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Heparin Diversification

Sheep Heparins as an Alternate for Porcine Heparins

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- Porcine, Ovine and Bovine Heparin Structural Difference
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Heparin Usage Worldwide

- Heparin, especially LMWH, is used extensively in developed countries.
- Historic, heparin is extracted from porcine, bovine and ovine mucosa or lung.
- Since the outbreak of BSE in 1990s, porcine heparin is the sole source for US and Europe.
- Bovine is still used in South America and some Muslin countries.

- Heparin is significantly under-used in Muslin countries.
  - Lower income, lower health spending
  - Porcine source is an issue for Muslin population

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Why Need Heparin Diversification?

- 90% of porcine intestines have been used for heparin production.
- Single source and 70% from China
- Heparin usage increases as world population income grow.
- Muslin countries are seeking alternative heparin sources.
- Worldwide ovine and bovine population are significant and 80% are farmed outside of China.

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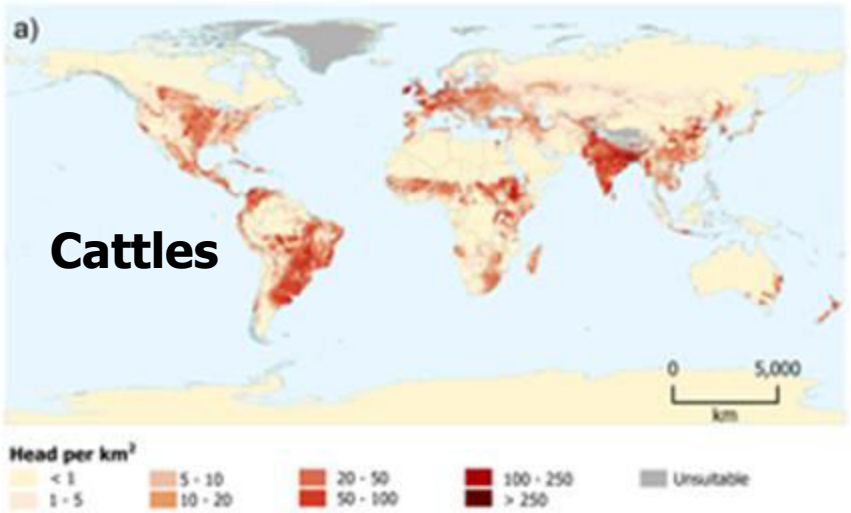
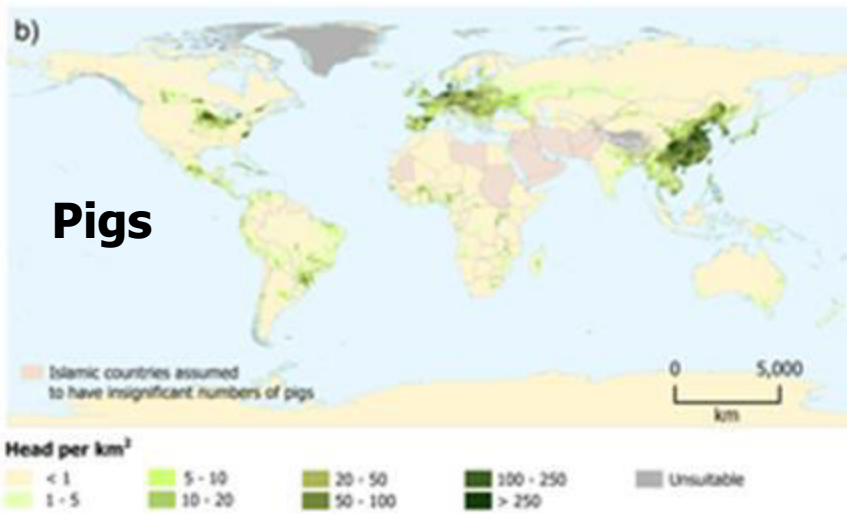
Worldwide Livestock Population



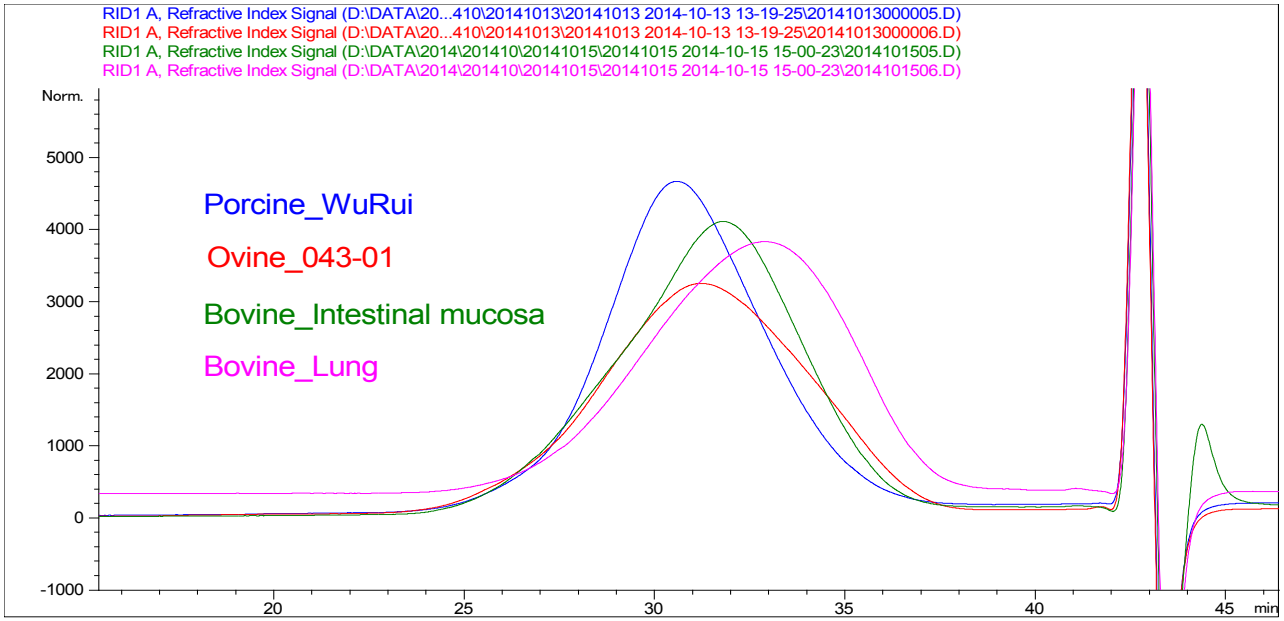


2010 World Sheep Inventory - Top 10 countries

Country	Number of head
China	134,021,213
India	73,991,000
Australia	68,085,500
Iran	54,000,000
Sudan	52,014,100
Nigeria	35,519,800
New Zealand	32,562,600
United Kingdom	31,000.000
Pakistan	27,800,000
Ethiopia	25,979,900
Total	**1,078,948,201**

Worldwide, there are 1.4 billion cattle, 1.9 billion sheep and goats, 980 million pigs.

Heparin API MW Distribution



Item	Mw(Da)	M_{24000}	$M_{8000-16000}$/ $M_{16000-24000}$
Specifications (EP8.0)	15,000-19,000	NMT 20%	NLT 1.0
Porcine_HS	17,685 Da	16.6 %	1.2
Ovine_HS	16,804 Da	17.2 %	1.7
Bovine intestinal mucosa_HS	16,417 Da	16.6 %	2.1
Bovine lung_HS	13,588 Da	8.9 %	2.7

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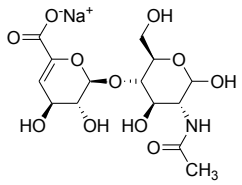
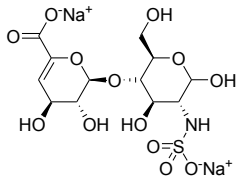
Disaccharide Analysis



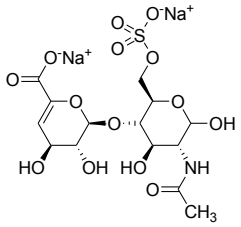
1#

△ ⅣA



2#

△ ⅣS



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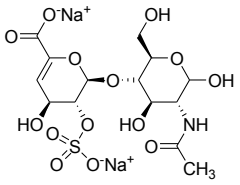

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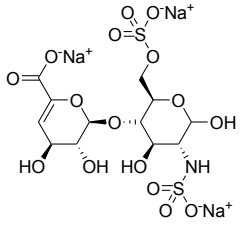
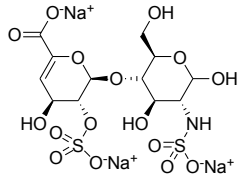


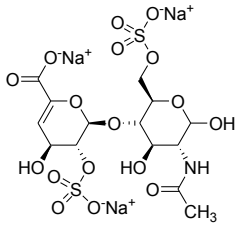
6#

△ ⅡS



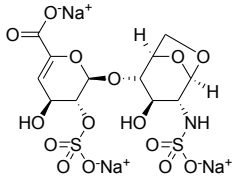
7#

△ ⅢS



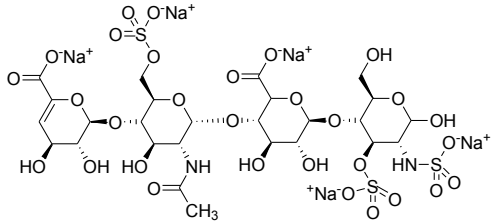
8#

△ ⅠA



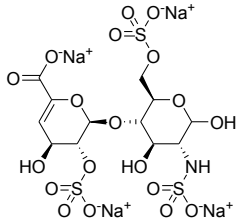
9#

1,6-Anhydro △ ⅠS or
1,6-Anhydro △ ⅠS glucose



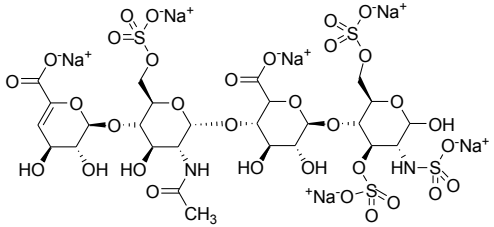
10#

△ ⅡA-△ ⅣSglu



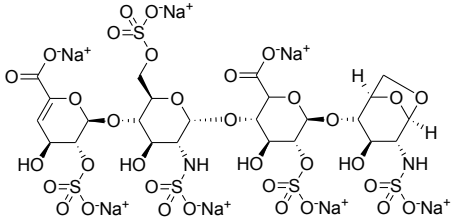
11#(Main peak)

△ ⅠS



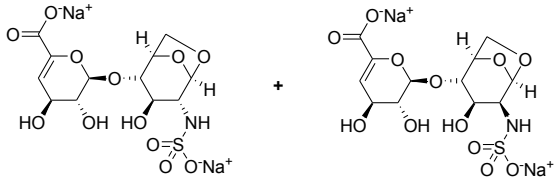
13#

△ ⅡA-△ ⅡSglu



14#

1,6-Anhydro △ ⅠS-ⅠS epi or
1,6-Anhydro △ ⅠS-ⅠS mannose

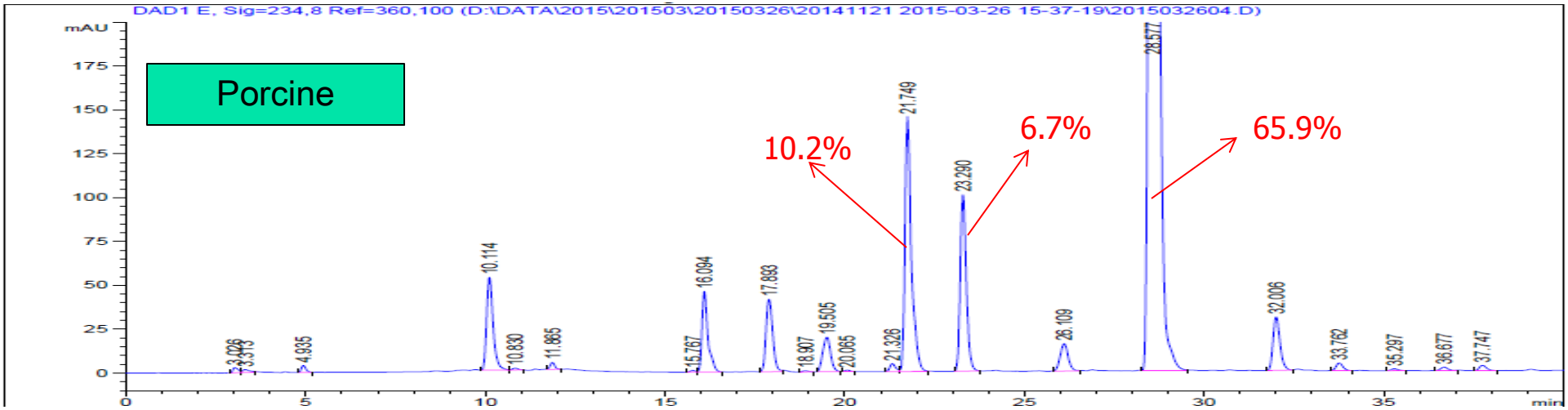


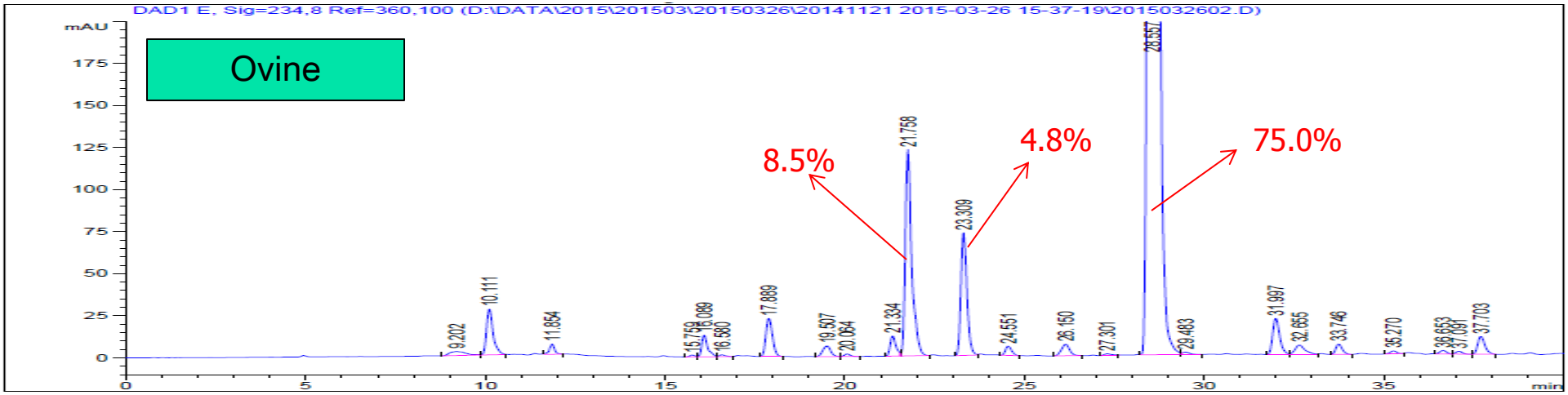
4#

1,6-Anhydro △ ⅡS or
1,6-Anhydro △ ⅡS glucose

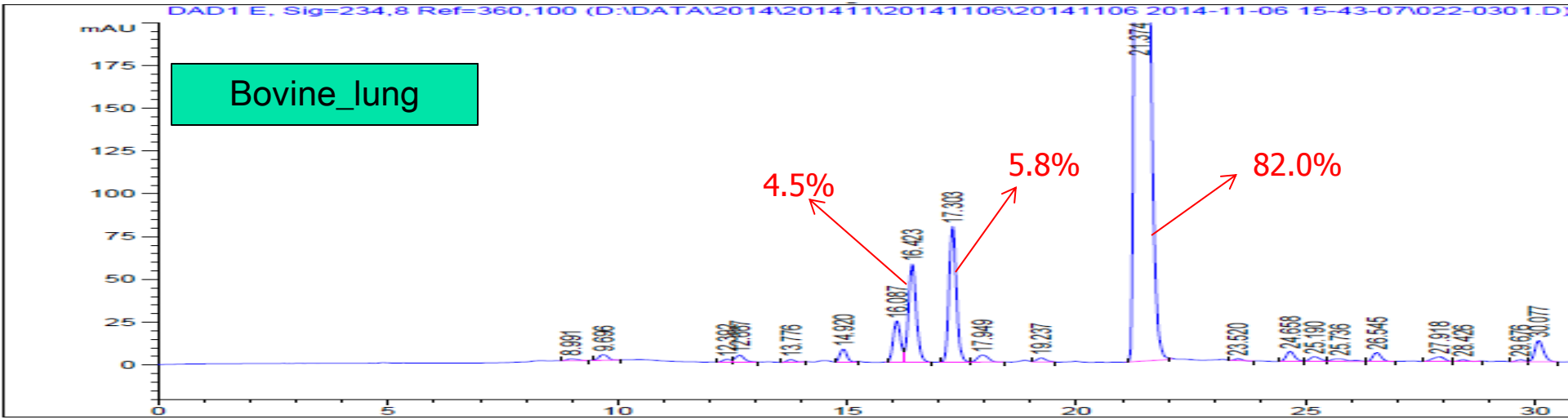
1,6-Anhydro △ ⅡS epi or
1,6-Anhydro △ ⅡS mannose

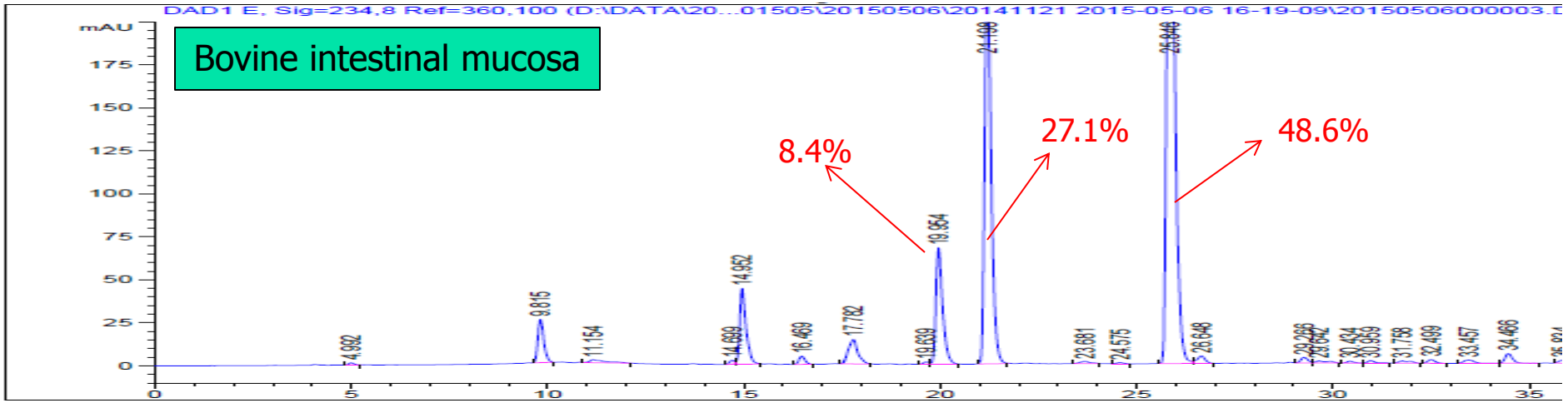
Disaccharide Analysis





Disaccharide Analysis





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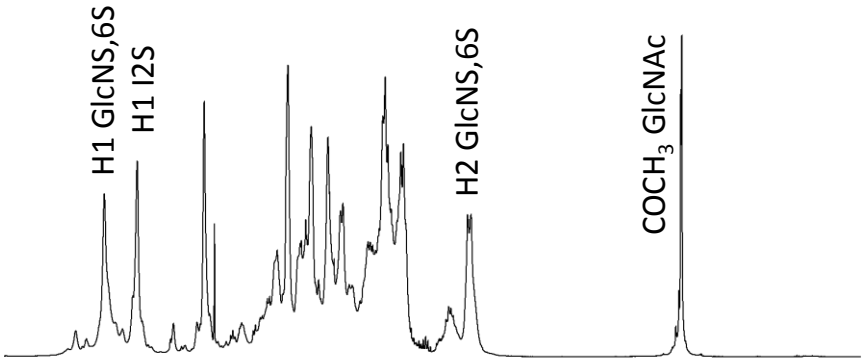
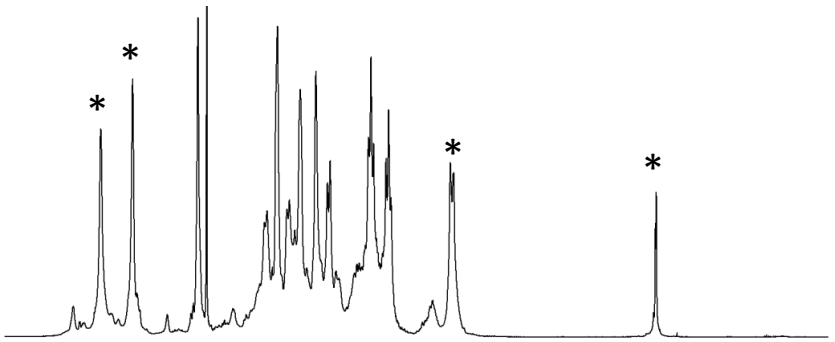
^{1}H-NMR

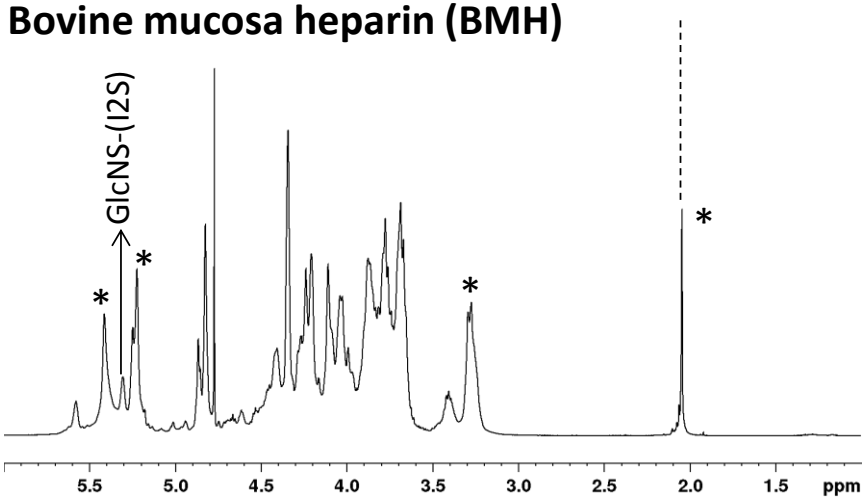
Porcine mucosa Heparin (PMH)



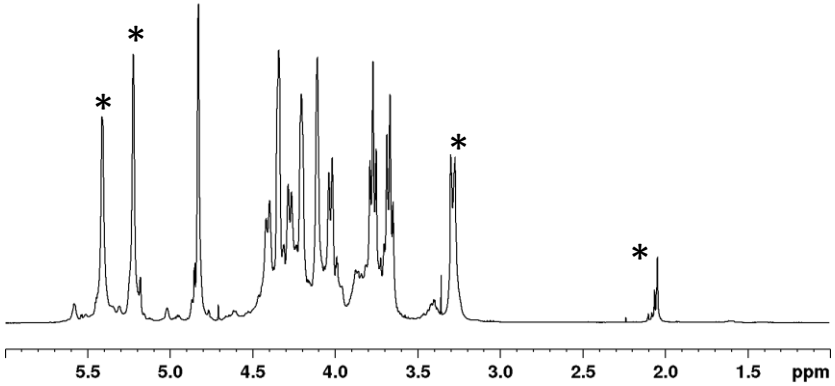
Ovine mucosa heparin (OMH)



Bovine mucosa heparin (BMH)

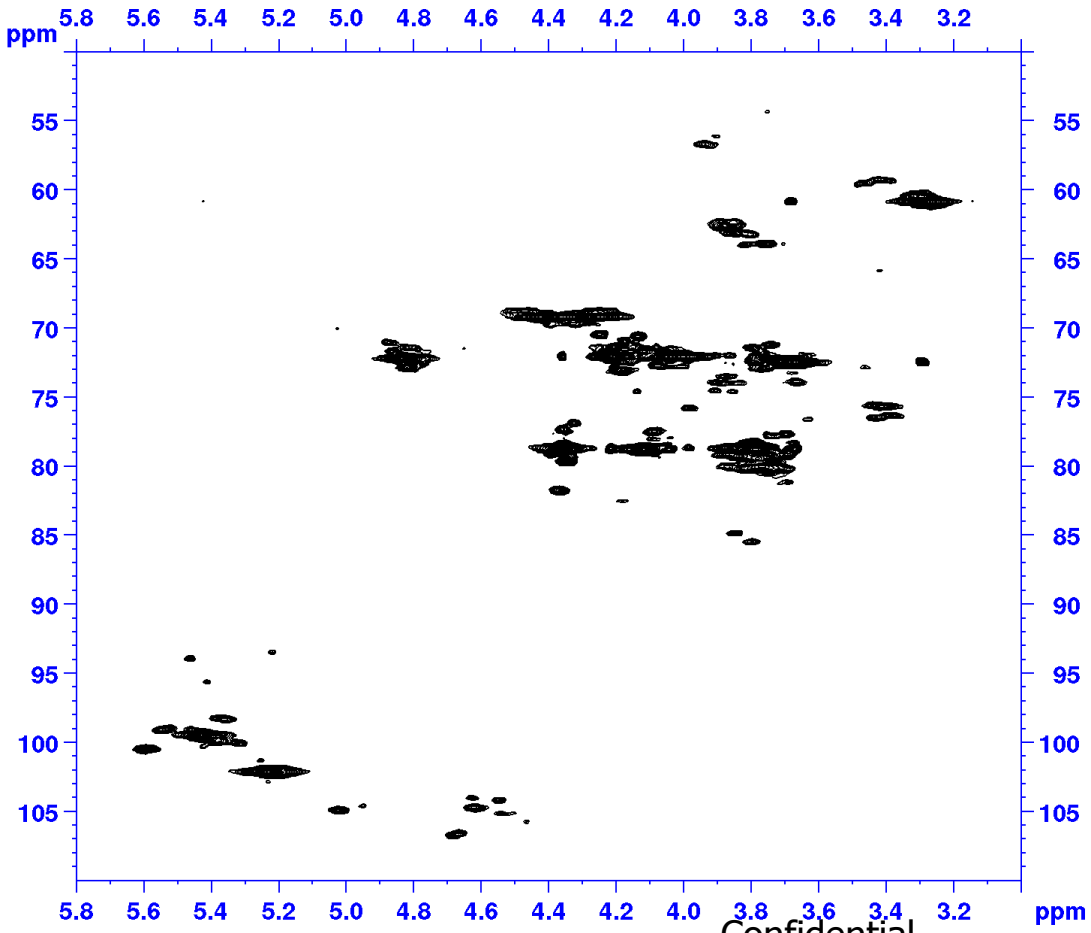


Bovine lung heparin (BLH)



2D-HSQC-NMR

campione G11480_OMH
temperatura 298 K
analisi PCA
SR -45.32 -435.9
data 12/06/2015




```
Current Data Parameters
NAME          G11480_OMH
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150614
Time               3.46
PROBHD   5 mm CPTCI 1H-
PULPROG  hsqcetgpsisp2.2
TD                  1024
SOLVENT              D2O
NS                     8
DS                    16
TE                298.0 K
CNST2         150.0000000
CNST17         -0.5000000
D0              0.00000300 sec
D1              2.50000000 sec
D4              0.00166667 sec
D11             0.03000000 sec
D16             0.00020000 sec
D24             0.00086207 sec
IN0             0.00004150 sec

======== CHANNEL f1 ========
SFO1          600.1328208 MHz
NUC1                    1H
P1                   11.12 usec
P2                   22.24 usec
P28        0 usec
PLW1           7.00000000 W

======== CHANNEL f2 ========
SFO2          150.9148812 MHz
NUC2                    13C
CPDPRG[2              garp4
P3                   13.00 usec
P14                 500.00 usec
P24                2000.00 usec
PCPD2                60.00 usec
PLW0       0 W
PLW2         123.00000000 W
PLW12          5.77419996 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3                0.500
SPOFFS3    0 Hz
SPW3          31.76000023 W
SPNAM[7]     Crp60comp.4
SPOAL7                0.500
SPOFFS7    0 Hz
SPW7          31.76000023 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPNAM[4]      SMSQ10.100
GPZ1                80.00 %
GPZ2                20.10 %
GPZ3                11.00 %
GPZ4                -5.00 %
P16               1000.00 usec
P19                600.00 usec

F1 - Acquisition parameters
TD                   320
SFO1            150.9149 MHz
FIDRES         37.650604 Hz
SW                79.834 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                  4096
SF            600.1299547 MHz
WDW                 QSINE
SSB                     2
LB         0 Hz
GB         0
PC                   1.40

F1 - Processing parameters
SI                  1024
MC2         echo-antiecho
SF              150.9023731 MHz
WDW                 QSINE
SSB                     2
LB         0 Hz
GB         0
```

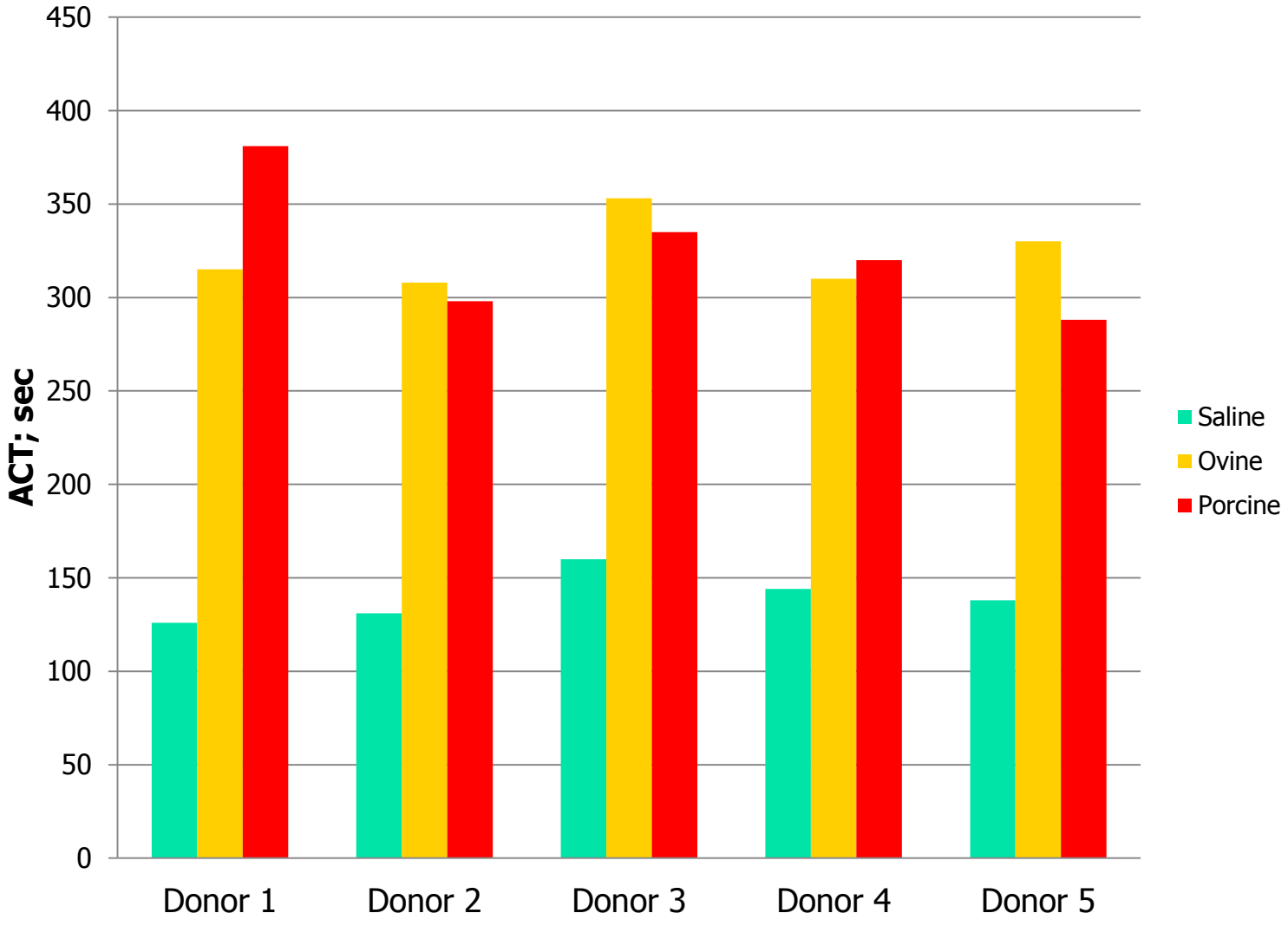
2D Quantitative HSQC NMR

Amines												
Ronzoni code	***Ronnsi code***	**A1NS-(I2S)**	**A1NS-I**	**A1NS-G**	**A***	**ANAc-G**	**ANAc-I**	**ANH2**	**ANSared**	**ANAc ared**	**A-(GalA)+A?**	**A6S**
G11480	RXJL-0009-SJT-108-01	69.8	6.0	8.9	6.5	4.2	*0.3*	*0.4*	1.4	*1.0*	1.1	86.6
G11481	RXJL-0009-SJT-105-01	68.7	5.5	10.6	6.3	4.2	*0.2*	*0.0*	1.0	*1.2*	1.8	84.7
G11482	RXJL-0009-SJT-108-02	67.6	5.4	10.5	6.6	5.3	*0.3*	*0.5*	1.6	*0.9*	0.8	85.5
mean		***68.7***	***5.7***	***10.0***	***6.5***	***4.6***	***0.3***	***0.3***	***1.3***	***1.0***	***1.2***	***85.6***
Porcine mean		59.0	8.4	9.5	6.7	12.2	0.6	1.1	0.4	0.6	1.3	77.2
Uronic acid		**I2S**	**I-A6S**	**I-A6OH**	**G-A***	**G-ANS**	**G-Anac**	**Epox**	**GalA**	**G2S**		
G11480	RXJL-0009-SJT-108-01	83.2	4.8	*1.2*	1.5	5.5	2.4	*0.4*	*0.4*	*0.6*		
G11481	RXJL-0009-SJT-105-01	83.0	4.9	*1.2*	1.5	5.2	2.6	*0.3*	*0.5*	*0.7*		
G11482	RXJL-0009-SJT-108-02	83.7	4.6	*1.1*	1.7	5.6	2.0	*0.5*	*0.5*	*0.4*		
mean		***83.3***	***4.8***	***1.2***	***1.6***	***5.5***	***2.3***	***0.4***	***0.4***	***0.6***		
Porcine mean		72.3	5.6	*2.9*	2.4	6.7	6.3	*0.3*	*1.2*	*0.0*		
LR		**(Gal1+G)/2 (LR)**	**% Ser ox**			**Sulf deg.**						
G11480	RXJL-0009-SJT-108-01	2.8	96.1			2.71						
G11481	RXJL-0009-SJT-105-01	2.9	97.7			2.69						
G11482	RXJL-0009-SJT-108-02	2.5	94.1			2.69						
mean		***2.7***	***96.0***			***2.69***						
Porcine mean		1.3	95.0			2.42						

Anticoagulant activity

Item	Specifications (EP8.0)	Porcine	Ovine	Bovine lung	Bovine intestinal mucosa
Batch no.	/	/	**RX0009-SJT-105-1**	RX0014-JCJ-045	RX0014-JCJ-046
Anti-Xa	NLT 180 IU/mg Calculated on a dry basis	>180	**181.5**	135.1	123.8
Anti-IIa	/	>180	**180.9**	126.2	168.7
Anti-Xa/ Anti-IIa	/	~1:1	**1.00**	1.07	0.73
Anticoagulant activity (by sheep plasma)	/	>180	**179**	152	167

Heparins in Human Whole Blood ACT



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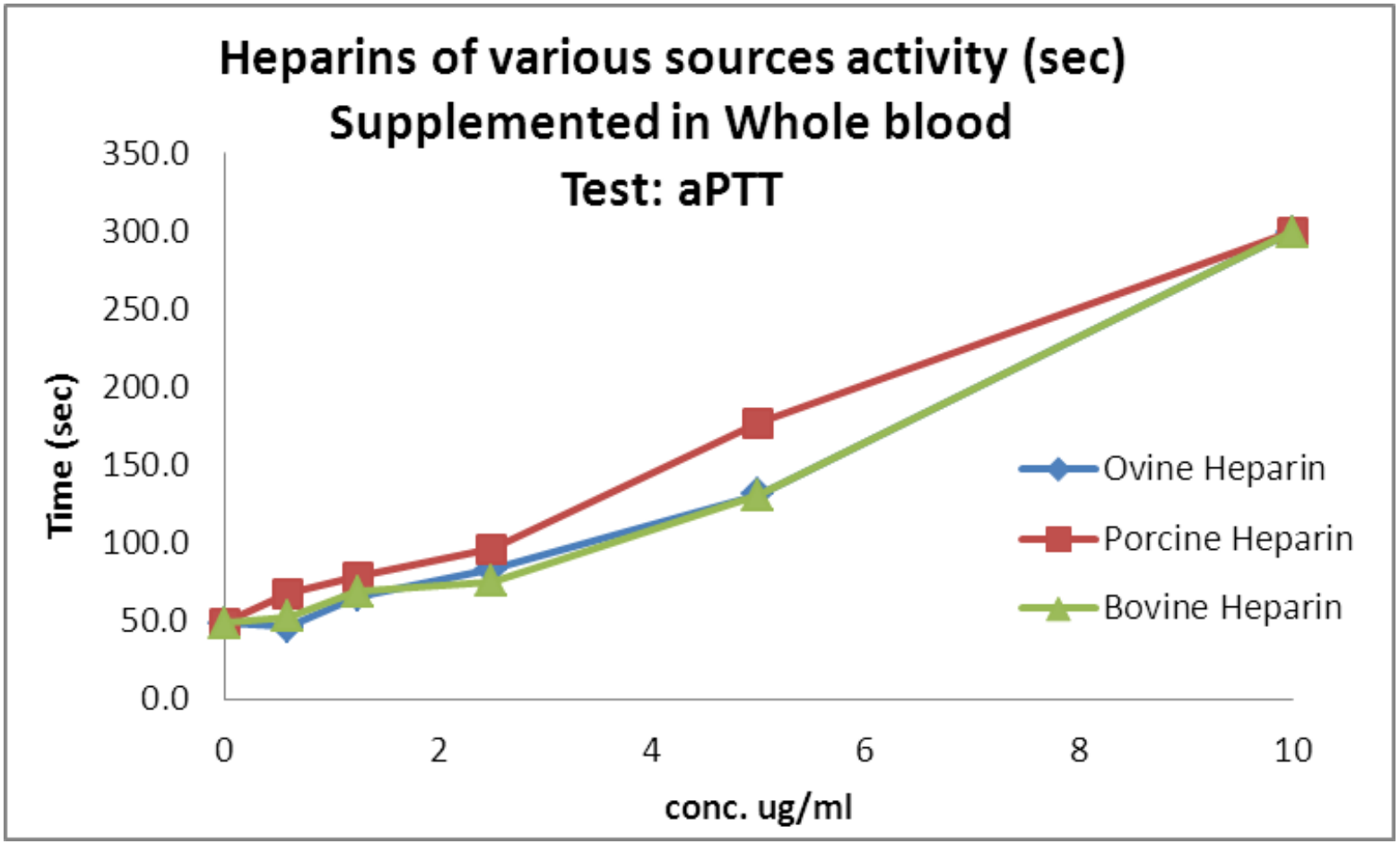
Whole Blood Clotting Times



At an equigravimetric level all agents produced similar effects.

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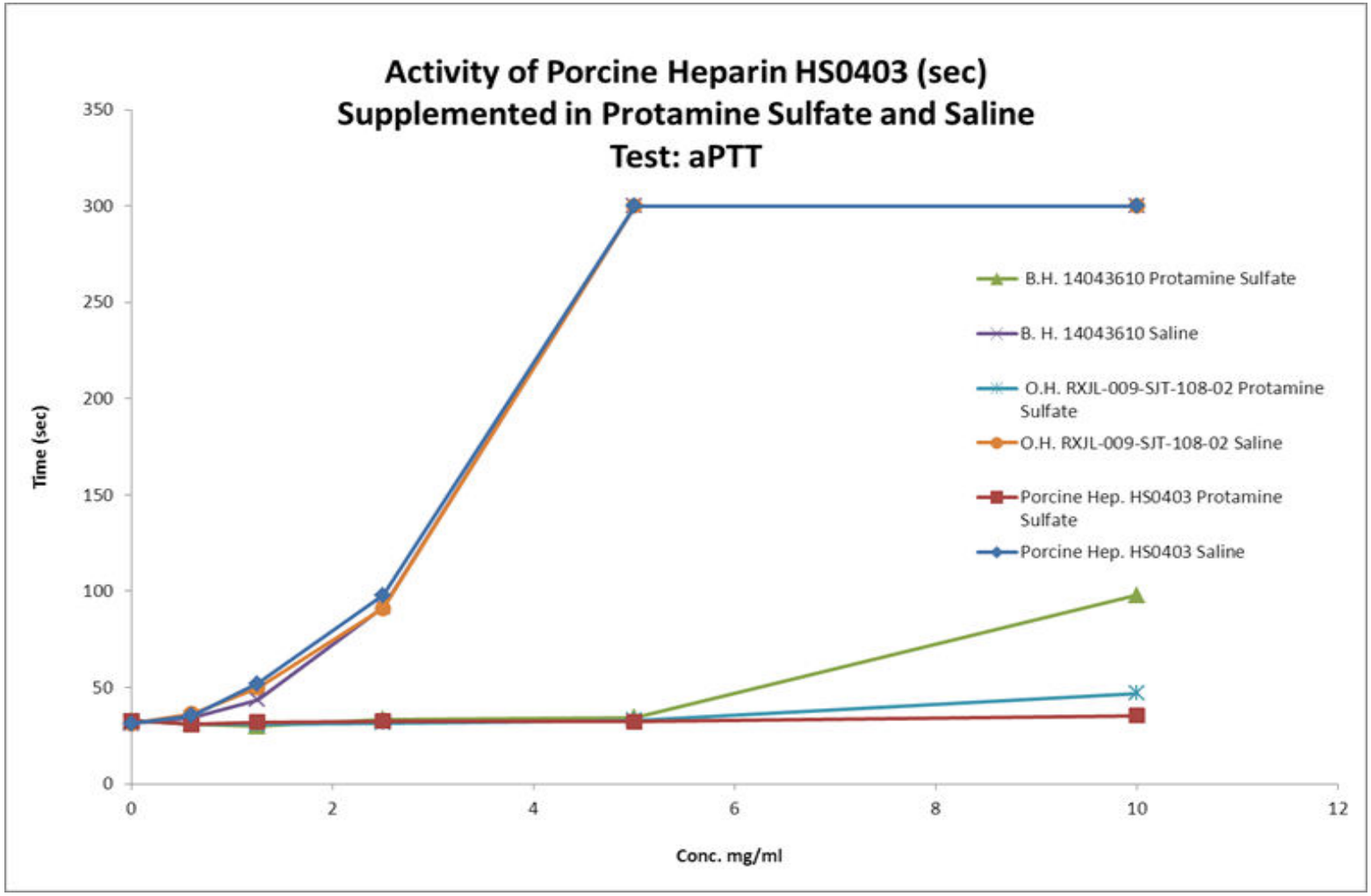
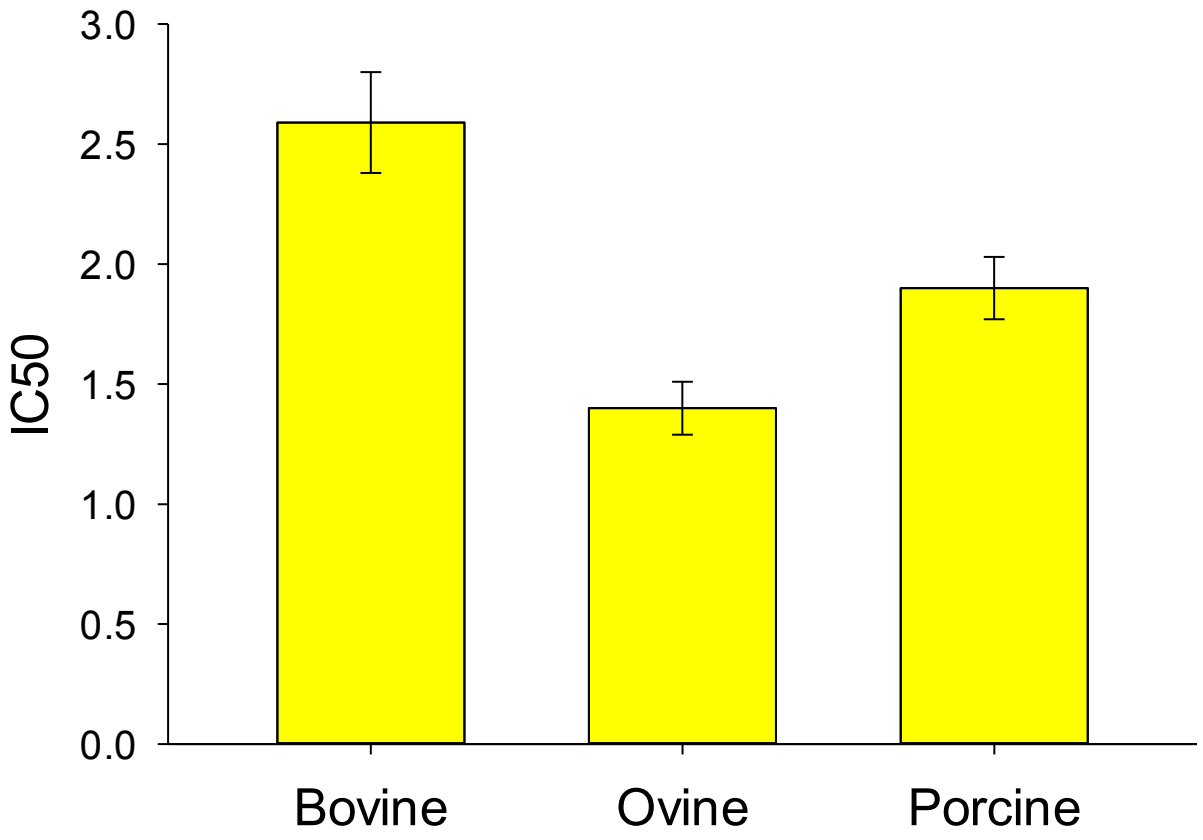
Neutralization by Protamine Sulfate



Relative Inhibition of Factor Xa in an AT Supplemented System



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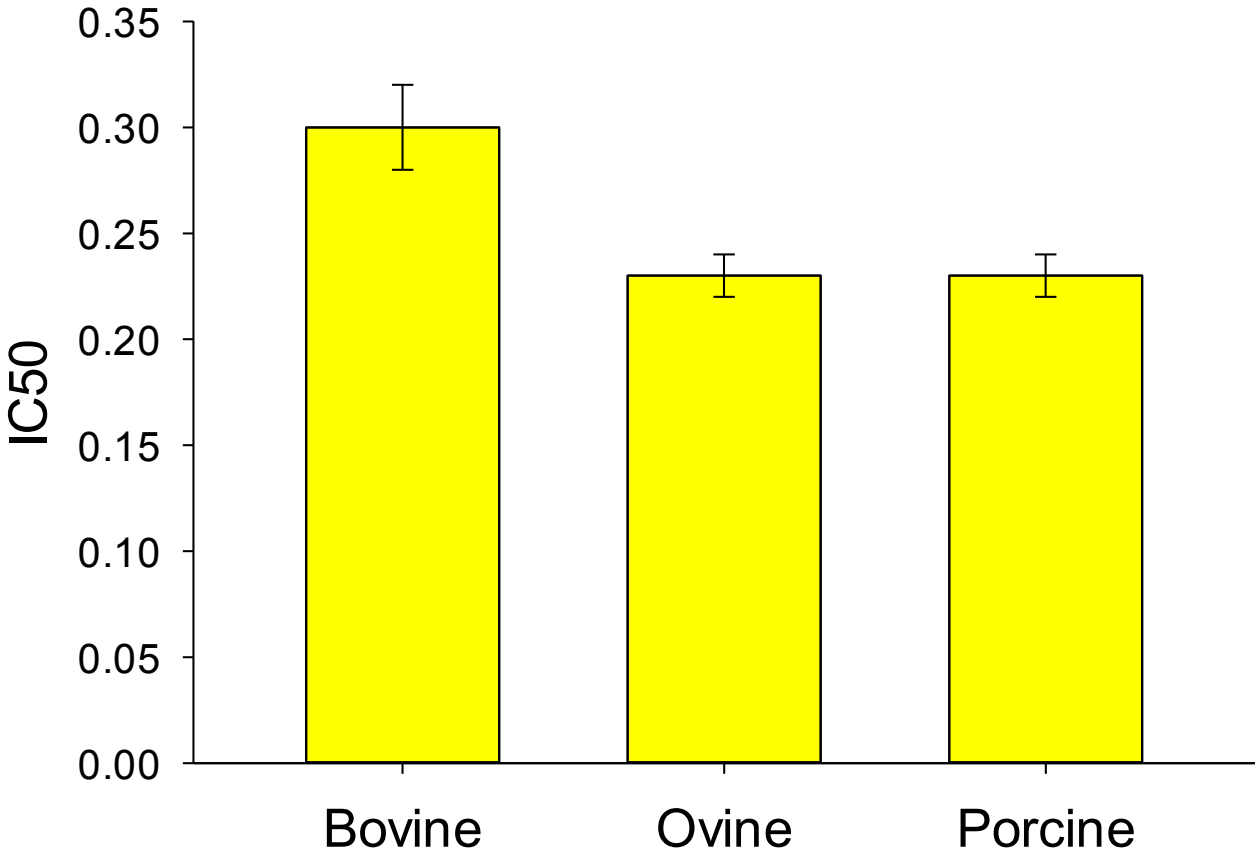
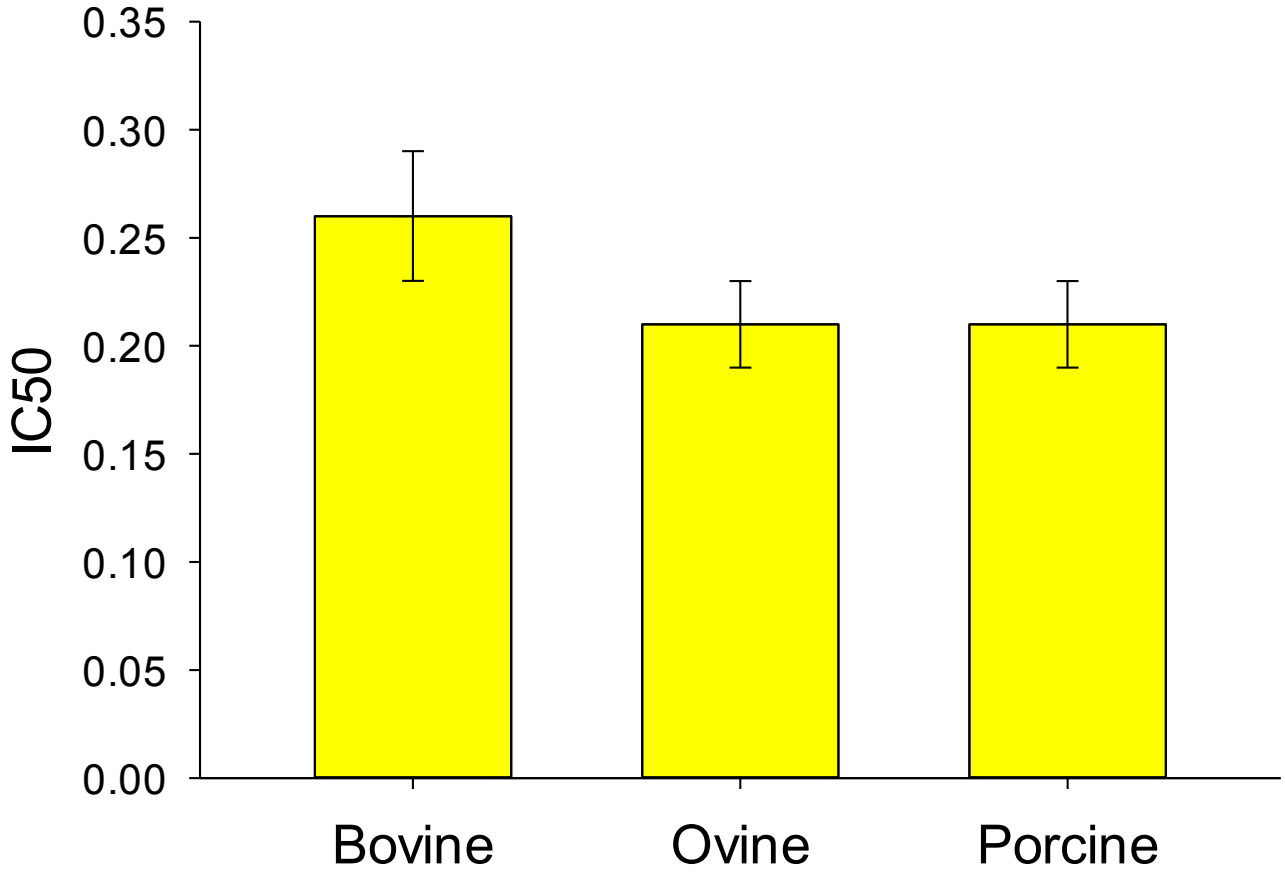
Relative Inhibition of Factor IIa in an AT Supplemented System



Thrombin Generation Inhibition
Technoclone Thrombin Generation Assay



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Conclusions

1.MW, disaccharide analysis and 1D, 2D NMR were performed for porcine, bovine and ovine purified heparin.

2.Overall ovine heparin is structurally closer to porcine. Significant difference was observed for bovine heparin in disaccharide and NMR.

3.In the whole blood supplementation studies the bovine, ovine and porcine exhibited similar anticoagulant activity as measured by ACT, APTT.

1.Protamine sulfate neutralization was comparable except the bovine heparins were only partially neutralized at higher concentrations.

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Conclusions

6. The anti-Xa and anti-lia activity of ovine and porcine heparins were comparable and were much higher than bovine heparin.

6. The AT supplemented systems ovine and porcine heparins exhibited stronger inhibition of both Xa and IIa in comparison to bovine heparin.

7. These studies suggest that ovine heparin is functional comparable to porcine heparin.

Acknowledgments

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Ronzoni Institute

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