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DIURNAL AND SEASONAL VARIATIONS OF SERUM ENZYME ACTIVITY IN CATTLE AND SHEEP

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Variations in serum enzyme activity during the day and changes due to environmental and dietary conditions are sparsely investigated both in man and animals. Knowledge about variations of this kind is of significance for the interpretation of serum enzyme changes due to pathological conditions, and particularly in studies on physiological factors affecting these enzymes.

It has previously been shown in cattle and sheep that highly significant variations between individuals exist in some serum enzymes increasingly used in clinical diagnostic work, such as aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), total lactate dehydrogenase (EC 1.1.1.27), and α-hydroxybutyrate dehydrogenase (*Tollersrud* 1969).

The present paper includes results from further two investigations on physiological variations of these enzymes in cattle and sheep:

A. Variations during the day under practical indoor feeding conditions.
B. Changes following the transfer from indoor feeding to spring pasture.

MATERIAL AND METHODS

Investigation A

Cattle. Blood was taken from six adult, lactating cows at three hrs. interval from 6 a.m. to 9 p.m. during one day. The feeding schedule was:

5.30 a.m. Concentrates, carbohydrates
6.00 a.m. Swedes
6.30 a.m. Alkali treated straw
7.30 a.m. Grass silage
2.30 p.m. Concentrates, protein-rich
3.00 p.m. Swedes
3.30 p.m. Hay

All animals were fed the same amounts of roughages; concentrates were given according to milk yields.

Sheep. Six adult non-pregnant, penned ewes were bled at the same times as described for cows. The daily feeding was as follows:

6 a.m. Concentrates
7 a.m. Grass silage
3 p.m. Hay

Investigation B

Cattle. Blood was collected from 16 dairy cows at 9 a.m. on the day before putting the animals out to pasture. Blood was further taken from the same animals and at the same time of the day after two weeks of acclimatization at pasture. During the first week the animals were partly fed indoors, while the next week they were kept on pasture except during milking.

Sheep. Blood from 23 adult ewes and 36 nursing lambs at the age of about four weeks was taken on the day before turning out to pasture and after one and two weeks outdoors. The sheep were kept full time on pasture after the transfer.

Analytical procedures

The enzyme analyses were performed within two days, the dehydrogenases within the day of sampling and the transferases on the following day.

Aspartate aminotransferase (AspAT = GOT) and alanine aminotransferase (AlAT = GPT) were analysed according to Technical Bulletin No. 505 (1964), from Sigma Chemical Company. The activity is expressed as Sigma-Frankel units. Total lactate dehydrogenase (LDH) was analysed according to Sigma Technical Bulletin No. 500 (1960). The activity is expressed as Berger-Broida units. α-hydroxybutyrate dehydrogenase (HBD) was analysed according to Sigma Technical Bulletin No. 495 (1964), and the activity expressed as Sigma units of HBD.

Statistical procedures

Statistical procedures were according to standard techniques. In Investigation A analyses of variance based in principle on a model including animals with random effects and time of the day with fixed effects were used. Interaction between these factors was presumed negligible according to the results of *Hagemeister & Unshelm*

(1968). (Both coefficients in the component analyses were 6). Intraclass correlations used as repeatabilities as well as the coefficients of variation were calculated to investigate the precision of laboratory methods.

In Investigation B the t-test of paired observations was used.

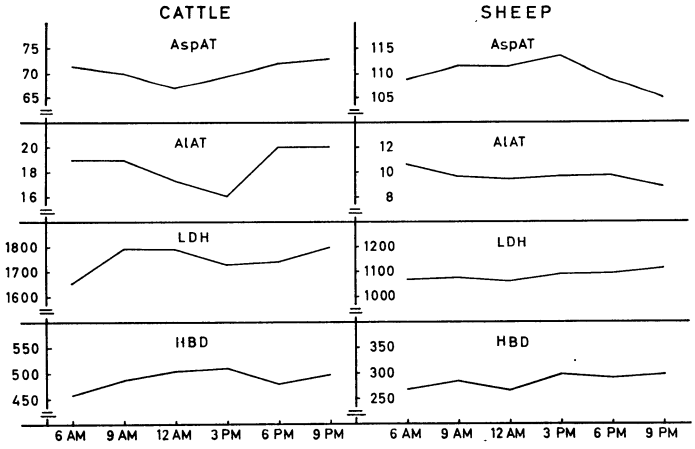
RESULTS

Investigation A

The results of serum enzyme variations of cattle and sheep during the day are given in Tables 1 and 2. The tables show that the enzyme variations due to the time of the day were very moderate compared with the existing variations among individuals.

T a b l e 1. Serum enzyme values ($\bar{x} \pm s$) of cows from 6 a.m. to 9 p.m. n = 6.

Enzyme	6 a.m.	9 a.m.	12 a.m.	3 p.m.	6 p.m.	9 p.m.
AspAT	71.5±8.7	70.2±8.2	67.0±10.9	69.5±8.8	72.3±10.2	73.0±7.7
AlAT	19.0±1.6	19.5±2.9	17.3± 1.6	16.0±2.4	20.8± 1.6	20.5±1.0
LDH	1654±213	1796±253	1788±314	1728±253	1738±275	1800±275
HBD	461± 98	487±101	504±106	509± 95	477±114	497±115



F i g u r e 1. Mean serum activities of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and α-hydroxybutyrate dehydrogenase in cattle and sheep during the day. For units, see text.

Table 2. Serum enzyme values ($\bar{x} \pm s$) of ewes from 6 a.m. to 9 p.m. n = 6.

Enzyme	6 a.m.	9 a.m.	12 a.m.	3 p.m.	6 p.m.	9 p.m.
AspAT	108.5±38.0	111.5±42.0	110.3±39.5	113.5±41.0	108.7±38.0	104.8±34.6
AlAT	10.7± 2.1	9.8± 1.5	9.5± 2.4	9.7± 2.1	9.7± 1.6	8.8± 2.3
LDH	1065±209	1075±211	1055±199	1087±216	1088±203	1103±198
HBD	268± 87	282± 92	264± 87	292± 93	281± 88	291± 92

Fig. 1 indicates that the mean transferase levels in cattle were lowest between the feeding times, in the middle of the day, whereas the dehydrogenases were lowest early in the morning. The diurnal enzyme variations in sheep were generally less pronounced than in cattle. This was confirmed in the following analyses of variance, Tables 3 and 4, where the contributions of time and animals to the total variation in serum enzymes are given.

Table 3. Serum enzymes of cows. Components of variance (comp.) due to animals and time of the day.

Cause of variation	Degrees of freedom	AspAT		AlAT		LDH		HBD	
		mean square	comp. in %	mean square	comp. in %	mean square	comp. in %	mean square	comp. in %
Animals	5	434***	80.8	14.36**	30.3	355917***	79.7	64578***	94.6
Time	5	29	2.8	21.03***	46.1	19197	1.3	1953**	2.4
Residual	25	14	16.4	1.65	23.6	13575	19.0	341	3.0

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Table 4. Serum enzymes of ewes. Components of variance (comp.) due to animals and time of the day.

Cause of variation	Degrees of freedom	AspAT		AlAT		LDH		HBD	
		mean square	comp. in %	mean square	comp. in %	mean square	comp. in %	mean square	comp. in %
Animals	5	8968***	98.3	21.29***	79.0	250906***	97.8	47749***	97.4
Time	5	53*	0.4	2.09*	5.5	1718	0.4	776***	1.4
Residual	25	19	1.3	0.67	15.5	778	1.8	98	1.2

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

For alanine aminotransferase and for α-hydroxybutyrate dehydrogenase statistical significant time-of-day effects were found in both species.

However, the relative components of variance due to the time of the day were for all enzymes, except for one case, less than 6 %, whereas the animal components varied from 80 to 98 %.

Investigation B

The changes of serum enzyme activity occurring in cattle and sheep by transfer from indoor feeding to pasture are given in Tables 5, 6, and 7.

Table 5. Serum enzyme activity ($\bar{x} \pm s$) of cows on indoor feeding and after two weeks at pasture. n = 16.

Enzyme	On indoor feeding	After 2 weeks at pasture	P
AspAT	77.8 ± 9.3	118.8 ± 18.4	< 0.001
AlAT	21.8 ± 4.9	33.2 ± 4.6	< 0.001
LDH	1953 ±352	1954 ± 451	> 0.25

Table 6. Serum enzyme activity ($\bar{x} \pm s$) of adult ewes on indoor feeding v. after one and two weeks at pasture, respectively. n = 16.

Enzyme	On indoor feeding	After 1 week at pasture	P	After 2 weeks at pasture	P
AspAT	81.6±16.5	105.8±58.8	= 0.1	111.1±37.5	< 0.01
AlAT	12.3± 2.3	15.5± 3.5	< 0.01	14.0± 2.4	< 0.1
LDH	830±202	835±231	> 0.25	846±232	> 0.25

Table 7. Serum enzyme activity ($\bar{x} \pm s$) of lambs kept indoors v. after one and two weeks at pasture, respectively. n = 26.

Enzyme	On indoor feeding	After 1 week at pasture	P	After 2 weeks at pasture	P
AspAT	69.9±10.6	70.9±10.7	> 0.25	82.4±14.5	= 0.002
AlAT	16.6± 4.0	16.3± 4.0	> 0.25	13.0± 2.8	= 0.001
LDH	988±137	935±161	< 0.25	917±145	= 0.05

It appears from the tables that the transferases in serum were significantly increased in both cows and ewes when transferred from indoor feeding to pasture, whereas no significant changes

occurred in lactate dehydrogenase. In ewes as well as in lambs the aspartate aminotransferase activity was greater after two than after one week on pasture.

Precision of laboratory methods

The precision expressed as the coefficient of variation was estimated from the components in Tables 3 and 4. Duplicates of a number of samples previously taken from other animals were used for comparison. The results are given in Table 8.

The repeatabilities of duplicates are close to those found by *Hagemeister & Unshelm* (1968).

Table 8. The precision of analytical methods as estimated by duplicated analyses of samples from 15 animals of each species (C) and from the residual and animal components in Investigation A. CV = coefficient of variation, r = repeatability.

		Cows		Sheep	
		C	A	C	A
AspAT	$\bar{x}$	59.4	70.6	91.5	109.6
	CV	1.47	5.34	1.23	4.00
	r	0.997	0.831	0.993	0.987
AlAT	$\bar{x}$	15.8	18.9	15.8	9.7
	CV	2.00	6.82	2.00	8.42
	r	0.995	0.561	0.976	0.836
LDH	$\bar{x}$	1500	1750	978	1079
	CV	1.05	6.66	1.24	2.59
	r	0.995	0.808	0.986	0.982
HBD	$\bar{x}$	519	489	315	280
	CV	1.03	3.77	2.61	2.55
	r	0.997	0.969	0.981	0.988

DISCUSSION

The results shown in Table 8 indicate the lower and upper limits of the precision of the laboratory methods. For alanine aminotransferase the figures may imply an existing interaction between animals and the time of the day. As duplicates usually are subjected to correlated errors (*Gedde-Dahl* 1966; *Unshelm & Rappen* 1968), the figures from Investigation A may be more realistic. Repeatabilities or similar statistics are useful when

comparing different methods on the same set of samples, whereas coefficients of variation may be preferred when comparing different materials (*Gedde-Dahl*).

Our results concerning the great variations between animals relative to the time-of-day variation are in agreement with those of *Hagemeister & Unshelm* (1968). These authors, however, observed an increased activity of about 20 % in aspartate- and alanine aminotransferases as well as of lactate dehydrogenase from 8 a.m. to 4 p.m. *Page et al.* (1960) found in cattle exposed to high temperatures a rise of the transferase levels up to 75 % from 4 a.m. to 4 p.m.

Our data showed no consequent rise or fall of serum enzymes during the day, and are thus more in accordance with recent results obtained in goats (*Aas Hansen* 1970). The discrepancy in results, and also the smaller time-of-day effect in sheep than in cattle, may perhaps partly be due to different composition of the diets.

Increased aminotransferase levels on pasture are reported by other authors. *Young et al.* (1965) found significant higher serum aspartate aminotransferase activity in sheep when pastured than when they were penned. *Hagemeister & Unshelm* observed a significant increase of alanine aminotransferase, but not of aspartate aminotransferase, in cattle on pasture compared with on stall-feeding. For lactate dehydrogenase these authors and the present investigation showed a decrease or no change.

The effect does not seem to be limited to the spring pasture period. *Aas Hansen* showed significantly raised transferase values in cattle and sheep up to three and a half months at pasture. No significant increase was observed after one day.

The difference in serum enzyme changes of lambs and ewes in the present investigation suggests an interaction between age and environmental factors. If an increased physical activity were the main cause, the enzyme release might have been expected to occur during the first days, and also include elevations of total LDH (*Tollersrud et al.* 1971).

The diurnal variations, and the differences in serum enzyme activity under the environmental or feeding conditions reported here, will be of minor significance for the interpretation of enzyme results in individual diagnoses. They contribute, however, to emphasize the need for consistency and specification of the conditions for a valid comparison of results.

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SUMMARY

In order to test the variation of enzyme activity in serum of cattle and sheep during the day, blood samples were taken at three hrs. interval from 6 a.m. to 9 p.m. The following enzymes were assayed: Aspartate aminotransferase (AspAT = GOT), alanine aminotransferase (AlAT = GPT), total lactate dehydrogenase (LDH), and α-hydroxybutyrate dehydrogenase (HBD). The variation between animals contributed by far to the greatest part of the total variation in clinical healthy animals. The time-of-day-dependant variation was less than 3 %, except for alanine aminotransferase.

During the first two weeks of spring pasture serum aspartate and alanine aminotransferase levels were significantly raised in both cows and ewes, compared with serum levels of the same animals on indoor feeding. No such increase occurred in total lactate dehydrogenase.

SAMMENDRAG

Døgnvariasjoner og sesongmessige forandringer i serumenzymaktiviteten hos storfe og sau.

For å undersøke i hvilken grad aktiviteten av visse diagnostisk betydningsfulle enzymer i serum varierer i løpet av dagen, ble blodprøver tatt av seks storfe og seks sauer med tre timers mellomrom fra kl. 6 til kl. 21. Følgende enzymer ble undersøkt: Aspartataminotransferase (AspAT = GOT), alaninaminotransferase (AlAT = GPT), total laktatdehydrogenase (LDH) og α-hydroxybutyratdehydrogenase (HBD).

Forskjellen mellom dyr bidro med langt den største andelen av den totale variasjon. Den delen som kunne tilskrives tidspunktet på dagen, utgjorde mindre enn 3 % for alle enzymer, unntatt for alaninaminotransferase.

Ved overgang fra innefôring til beite om våren, ble det funnet en signifikant stigning i begge de undersøkte transferaser både hos kyr og søyer, mens det ikke var noen forandring av LDH. Lam viste et noe annet bilde.

Variasjonene i løpet av dagen, og forandringene ved overgang fra innefôring til beite, vil sjelden spille noen praktisk rolle for vurderingen av enzymverdier i den kliniske diagnostikk. For mer vitenskapelige undersøkelser på dette felt bør en imidlertid nøye presisere under hvilke betingelser forsøkene er utført, for å kunne sammenlikne sine resultater med andres.

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