfate on collagenolytic activity

Only serum samples from calves were assayed to evaluate the effects of metal ions on collagenolytic activity. Cupric sulfate (CuSO4) was added in aqueous solution to the serum samples, resulting in a final concentration of 10 mM. Collagenolytic activity was expressed as units per 100 mL of serum. Controls contained an equal volume of water.

Statistical analysis

All enzyme activity measurements were performed in duplicate, and the mean results per sample were used for data analysis. Statistical analysis was performed separately for each species. However, data from cows were not analyzed due to the low replication and the lack of treatment. The cow's data were used solely to describe the enzyme activity. The Generalized Linear Models (GLM) procedure of SAS® (SAS Institute Inc., ver 9.4, 2021 license, Cary, NC, USA) was used for statistical analysis. When a significant ($P<0.05$) main effect was detected, means were separated using the Tukey's studentized range test (SAS®). Additionally, the correlation between collagenolytic activity and certain characteristics, such as age, final weight, and daily gain in the experimental animals, was estimated using the CORR procedure of SAS®. The correlation coefficient was then tested using the Student's t-test.

A 2×2 factorial design was employed to assess the impact of two independent variables, Factor A (sheep breeds in first experiment and calves sex in second experiment) and Factor B (two type rations, high and low RDP in first experiment and control and control + feed block in second experiment), on the dependent variable using GLM procedure using the following model:

$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$

where, Y_{ijk} represents the observed collagenase activity for the ith level of factor A, the jth level of factor B, and the kth replicate. µ is the overall mean of the observed collagenase activity. α_i represents the effect of the ith level of Factor A, β_j represents the effect of the jth level of Factor B, $(\alpha\beta)_{ij}$ represents the interaction effect between the ith level of Factor A, the jth level of Factor B, ε_{ijk} accounts for the random variability not explained by the factors in the model.

Analysis of variance was performed to assess the significance of the main effects and interactions.

Table 1. Composition of mixed rations and feed block used for feeding sheep, goats and calves

	Sheep and goats rations		Calves ration	
	Low RDP	High RDP	TMR	Feed Block
Feedstuff (g/kg DM basis)				
Alfalfa hay	249	248	178	-
Wheat straw	249	248	59	-
Corn silage	-	-	132	-
Barley grain	199	298	111	-
Corn grain	-	-	241	-
Wheat bran	89	30	132	257
Soybean meal	121	40	123	-
Molasses	-	-	-	406
Urea	-	11	-	114
Limestone	11	11	3	-
Cement	-	-	-	113
Calcium carbonate	-	-	12	-
Salt	7	7	-	110
Mineral and vitamin mixture	4	4	9	-
Chemical and nutritional composition (g/kg DM)				
Dry matter[1]	895	896	700	867
Crude protein[1]	158	157	160	399
RDP[2]	102	121	105	386
UDP[2]	56	36	55	13
Crude fat[1]	26	21	35	12
NDF[1]	451	446	340	131
Ash[1]	80	80	65	246
Metabolizable energy[2] (MJ/kg)	9.577	9.531	11.338	7.782

1) Laboratory measurement
2) Calculated from tables (NRC, 2001; NRC,2007)
RDP: rumen degradable protein; UDP: undegradable rumen protein; NDF: neutral detergent fiber.

Results

Descriptive statistics for some measures are presented in Table 2. The mean concentration of blood free HYP in rams and goat bucks was approximately 6.5 times higher compared to calves and cows, indicating potential species-specific differences in collagen turnover. Moreover, collagenolytic activity exhibited age-related variations, with younger ruminants (calves) showing significantly higher activity compared to elder ruminants. Notably, rams and goat bucks exhibited at least 4 times higher collagenolytic activity compared to lactating cows, suggesting potential hormonal or metabolic influences.

Table 2. Descriptive statistics of some parameters in experimental animals

Variable	n	Mean	SD[1]	Minimum	Maximum
Calves (Male)					
Age (day)	5	202	31.1	163	243
Final weight (kg)	5	165	36.3	127	224
Daily gain (g/day)	5	719	230.1	437	977
Serum free HYP[2] (µmol/dL)	5	1.387	0.341	1.022	1.811
Collagenase activity (U/dL)	5	26.891	7.093	19.408	35.771
Calves (Female)					
Age (day)	7	237	41.8	177	289
Final weight (kg)	7	182	36.1	157	253
Daily gain (g/day)	7	736	146.1	597	1006
Serum free HYP (µmol/dL)	7	1.053	0.188	0.811	1.265
Collagenase activity (U/dL)	7	20.695	3.734	15.446	25.221
Calves (Both sexes)					
Age (day)	12	222	40.4	163	289
Final weight (kg)	12	175	35.6	127	253
Daily gain (g/day)	12	729	172	437	1006
Serum free HYP (µmol/dL)	12	1.192	0.302	0.811	1.811
Collagenase activity (U/dL)	12	23.276	6.006	15.446	35.771
Sheep (Baluchi)					
Age (day)	7	457	65.9	375	511
Final weight (kg)	7	65.3	6.77	55.7	72.2
Daily gain (g/day)	7	176	45.1	129	257
Plasma free HYP (µmol/dL)	7	7.219	3.748	2.709	11.327
Collagenase activity (U/dL)	7	0.626	0.313	0.070	0.982
Sheep (Kurdi)					
Age (day)	9	46.8	66.9	377	514
Final weight (kg)	9	69.8	9.34	58.4	83.9
Daily gain (g/day)	9	166	40.5	100	211
Plasma free HYP (µmol/dL)	9	6.237	2.866	2.734	11.103
Collagenase activity (U/dL)	9	0.594	0.232	0.279	0.920
Sheep (Both breeds)					
Age (day)	16	463	64.5	375	514
Final weight (kg)	16	67.8	8.38	55.7	83.9
Daily gain (g/day)	16	170	41.4	100	257
Plasma free HYP (µmol/dL)	16	6.696	3.223	2.709	11.327
Collagenase activity (U/dL)	16	0.609	0.263	0.070	0.982
Native goat bucks					
Age (month)	6	55.9	2.53	52.8	60.2
Final weight (kg)	6	76.6	5.67	70.0	86.5
Daily gain (g/day)	6	80	67.5	0.0	179
Plasma free HYP (µmol/dL)	6	5.979	2.739	3.866	10.956
Collagenase activity (U/dL)	6	0.443	0.363	0.086	0.933
Lactating cows					
Age (month)	3	62	2	60	64
Final weight (kg)	3	679	33.5	645	712
Daily gain (g/day)	3	0.0	0.0	0.0	0.0
Plasma free HYP (µmol/dL)	3	0.686	0.203	0.496	0.899
Collagenase activity (U/dL)	3	0.123	0.213	0.0	0.368

[1] Standard deviation.
[2] Hydroxyproline.

In sheep (Table 3), no significant differences were found for age, final weight, daily gain, plasma free HYP, and collagenolytic activity between breeds and rations. However, a notable finding was that rams on a high RDP ration displayed 33% lower collagenolytic activity compared to rams on the control ration (P=0.0769),

indicating a potential effect of diet on collagen metabolism. No significant interactions were found between ration and breed for any of the measured characteristics.

Table 3. Some characteristics measured in sheep on different treatments

Item	Breed		Ration		Treatment				Probability		
	Baluchi	Kurdi	Control	High RDP	BC[1]	BHR[2]	KC[3]	KHR[4]	B[5]	R[6]	B×R[7]
Age (day)	457	468	450	478	433	476	459	480	0.6800	0.3940	0.7622
Final weight (kg)	65.3	69.8	67.5	68.2	64.6	65.8	69.2	70.6	0.3241	0.7788	0.9812
Daily gain (g/day)	176	166	165	176	174	178	160	174	0.7186	0.7148	0.8335
Plasma free HYP[8] (µmol/dL)	7.219	6.237	7.409	5.880	7.204	7.231	7.533	4.078	0.4346	0.3462	0.3388
Collagenolytic activity (U/dL)	0.626	0.594	0.721	0.482	0.831	0.473	0.654	0.495	0.5704	0.0769	0.4700

[1]Baluchi on control ration; [2]Baluchi on high rumen degradable protein (RDP); ration; [3]Kurdi on control ration; [4]Kurdi on high RDP ration; [5]Breed; [6]Ration; [7]interaction.
[8]Hydroxyproline.

Table 4 provides the effects of different treatments on age, final weight, daily gain, and some biochemical parameters for goats. Goats on a high RDP ration exhibited significantly higher daily weight gain compared to goats on the control ration (P=0.0151). However, no significant differences were found for other measurements in goats, suggesting that the observed effect may be specific to growth parameters.

Table 4. Some characteristics measured in goat bucks on different treatments

Item	n	Ration		Probability
		Control	High RDP[1]	
Age (month)	6	54.5	57.3	0.2094
Final weight (kg)	6	72.8	80.4	0.1010
Daily gain (g/day)	6	25	136	0.0151
Plasma free HYP[2] (µmol/dL)	6	4.295	7.663	0.1426
Collagenolytic activity (U/dL)	6	0.242	0.645	0.1997

[1]Rumen degradable protein; [2] Hydroxyproline.

In calves (Table 5), no significant differences were found for age, final weight, daily gain, plasma free HYP, and collagenolytic activity between the two rations. However, notable distinctions emerged in age (P=0.0455), serum free HYP (P=0.0506), and collagenolytic activity (P=0.0546) between male and female calves on different rations. Additionally, a noteworthy interaction between sex and ration was discerned concerning final weight (P=0.0436) and daily weight gain (P=0.0480).

Table 5. Some characteristics measured in calves on different treatments

Item	Sex		Ration		Treatment				Probability		
	Male	Female	Control	C+F[1]	MC[2]	MCF[3]	FC[4]	FCF[5]	S[6]	R[7]	S×R[8]
Age (day)	202	237	209	241	209	191	210	274	0.0455	0.2267	0.4620
Final weight (kg)	165	182	169	183	179	142	161	210	0.2136	0.7455	0.0436
Daily gain (g/day)	719	736	765	693	893	544	680	792	0.8558	0.2508	0.0480
Serum free HYP[9] (µmol/dL)	1.387	1.053	1.162	1.235	1.272	1.559	1.080	1.018	0.0506	0.4997	0.3061
Collagenolytic activity (U/dL)	26.891	20.695	22.140	24.867	23.882	31.403	20.834	20.509	0.0546	0.2790	0.2411

[1]Control+Feed block; [2] Male on control ration; [3] Male on control+feed block ration; [4] Female on control ration; [5] Female on control+feed block ration; [6] Sex; [7] Ration; [8] Interaction.
[9] Hydroxyproline.

Table 6 demonstrates the inhibitory effect of copper ion, specifically cupric sulfate in aqueous solution, on collagenolytic activity. The results indicated a strong inhibition of more than 90% of collagenolytic activity by copper ion. Notably, a significant tendency for inhibition (P=0.0752) was found in calves on feed blocks included in their ration.

Correlation coefficients between collagenolytic activity and other measures within each ruminant species are shown in Tables 7. Strong significant positive

correlations were found between collagenolytic activity and blood free HYP for all ruminant species. Specifically, in rams, a significant positive correlation (r=0.54; P=0.0398) was found between collagenolytic activity and daily weight gain. In contrast, in calves, a significant negative correlation (r=-0.69; P=0.0133) was observed between collagenolytic activity and age.

Table 6. Effect of heavy metal ions on collagenolytic activity (U/dL) in calves on different treatments

	Sex		Ration		Treatment				Probability		
Item	Male	Female	Control	C+F[1]	MC[2]	MCF[3]	FC[4]	FCF[5]	S[6]	R[7]	S×R[8]
Serum control	21.689	16.739	17.903	20.059	19.278	25.306	16.872	16.561	0.0507	0.2728	0.2280
Serum + 10 mM cupric sulfate	1.085	0.815	0.945	0.903	1.064	1.116	0.855	0.761	0.0386	0.8593	0.5406
Activity inhibition (%)	94.9	95.1	94.7	95.4	94.5	95.5	94.9	95.3	0.7837	0.0752	0.4183

[1]Control+Feed block; [2] Male on control ration; [3] Male on control+feed block ration; [4] Female on control ration; [5] Female on control+feed block ration; [6] Sex; [7] Ration; [8] Interaction.

Table 7. Correlation of collagenolytic activity (U/dL) with some characteristics in experimental animals

Species	n	Age (day)	Final weight (kg)	Daily gain (g/day)	Blood free HYP[1] (µmol/dL)
Sheep	16	-0.31	-0.17	0.54	0.70
Probability		0.2678	0.5330	0.0398	0.0036
Goat bucks	6	0.42	0.08	0.56	0.89
Probability		0.4090	0.8788	0.2485	0.0181
Calves	12	-0.69	-0.46	-0.39	0.98
Probability		0.0133	0.1346	0.3735	<0.000
Lactating cows	3	0.87	0.03	-	0.91
Probability		0.3333	0.9835	-	0.2717

[1] Hydroxyproline.

Discussion

In the present study, collagenolytic activity was measured based on the difference in concentration of HYP in the media containing bovine Achilles collagen before and after the addition of samples. Collagen is known for its resistance to other proteases due to its tightly packed structure (Ramachandran and Kartha, 1955; Orgel et al., 2006; Rosenblum et al., 2010). Bannister and Burns (1972) reported that serum collagenase enzyme(s) differ from bacterial collagenase in that they are not inhibited by cysteine or EDTA, as is the case with bacterial collagenase (Seifter et al., 1970), suggesting that the enzyme(s) does not contain a metal ion essential for activity. Also, in this context, for the sake of data simplification, enzyme activity was quantified as units per 100 mL of the sample, yielding values roughly an order of magnitude greater than those documented by Gries et al. (1970) for human serum.

The HYP is predominantly present in collagen, representing 13% of the amino acids, while other animal proteins contain insignificant quantities of HYP (Rosol and Capen,1997). During collagen degradation, HYP is released and not reutilized (Rosol and Capen, 1997; Neuman and Logan, 1950). In the present study, sheep and goats exhibited higher HYP concentrations in their plasma compared to cattle species (calves or lactating cows). Varghese et al. (1981) reported that sheep plasma contains at least three fractions of HYP: free, protein-bound, and peptide-bound. However, Siddiqi and Alhomida (2000) reported higher concentrations of free HYP in sheep plasma (9.517 ± 1.81), which is 42% higher than the HYP concentration observed in sheep in the present study. It is important to note that Siddiqi and Alhomida did not provide information regarding the age and weight of their sheep. The considerable variation in HYP observed in the present study may be attributed to species differences, as described earlier by Siddiqi et al. (2001). Black and Capen (1971) reported that plasma HYP levels in cows increased from 0.41 ± 0.06 µg/mL at 4 weeks prepartum to 1.01 ± 0.14 µg/mL at 3 days prepartum. The HYP value in their study closely aligns with the findings of the present study (Table 2). Almeida et al. (2006) reported that in six-month-old Boer goat bucks, free HYP in plasma ranged from 2.54 to 7.53 µmol/dL. The HYP values in the present study fall within the reported range. Furthermore, Almeida et al. (2006) found that nutritionally restricted feeding led to significantly higher HYP concentrations in goat bucks compared to those on a normal feeding.

Notable differentiations surfaced in age (P=0.0455), serum free HYP (P=0.0506), and collagenolytic activity

(P=0.0546) between male and female calves subjected to distinct rations. Moreover, a pronounced interaction between sex and ration was observed concerning final weight (P=0.0436) and daily weight gain (P=0.0480), underscoring a nuanced, sex-dependent responsiveness to varying dietary factors.

In some enzymes, cysteine amino acid residues possess sulfhydryl groups. These sulfhydryl groups play a role in the catalytic effect of enzymes and in the binding of substrates, coenzymes, and metal ions. Collagenolytic activity was strongly inhibited by Cu^{2+} ions, suggesting the involvement of sulfhydryl groups, and to a lesser extent, histidine (Bannister and Burns, 1972). In present study, notably, a tendency for inhibition (P=0.0752) was observed in calves on feed blocks included in their ration, indicating a potential impact of dietary components on collagen metabolism. The establishment of a copper-sulfhydryl linkage results in the suppression of collagenase activity, leading to low collagenolytic activity in the samples. These results are in agreement with Bannister and Burns (1972) who have reported more than 90% inhibition in collagenolytic activity in avian serum by adding 10 mM cupric sulfate.

A significant positive correlation was found between collagenolytic activity and free HYP in the blood of different ruminant species in the present study. Collagenase action leads to the release of HYP during collagen degradation, and HYP is not reused (Rosol and Capen, 1997; Neuman and Logan, 1950). The presence of enzymes associated with collagen degradation in the blood offers the possibility of using an easily measured parameter to study this important component of connective tissue. Bannister et al. (1970) demonstrated the high concentration of HYP in the blood of domestic fowl. It appears that the concentration of HYP, as a product of collagenase activity, does not suppress its activity. In sheep, a positive significant correlation was found between daily weight gain and collagenolytic activity. This finding is in agreement with Bannister and Burns (1972), who demonstrated a relationship between the concentration of a serum collagen-degrading enzyme and growth rate, and thus age, in domestic fowl. In present study, strong significant positive correlations were found between collagenolytic activity and blood free HYP for all ruminant species, indicating a consistent association between collagen turnover and HYP release. Specifically, in rams, a significant positive correlation (r=0.54; P=0.0398) was found between collagenolytic activity and daily weight gain. In contrast, in calves, a significant negative correlation (r=-0.69; P=0.0133) was observed between collagenolytic activity and age, suggesting an age-dependent modulation of collagen turnover. In contrast, a negative significant correlation was observed between age and collagenolytic activity in calves. Aging affects the body's collagen in two ways: first, as animals age, the body's ability to produce collagen protein slows down, resulting in insufficient new collagen for the skin, joints, and other body parts; second, the inhibition of cross-linking within collagen fibrils leads to a drastic reduction in the tensile strength of the fibrils, making collagenous tissues fragile and prone to tearing, such as skin, tendons, and blood vessels (Patel, 2004). Bannister and Burns (1972) reported that non-growing birds had no measurable collagenolytic activity in their serum, suggesting that the enzyme(s) may be linked to different degrees of growth. Another possibility is the presence of an enzyme inhibitor in the serum of older animals that masks the activity. Rajabi et al. (1991) demonstrated a marked increase in collagenase inhibitory activity, indicating the presence of a strong regulatory mechanism to protect against extensive collagen degradation beyond what is necessary. However, further investigation is needed to explore these hypotheses, which would require the purification of the enzyme(s).

Conclusion

In conclusion, the present study demonstrates the presence of enzyme(s) in the blood plasma or serum of sheep, goats, calves, and lactating cows that exhibit collagenolytic activity, as measured using bovine Achilles' tendon collagen as a substrate. The collagenolytic activity of the enzyme(s) in calves' serum was strongly negatively correlated with age and significantly inhibited by the presence of cupric sulfate as a metal ion. However, no detectable collagenolytic activity was observed in the blood of non-growing sheep, goats, and lactating cows under the conditions of the present study.

Future research should focus on purifying the enzyme(s) responsible for collagen degradation and investigating the factors that influence the inhibition of these enzyme(s). Understanding the specific properties and mechanisms of these enzyme(s) will provide valuable insights into the regulation of collagen metabolism in different species and may have implications for various physiological processes and diseases related to collagen homeostasis.

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