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Activities of Proteolytic Lysosomal Enzymes in Blood and Liver of Growing Cattle

Abstract

The activities of proteolytic lysosomal enzymes were investigated in serum, leucocytes, liver and muscle of German-Holstein heifers. Altogether there were 119 investigations: 19 9 months, 80 12 months and 20 16 months old female young cattle. Lysosomal enzymes in leucocytes were proven inappropriate for investigation because of very high variation coefficients. The enzymes investigated in serum, liver and muscle only had variation coefficients ranging from 20 to 40% in most cases. The closest connection between activities of individual enzymes was observed in the liver where activities, except for that of lysosomale esterase (EL), were highly correlated with each other. High correlations were calculated between 9 and 16 months old heifers.

Key Words: lysosomal enzyme, proteolysis, growing cattle

Zusammenfassung

Titel der Arbeit: **Aktivitäten der proteolytischen lysosomalen Enzyme im Blut und in der Leber wachsender Rinder**

Die Aktivitäten der proteolytischen lysosomalen Enzyme wurden im Serum, in den Leukozyten, in der Leber und im Muskel von Holstein Friesian Färsen bei einem Alter von 9 Monaten 19, von 12 Monaten 80 und von 16 Monaten 20 Tiere in insgesamt 119 Untersuchungen ermittelt. Die lysosomalen Enzymaktivitäten in den Leukozyten sind für diese Untersuchung ungeeignet, da sie überhöhte Variationskoeffizienten auswiesen. Die untersuchten Enzymaktivitäten im Serum, in der Leber und im Muskel variieren zwischen 20 bis 40 %. Die engsten Beziehungen zwischen den individuellen Enzymen wurden in der Leber beobachtet. Die lysosomale Esterase z.B. korrelierte eng mit fast allen anderen. Die engsten Korrelationen wurden zwischen dem 9. und 16. Lebensmonat für fast alle Enzymaktivitäten mit r= 0,6 – 0.9 ausgewiesen.

Schlüsselwörter: Lysosomale Enzyme, Proteolyse, Wachstum, Rind

Introduction

Differences between the protein yield in milk, or growth, can be observed between the periods of lactation or at a particular age and can be accounted for via the action of hormones (DAY et al., 1986; PANICKE, 1991; STAUFENBIEL, 1993; LACHMANN, 1994). The protein yield is the result of protein biosynthesis as well as proteolytic degradation. Degradation also determines the protein level in biological units. According to PFEIFER (1981), there is a close correlation between the activity of lysosomal enzymes and the concentration of protein in the cell. The proteolytic activity at a higher protein yield is restrained in all cells of the growing organism (SCHMIDT et al., 1992). Because this also holds true for leucocytes, the proteolytic

activity could explain the different protein yields as well as the stability of the performance.

The aim of investigation was to determine the correlation between the activities of individual lysosomal enzyme activities in serum, leucocytes, liver, and muscle as well as to correlate individual enzyme activities in the same compartment with different stages of cattle development.

Materials and methods

The proteolytic activities of lysosomal enzymes as well as their performance are influenced by systematic environmental factors. This is realized to a large extent by the simultaneousness and limited by the equipartition of the investigated young heifers of German-Holstein. Animals in groups 1, 3 and 4 were 10 to15 months old in different years. Heifers in group 2a were 9 months old , and those in group 2b were 16 months old. Groups 2a and 2b consisted of the same animals at different ages. Altogether there were 119 investigations in 99 female young cattle (Table 1). All animals lived in the same farm in different years.

Samples of liver and muscle were obtained under sterile conditions by specific needle biopsy in conjunction with local cryoanesthesia (DIRKSEN, 1990). The tissue samples were washed free of blood with 0.9% NaCl at 4ºC. The tissue was transferred to a cooled homogeniser containing 3 ml 0.9% NaCl + 0.1% TRITON-X 100 and homogenised with a motor-driven Teflon-piston (800 rpm) at 4ºC for 3 minutes. The resulting homogenate was spun at 20,000 *g* for 20 minutes. The supernatant was stored at -20ºC for later enzymatic determination. The protein concentration of each supernatant was estimated by using the micro-Lowry-based Protein Assay provided by Sigma.

Table 1
Characteristics of investigated groups (Untersuchungsmaterial)

No. of group	1		2a		3		4		2b	
Age in months by investigation	15		9		12		10		16	
Months of investigation	April		November		April		April		April	
n	20		19		20		40		20	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Weight at birth (kg)	39.1	6.1	41.3	3.9	39.6	5.5	41.1	5.6	41.6	4.0
Weight at investigation (kg)	339.4	13.2	268.4	12.3	315.7	37.8	302.6	30.0	383.8	20.4
days of life	465.1	27.4	275.3	15.5	353.3	52.2	296.7	17.0	512.3	14.4
Daily gain (g)	646.5	29.0	827.3	61.5	784.0	43.9	870.0	66.5	486.9[+]	39.2

[+] daily gain (g) during 9-16 months old

Blood was taken from the vena jugularis of female young cattle and preserved with heparin. The isolation of the leucocytes from blood was performed using density gradient centrifugation (ZEMAN et al., 1988). A 1.5 ml volume of gradisol was added to a siliconized 12.5 ml centrifuge tube at 22 to 25°C, followed by 2.5 ml of preserved blood. This was centrifuged at 400 *g* for 20 minutes in a swing-out centrifuge. The plasma phase was then poured off and the phase containing a mixed population of lymphocytes and granulocytes was removed using a Pasteur pipette. This was washed

with 5 ml 0.9 % NaCl, suspended in 3 ml 0.1 % Triton X-100 and frozen. After thawing, the leucocytes were centrifuged at 20,000 *g* for 20 minutes and the clear phase was saved for enzymatic analysis.
The activities of individual enzymes were determined using a sensitive spectrofluorimetric method using specific substrates (BARRETT, 1972). The activities of the enzymes in leucocytes, liver, and muscle were expressed as nmoles mg protein^{-1} h^{-1}, whereas the activity of cathepsin was expressed as mg degraded casein mg protein^{-1}h^{-1}. In serum the activity was expressed as moles released l^{-1} h^{-1}. Investigated enzymes are listed below:

1. arginyl-aminopeptidase	=	ARG	(EC3.4.11.6)
2. alanyl-aminopeptidase	=	ALA	(EC3.4.11.14)
3. leucyl-aminopeptidase	=	LEU	(EC3.4.11.1)
4. N-acetyl-glucosaminidase	=	NAGL	(EC3.2.1.30)
5. α-glucosidase	=	AGLD	(EC3.2.1.20)
6. lysosomale esterase	=	EL	(EC3.1.1.2)
7. cathepsin D+L	=	CATH	(EC3.4.23.5+3.4.22.15)
SAAL	=	ARG+ALA+LEU	

The means and standard deviations were computed with the MEANS procedure, and the correlations with the CORR procedure of the SAS System, version 8.2 (SAS, 1999).

Results

The means and standard deviations of enzyme activities in serum, leucocyte, liver, and muscle were determined for each group investigated (Table 2). Leucocyte enzymes showed the largest standard deviations.
No significant correlations were found between AGLD and other lysosomal enzymes in serum. LEU and SAAL activities were significantly correlated with only some enzymes in serum and leucocytes (Table 3). Medium correlation were found between daily gain and ALA and AGLD. In leukocytes ALA, LEU, and NAGL enzymes were correlated with each of the other lysosomal enzymes. The correlations were high in some cases. The enzyme most highly correlated with daily gain was ALA (Table 3).
The statistical evaluation of enzyme activities in isolated tissues has limited implications as different reference systems are used. In the case of solid tissue or cell populations, the activity is expressed in terms of nmol or ng of degraded substrate per mg cytosolic protein. The reference system is the concentration of cytosolic protein, which can vary markedly between tissues and is affected by physiological conditions such as feeding, starvation, and diurnal fluctuations. These factors make precise evaluation of enzyme activities difficult. In serum, enzyme activity is expressed in nmol l^{-1} fluid^{-1}h^{-1}. Comparison of enzymatic activities between two different reference systems must be done with care.
Correlations between the activities of eight lysosomal enzymes in liver and in muscle were estimated (Table 4). Both EL and daily gain were not significantly correlated with many of the enzymes. Other enzymes had correlation with each other enzyme in the liver and a negative correlation was found in case of EL with ARG and daily gain. Lysosomal enzymes were not significantly correlated with daily gain of the heifers in muscle. There were no high correlation found between enzymes in muscle except SAAL, which is the sum of ARG+ALA+LEU.

Table 2
Activity of lysosomal enzymes in serum (nmol l^{-1} h^{-1}),leucocyte, liver, and muscle (nmol mg protein $^{-1}$ h^{-1})* (Aktivitäten der lysosomalen Enzyme in Serum (nmol l^{-1} h^{-1}), Leukozyten, Leber und Muskel (nmol mg protein $^{-1}$ h^{-1})*

No. of group	1		2a		3		4		2b	
n	20		19		20		40		20	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Serum-enzymes										
ARG	1901	296	2749	308	2312	536	1267	327	2780	637
ALA	2500	351	3201	344	3133	481	3846	804	3210	1083
LEU	2988	312	3407	792	3099	459	3166	870	2892	1157
SAAL	7388	447	9357	1179	8544	1142	8279	1455	8882	2627
NAGL	3269	1167	1889	1144	1847	1027	1585	984	1760	581
AGLD	822	301	-	-	-	-	-	-	-	-
EL	736	147	875	242	702	42	622	76	453	120
Leucocyte-enzymes										
ARG	-	-	59,888	34,424	139,863	66,243	-	-	124,871	133,691
ALA	10,869	4841	339,883	236,995	198,798	160,137	-	-	195,013	86,604
LEU	12,041	5616	150,018	94,591	307,790	153,336	-	-	81,336	38,669
SAAL	-	-	549,790	326,168	646,451	342,670	-	-	401,220	169,333
NAGL	54,914	36,872	411,185	136,931	540,727	310,729	-	-	504,230	242,854
EL	24,988	10,577	75,860	36,343	190,910	52,307	-	-	187,707	163,436
Liver-enzymes										
ARG	239	114	328	135	374	61	613	96	439	96
ALA	798	203	651	174	690	123	904	110	800	150
LEU	573	197	458	126	485	97	719	89	559	118
SAAL	1611	461	1437	406	1549	222	2236	257	1797	317
NAGL	717	184	520	126	540	79	702	103	636	106
AGLD	178	79	194	66	298	79	347	65	271	45
EL	681	239	479	119	389	70	401	78	479	91
CATH	1072	393	928	369	898	119	2278	495	1207	321
Muscle-enzymes										
ARG	-	-	4521	1758	6318	2042	-	-	6844	2373
ALA	-	-	7171	2179	8937	2759	-	-	8512	1898
LEU	-	-	2341	1024	3888	682	-	-	4462	1290
SAAL	-	-	14,032	3815	19,143	3901	-	-	19,817	3162
AGLD	-	-	2392	440	1539	378	-	-	1484	250
CATH	-	-	1806	690	2708	426	-	-	3137	856

* CATH in ng/mg protein per h

Table 3
Correlation between lysosomal enzyme-activities in serum and leucocyte (above leucocyte, below serum) (Korrelationen zwischen den lysosomalen Enzymaktivitäten im Serum und in den Leukozyten (oberhalb Leukozyten, unterhalb Serum))

	ARG	ALA	LEU	SAAL	NAGL	EL	AGLD	Daily gain
ARG	-	NS	0.84***	0.63***	0.40*	0.62***	-	NS
ALA	-0.23*	-	0.58***	0.80***	0.48***	0.31*	-	0.63***
LEU	0.20*	0.24*	-	0.83***	0.73***	0.75***	-	0.52***
SAAL	0.49***	0.59***	0.77***	-	0.39*	NS	-	NS
NAGL	NS	-0.36***	NS	-0.27**	-	0.69***	-	0.56***
EL	0.39***	-0.21*	NS	NS	0.23*	-	-	0.45***
AGLD	NS	NS	NS	NS	NS	0.45*	-	-
Daily gain	-0.26*	0.50***	NS	NS	-0.40***	NS	0.60**	-

*P <0.05; ** P <0.01; *** P <0.001 statistically confirmed

The correlations between enzymes of different tissues are shown in Table 5 and 6. Where there were no correlations which differed significantly from zero, computed correlations were not reported in the tables. Activity of NAGL in serum resulted in

negative correlation with enzymes in leucocyte. ARG, ALA and EL in serum had correlations with almost each enzyme in liver, but SAAL, NAGL and AGLD resulted in no correlations with liver enzymes. Lysosomal enzymes in muscle were not correlated with many of serum- and liver-enzymes, but most of its enzymes had correlations with ARG, LEU and EL in leucocyte. ARG and EL enzymes showed middle correlation with most lysosomal enzymes between serum-liver (negative correlations) and leucocyte-muscle. Some low correlations were determined between enzymes in leucocyte and in liver.

Table 4
Correlation between lysosomal enzyme-activities in liver and muscle (above muscle, below liver) (Korrelationen zwischen den lysosomalen Enzymaktivitäten in der Leber und im Muskel (oberhalb Muskel, unterhalb Leber))

	ARG	ALA	LEU	SAAL	NAGL	AGLD	EL	CATH	Daily gain
ARG	-	0.40*	0.54**	0.81***	-	NS	-	0.50**	NS
ALA	0.69***	-	NS	0.82***	-	NS	-	0.41*	NS
LEU	0.78***	0.84***	-	0.68***	-	-0.52**	-	0.64***	NS
SAAL	0.90***	0.92***	0.95***	-	-	-0.40*	-	0.62***	NS
NAGL	0.53***	0.65***	0.68***	0.67***	-	-	-	-	-
AGLD	0.67***	0.40***	0.51***	0.57***	0.39***	-	-	-0.49**	NS
EL	-0.24*	NS	NS	NS	NS	NS	-	-	-
CATH	0.80***	0.75***	0.81***	0.86***	0.63***	0.56***	NS	-	NS
Daily gain	0.43***	NS	NS	0.26*	NS	0.28**	-0.49***	0.39***	-

*P <0.05; ** P <0.01; *** P <0.001 statistically confirmed

Table 5
Correlation between lysosomal enzyme-activities of serum-leucocyte, serum-liver, and serum-muscle (Korrelationen zwischen den lysosomalen Enzymaktivitäten des Serums und der Leukozyten, der Leber sowie des Muskels)

	Serum					
	ARG	ALA	SAAL	NAGL	EL	AGLD
Leucocyte						
ALA	0.42***	0.38**	0.36**	-0.34**	0.28*	
LEU	0.37**	0.46***	0.36**	-0.39**		
NAGL	0.29*	0.41**	0.32*	-0.26*		0.49*
EL		0.30*		-0.40**		
Liver						
ARG	-0.56***	0.47***		-0.30**	-0.49***	
ALA	-0.57***	0.25*			-0.25*	
LEU	-0.59***	0.30**			-0.33**	
SAAL	-0.62***	0.37***			-0.39***	
NAGL	-0.46***		-0.34**		-0.31**	
AGLD	-0.41***	0.25*			-0.46***	
EL		-0.33**		0.22*		
CATH	-0.67***	0.40***			-0.43***	
Muscle						
LEU	-0.36*					
AGLD	0.49**					
CATH	-0.45**		-0.35*			

*P <0.05; ** P <0.01; *** P <0.001 statistically confirmed

Table 7 shows only significant correlations of same enzyme in same tissue between the same heifers of different ages. Enzymes in serum, leucocytes, and muscle had no correlations between 9 and 16 months old growing cattle, but all lysosomal enzyme-activities in liver were correlated and each correlation was high except for AGLD which showed only a moderate correlation.

Table 6
Correlation between lysosomal enzyme-activities of leucocyte-liver, leucocyte-muscle, and liver-muscle (Korrelationen zwischen den lysosomalen Enzymaktivitäten in den Leukozyten und in der Leber sowie im Muskels und zwischen Leber und Muskel)

	Leucocyte					Liver	
	ARG	ALA	LEU	NAGL	EL	AGLD	EL
Liver							
ARG			0.29*		0.31*		
LEU				-0.33*			
NAGL				-0.30*			
AGLD	0.37*		0.36*		0.58***		
EL			-0.40**	-0.44**	-0.47***		
Muscle							
ARG	0.48**				0.35*		
ALA	0.53**		0.39*		0.45**	0.41*	
LEU	0.59***		0.50**	0.39*	0.53**	0.37*	-0.41*
SAAL	0.66***		0.50**		0.55***	0.43*	
AGLD	-0.38*	0.35*			-0.49**		0.44*
CATH	0.53**		0.37*		0.53**		-0.48**

*P <0.05; ** P <0.01; *** P <0.001 statistically confirmed

Table 7
Correlation between lysosomal enzyme-activities of 9 and 16 month old heifers (group: 2a and 2b in Table 1) in liver, leucocytes,. serum, and muscle (Korrelationen zwischen den lysosomalen Enzymaktivitäten im Alter der Färsen von 9 und 16 Monaten in der Leber, den Leukozyten, dem Serum sowie im Muskel)

Component	Enzyme	r
Liver	ARG	0.94***
	ALA	0.89***
	LEU	0.97***
	SAAL	0.96***
	NAGL	0.93***
	AGLD	0.60***
	EL	0.85***
	CATH	0.84***
Leucocyte	-	-
Serum	NAGL	0.51*
Muscle	ALA	0.57*

*P <0.05; ** P <0.01; *** P <0.001 statistically confirmed

Discussion

The main site for the degradation of proteins is the lysosome compartment, which contains approximately 20 endo- and exopeptidases. Lysosomal proteolysis is strongly dependent on the type of cell and can account for 30 to 100% of the total proteolysis. Lysosomal degradation is somewhat specific, whereby proteins with longer half lives are preferred. Proteins with high turnover-rates are usually degraded by a ubiquitin-dependent mechanism in the proteosome. Various means of degradation are interconnected and strongly regulated. The assessment of proteolytic activity, particularly of lysosomal enzymes, provides insight on the level of protein synthesis. The factors affecting quality of cattle include growth, milk yield, fertility and health and are determined by genotype and environment. Consideration of proteinmetabolism is integral in assessing these parameters. Protein yield is the end result of proteinbiosynthesis and proteolysis. Proteolysis is restrained in all cells of developing organisms requiring higher protein yields. Due to external challenges, variances in proteolytic activities of leucocytes are especially high. There is high correlation between the quantity of protein in a given cell and the turnover rate. The rate of

degradation of a protein appears to determine its final concentration. Protein degradation can occur in one of three ways: (BIENKOWSKI, 1983) via basal degradation in the endosomal system, intralysosomal proteolysis, or ubiquitin-dependent degradation in the cytoplasm (SOMMER and SEUFERT, 1992). It can be assumed that intracellular proteins are subject to constant turnover and that this process is physiologically important. In comparison to present knowledge concerning protein synthesis, the biochemical processes of protein degradation are relatively unknown (PANICKE et al., 1996). In terms of kinetics, the turnover of individual proteins is often described as the result of two zero order reactions for synthesis and one first order reaction for degradation (MILLWARD, 1978; AMENTA and BROCHER, 1981). This implies that the rate of protein degradation in a tissue or a population of cells is proportional to the concentration. Furthermore, it means that all protein molecules in the cell are equally prone to degradation. As a result, proteolysis occurs at the same rate in all cells at all times (AMENTA and BROCKER, 1981). The degradation of various protein molecules regulates important cellular functions such as structure, control of many key-enzymes and other bio-regulators, the elimination of abnormal protein molecules resulting from errors in synthesis, and the destruction of multi-complexes such as the ribosome or mitochondria.

To date, little research has been performed on the activities of lysosomal enzymes responsible for protein synthesis and energy production in dairy cattle. Because of the genetic determination of protein growth and energetic growth as well as the protein and energy turnover, genetic-physiological investigations seem to be confirmed by protein synthesis and proteolysis even concerning dairy cattle. Protein growth in different tissues results from a dynamic balance between the rates of protein synthesis and proteolysis (BIENKOWSKI, 1983; PANICKE et al., 1996). A shift in this balance towards restricted proteolysis results in increased protein concentration in the cells. This increase requires less energy in comparison to de novo protein biosynthesis, which requires relatively large quantities of ATP (BIENKOWSKI, 1983; BALLARD et al., 1980). Reduced proteolysis in cells, measured in terms of activities of the proteolytic enzymes, is an important genetic feature in selection and breeding (BALLARD et al., 1980; LOBLEY, 1998). Mammals reduce protein much more intracellularly than they absorb by feeding (LOBLEY, 1998). The lysosomal degradative enzymes are largely responsible for these processes, especially the proteases like Cathepsine D, L, B, H, and the amino-peptidases. Proteolytic activity in the cytosol is so low that the relatively high protein turnover of the cell cannot be explained by this alone. This proteolysis underlies the mechanism of cellular autophagy (SEGLEN and BOHLEY, 1992) and is under strict hormonal control. Thus, the activities of different proteolytic enzymes may vary during growth. The aim of this study was to gain further knowledge of the lysosomal enzyme activities in different tissues as serum, leucocytes, liver, and muscle. The proteolytic activities in these tissues were tested on growing HF-cattle (Holstein Friesian) at the age of 9 to 16 month in groups.

The following insights can be drawn from the results:

1. The phenotypic variation of the proteolytic activities of the enzymes within the groups, which are measured in serum as nmol l plasma^{-1} h^{-1} and in leucocytes, liver and muscle as nmol mg protein^{-1} h^{-1}, had a variation coefficient from 10 to

30 %. Similar variation has been observed for the milk yield with 22 up to 28 %.

2. The lysosomal enzyme activities of the leucocytes did not provide further insight for subsequent genetic investigations. They show a lower heritability coefficient (h^2), according to our own estimations on dairy cows and have much higher phenotypic variation (PANICKE et al., 1999). The latter point could be explained by the methodical limits in the separation of the leucocytes.
3. The combination of the three aminopeptidase activities (ARG + ALA + LEU) as a common value (SAAL**)** seems to be justified as it decreases the variation coefficient to about 15%. The tight correlation coefficients between the individual values and their sum emphasize their equal importance in proteolysis as well as for their presentation in SAAL. In the serum, they reach about r=0.5-0.6, in the muscle and the leucocytes over r=0.8, and in the liver, over r=0.9. This fact may justify the application in breeding programs considerations.
4. The tight relationship between the lysosomal enzyme activities in the liver exceeds that in the blood, leucocytes, and muscle. Even the CATH, which splits the molecules for the aminopeptidases, shows consistently tight correlation coefficients (over r=0.8). Between the ninth and the sixteenth month of age the correlations were tight (over r=0.8) for the nearly all of the enzyme activities. These correlations, in addition to the ensured relations to the serum, especially for ARG, ALA, and LEU, at about r=0.6, r=0.3, and r=0.3 respectively, emphasize a superior qualification of the liver parameters. We recommend the further investigation of the liver in physiological and genetic studies. The estimation of these parameters must be done under standardized environmental conditions and with suitable animals.
5. The derivation of suitable metabolic characteristics concerning the performance stability of domestic animals, including further direct or indirect liver parameters, requires more scientific investigations.

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References

AMENTA, J.S.; BROCHER, S.C.:
Studies on protein turnover in cell culture: Evidence for the existence of a specific proteolyse state and its relation to cellular proliferation and cellular senescence. Acta biol.med.germ. **40** (1981), 1215-1226

BALLARD, F.J.; KNOWLES, S.E.; WONG, S.S.C.; BODNER, J.B.; WOOD, C.M.; GUNN, J.M.:
Inhibitation of protein breakdown in cultured cells is a consistent response to growth factors. FEBS Lett., **114** (1980), 209-212

BARRETT, A.:
Lysosomal enzymes. In: Dingle, J.T. (ed.), Lysosomes. A Laboratory Handbook. North-Holland Publ. Co., Amsterdam. 1972, 46-135

BIENKOWSKI, R.J.:
Intracellular degradation of newly synthetised secretory proteins. Bioch. Journ. (1983), 214

DAY, M.L.; IMAKAWA, K.; ZALESKY, D.D., KITTEK, R.J.; KINDER, J.E.:
Effect of restriction of dictary energy intake during the prepubertal period. J. Animal Science **62** (1986), 1641-1648

DIRKSEN, G.:
Leber. In: Rosenberger, G. Die klinische Untersuchung des Rindes. 3. Auflage, Verlag Paul Parey, Berlin, Hamburg (1990), 367-384
LACHMANN, J.:
Experimentelle Untersuchungen zum Einfluß der antepartalen Energieversorgung auf die Leistung und Gesundheit der Milchkuh in der Frühlaktation. Diss. Humboldt-Universität Berlin, Nat.-Math. Fak. I. (1994.)
LOBLEY, G.E.:
Nutritional and hormonal control of muscle and peripheral tissue metabolism in farm species. Livestock Product. Science **56** (1998), 91-114
MILLWARD, D.J.:
Regulation of muscle protein turnover in growth and development. Biochem. Soc. Trans. 1978) 6, 494-499
PANICKE, L.:
Wachstumsverlauf der Färse und Produktionsleistung der Milchkuh. Mh. für Veterinärmedizin **46** (1991) 18, 636-638
PANICKE, L.; SCHMIDT, M.; KOLATAJ, A.:
Degradationsrate und Proteinsynthes Kolloquium "Milchprotein und Proteinansatz" 11./12.04. Graal Müritz vom FBN Dummerstorf und Agrarwiss. Fakultät der Univ. Rostock. Schriftenreihe des FBN Dummerstorf, Heft 8 (1996)
PANICKE, L.; SCHMIDT, M.; KROL, T.; STAUFENBIEL, R.:
Proteolytische Aktivitäten der lysosomalen Enzyme bei Milchrindern. 1. Mitt.: Variation der lysosomalen Enzyme bei Milchkühen. Arch. Tierz., Dummerstorf **42** (1999) 4, 321-334
PANICKE, L.; SCHMIDT, M.; KROL, T.; STAUFENBIEL, R.:
Proteolytische Aktivitäten der lysosomalen Enzyme bei Milchrindern. 2. Mitt.: Lysosomale Enzymaktivitäten und die Milchleistung bei Kühen. Arch. Tierz., Dummerstorf **42** (1999) 5, 443-449
PFEIFER, U.:
Morphological aspects of intracellular protein degradation: Autophagy. Acta Biol. Med. Germ. **40** (1981), 1619-1624
SAS Procedures Guide,
Version 8. SAS Institute Inc., Cary, NC. (1999) 1729 pp.
SCHMIDT, M.; KOLATAJ, A.; BULLA, J.; KROL, T.; WITEK, B.; BANASIK, A.:
Activity of some lysosomal enzymes in plasma and leucocytes of rabbits exposed to effect of retinol and hydrocortisone Hormon. Metabol. Res. **24** (1992), 21-25
SEGLEN, P.O.; BOHLEY, P.:
Autophagy and other vacuolar protein degradation mechanism. Experimenta **48** (1992), 158-171
SOMMER, T.; SEUFERT, W.:
Genetic analysis of ubiqutin-dependent protein degradation. Experimentia **48** (1992), 172-178
STAUFENBIEL, R.:
Energie- und Fettstoffwechsel des Rindes. Habil-Schrift, Fachbereich Veterinärmedizin der Freien Universität Berlin (1993)
ZEMAN, K.; TEHORZEWSKI, H.; MAJEWSKA, E.; POKOCA, L.; PINKOWSKI R.:
A simple and rapid method for simultaneous purification of peripheral blood lymphocytes and granulocytes. Immunol. Polska, ssXIII (1988), 217-224

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