References

Allen, B. V. and Archer, R. K. (1976). Some haematological values in English Thoroughbred horses. *Vet. Rec.* **98**, 195–196.

Blackmore, D. J. (1975). Proceedings of the First International Symposium on Equine Haematology, Michigan, pp. 276–285. American Association of Equine Practitioners, Colorado, USA.

Blackmore, D. J. and Palmer, A. (1977). Phenylalanine inhibited p-nitrophenyl phosphatase activity in the serum as an indication of intestinal cellular disruption in the horse. *Res. Vet. Sci.* **23**, 146–152.

Blackmore, D. J., Campbell, Cliodhna, Dant, C., Holden, Jane E. and Kent, Joyce E. (1982). Selenium status of Thoroughbreds in the United Kingdom. *Equine Vet. J.* **14**(2), 139–143.

Butler, P. and Blackmore, D. J. (1982). Retinol values in the plasma of stabled Thoroughbred horses in training. *Vet. Rec.* **111**, 37–38.

Butler, P. and Blackmore, D. J. (1983). Vitamin E values in the plasma of stabled Thoroughbred horses in training. *Vet. Rec.* **112,** p. 60.

Cooley, W. W. and Lohnes, P. R. (1971). *Multivariate Data Analysis*. John Wiley & Sons Inc., New York. pp. 96–128.

Cotlove, E., Harris, E. K. and Williams, G. Z. (1970). Biological and analytic components of variation in long term studies of serum constituents in normal subjects. 3. Physiological and medical implications. *Clin. Chem.* **16**, 1028–1032.

Harris, E. K., Kanofsley, P., Shakarji, G. and Cotlove, E. (1970). Biological and analytic components of variation in long term studies of serum constituents in normal subjects. 2. Estimating biological components of variation. *Clin. Chem.* **16**, 1022–1027.

Krause, R. D., Anand, V. D., Gruemer, H. S. and Wilke, T. A. (1975). The impact of laboratory error on the normal range – a Bayesian Model. *Clin. Chem.* **21**, 321–324.

Leicester, C. (1964). *Bloodstock Breeding*. Cox and Wyman Ltd, London. pp. 17–95.

Rowlands, G. J., Payne, J. M., Dew, Sally M. and Manston, R. (1973). A potential use of metabolic profiles in the selection of superior cattle. *Vet. Rec.* **102**, 48–49.

Stubley, Deirdre, Campbell, Cliodhna, Dant, C. and Blackmore, D. J. (1983). Copper and zinc levels in the blood of Thoroughbreds in training in the United Kingdom. *Equine Vet. J.* **15** (in press).

Vessel, E. S. (1975). Pharmacogenetic aspects – what do we need to know about the individual in order to interpret the data? Abstracts IXth International Congress in Clinical Chemistry, Toronto, Canada. p. 14.

Williams, G. Z., Young, D. S., Stein, M. R. and Cotlove, E. (1970). Biological and analytic components of variation in long term studies of serum constituents in normal subjects. 1. Objectives, subject selected, laboratory procedures and estimation of analytical deviation. *Clin. Chem.* **16**, 1016–1021.

Williams, G. Z. (1971). Individual normal values and profiles of blood chemistry. *Clin. Chem.* **17**, p. 629 (Abstract).

Woolliams, J. A., Suttle, N. F., Weiner, G., Field, A. C. and Woolliams, C. (1982). *Animal Production* **35**, 299–307.

Young, D. S., Harris, E. K. and Cotlove, E. (1971). Biological and analytic components of variation in long term studies of serum constituents in normal subjects. 4. Results of a study designed to eliminate long term analytic deviations. *Clin. Chem.* **17**, 403–410.

Plasma Biochemical Values in Thoroughbred Horses in Training

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Summary

Fifteen biochemical tests were performed on plasma samples from Thoroughbred horses in training. The tests were conducted over a period of one year on horses selected from a large stable in the country and from six smaller stables in the Adelaide metropolitan area. Preliminary studies ascertained the stability of the biochemical constituents in stored blood and the effects of diurnal variations.

Of the 128 horses initially bled, only 63 were bled on a sufficient number of occasions during the two-month 'conditioning' period and the first two months of racing to be acceptable for statistical analysis. This analysis showed that stage of training had a significant effect on the majority of plasma constituents tested, although trends were apparent for only a few: increases in creatinine and bilirubin and decreases in urea, phosphorus and lactate dehydrogenase during the training period. Location-related influences also had a significant effect on the majority of plasma constituents, whereas sex-related influences and age did not have such a general effect. It was found that total protein, lactate dehydrogenase and aspartate aminotransferase differed between the sexes and that inorganic phosphorus, alkaline phosphatase and lactate dehydrogenase tended to decrease with age, whereas bilirubin and total protein tended to increase with age.

Introduction

It has become increasingly common among diagnostic laboratories to offer a panel of biochemical tests on blood. A number of these tests can assist in assessing the fitness and health of horses in training. Serum enzymes have a particular application in judging the status of the muscular system during training, and abnormalities in serum electrolytes have been noted by Williamson (1974) in horses with poor performance. Interpretation of these biochemical tests, however, has often been restricted by inadequate information on appropriate reference ranges.

The purpose of the present study was to examine the significance of stage of training on 15 biochemical constituents of the blood plasma of Thoroughbred horses in training. There have been several reports on the effect of training on multiple biochemical variables in blood plasma or serum of horses (Aitken *et al.*, 1974; Milne *et al.*, 1976; Snow and Mackenzie, 1977; Mullen *et al.*, 1979), but these studies have been restricted to the observations from a few horses or limited to the study of two-year-old horses.

Materials and Methods

The study of Thoroughbred horses in training commenced in August 1975 and was terminated in July 1976. Horses were from a large stable in the country (country horses) and six small stables in the Adelaide metropolitan area (city horses). Of the 128 horses selected for blood sample collection only 63 were in training for periods of time sufficient to permit sampling on four or five occasions at monthly intervals from the first week of training. Older horses were usually 'spelled' for at least two months at pasture, before re-entering a training programme.

Training procedures varied between stables and also within stables depending on whether the horse was a 'sprinter' or a 'stayer'. However, in general, horses were given slow work, usually a trot and canter over a distance of 3 to 6 km, for six days each week for the first two to four weeks of training. Over the next four to six weeks of training, horses were given slow work three days a week and half-pace work (200 m per 25 s) for the other three days. From seven to nine weeks of training, horses were worked at 'even time' (200 m per 15 s) twice a week with slow work on other mornings. Finally, horses were galloped (about 200 m per 12 s) on three or four 'fast' days before racing. Horses were usually ready to race nine to twelve weeks after entering the training programme.

Horses in training were given about 10 to 12 kg feed daily. The feed varied during the training programme. Typical of the daily feeds offered to horses in the first weeks of training were 3 to 4 kg grain with 2 kg cereal and lucerne chaff and 4 to 5 kg lucerne hay. At the racing stage a typical daily feed was 5 to 6 kg grain, 1 kg chaff and 4 to 5 kg hay.

The majority of horses accepted for the project were studied over the spring–summer period. The blood was collected from a jugular vein into lithium heparinized 'vacutainers' mid-morning after slow work. The plasma was separated from the blood cells within 4 to 5 hours of collection and the sample assayed the same day for sodium, potassium, chloride, bicarbonate, urea, glucose, total protein, calcium, inorganic phosphorus, cholesterol, creatinine, total bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LD), and aspartate aminotransferase (AST). Details of the analytical procedures have been given elsewhere (Judson *et al.*, 1983).

Only the results from horses bled on four or five occasions were considered for statistical evaluation. The statistical method was a split unit analysis using the computer programme 'Genstat System' which was devised at the Rothamsted Experimental Station, England. Missing values were calculated for variables in the blood sample missed in horses bled on only four of the five occasions during the training programme. The enzymes LD and AST were converted to their logarithmic value before statistical analysis.

Results

Stability and diurnal variation of constituents

Prior to the start of the survey, the stability of the 15 plasma constituents in blood stored for six days at 4°C and the diurnal variation of these plasma constituents in horses were examined. Most of the plasma constituents in blood samples collected from 12 horses were found to be stable during storage, although linear regression analysis indicated significant increases with time for potassium, urate and LD and decreases with time for sodium and glucose. The changes were more marked for glucose than for the other constituents. To minimize storage effects, plasma was thus separated from blood cells within 4 to 5 hours of collection of the blood sample.

Diurnal variation in blood composition was assessed by assaying constituents in blood samples collected from each of four horses at 05.00 h, 10.00 h, 16.00 h, 22.00 h and again at 05.00 h the following day. It was found that diurnal variation in plasma constituents can be neglected, except for sodium, potassium, bicarbonate, protein, calcium, bilirubin and LD. It is usually most convenient to collect blood samples mid-morning and this time was adopted because, of the seven constituents found to vary, only bilirubin in the 10.00 h sample differed markedly from the two 05.00 h samples, samples taken prior to feeding and slow work.

Number of horses and statistical comparisons

Table 1 gives details of the number of horses in each of the subgroups, i.e. location, sex, age and stage of training. A summary of the statistical findings of the effect of these subgroups on each of the 15 plasma biochemical constituents is given in Table 2.

TABLE 1. Subgroups and number of horses in each subgroup.

Subgroups	Geldings	Mares/fillies
Location		
City	18	9
Country	11	25
Sex		
Geldings	29	—
Mares/Fillies	—	34
Age*		
2 years	4 (0)	14 (0)
3 years	11 (6)	15 (6)
4 years	7 (6)	4 (2)
5 years	3 (2)	1 (1)
Aged	4 (4)	0 (0)
Stage of Training		
Week 1	26	31
Week 5	29	34
Week 9	29	33
Week 13	27	34
Week 17	24	23

* The number of horses located at the city stables is given in brackets

Effect of location of horses

Plasma constituents in city and country horses were compared after sex-related influences were removed. This exclusion was necessary because of the unequal distribution of the sexes between the two locations (see Table 1). With the exception of potassium and chloride, those plasma constituents shown to be affected by location were higher in the country horses than in the city horses (Table 3).

TABLE 2. Factors affecting plasma constituents: summary of statistical findings.

Constituent	Probability of similar subgroups*			
	City vs country[1]	Geldings vs mares/fillies[2]	Age[3]	Stage of training
Sodium	ns	ns	ns	<0.001
Potassium	<0.001	ns	ns	ns
Chloride	<0.001	ns	ns	<0.001
Bicarbonate	<0.05	ns	ns	ns
Urea	<0.001	ns	ns	<0.001
Glucose	ns	ns	ns	<0.05
Protein	ns	<0.05	<0.01	<0.001
Calcium	ns	ns	ns	ns
Phosphorus	<0.05	ns	<0.001	<0.01
Cholesterol	ns	ns	ns	<0.05
Creatinine	ns	ns	ns	<0.001
Bilirubin	<0.05	ns	<0.05	<0.001
ALP	<0.01	ns	<0.001	<0.001
LD	<0.05	<0.01	<0.05	<0.01
AST	<0.01	<0.01	ns	ns

*Significance level, ns: subgroups not significantly different ($p > 0.05$).
[1]Excluding effect due to sex of horses.
[2]Excluding effect due to location of horses.
[3]Excluding effects due to sex and location of horses.

Effect of sex-related influences

Sex-related influences on plasma constituents were tested after excluding location-related influences. Total protein and the enzymes LD and AST were the only constituents that differed between the sexes (see Table 2). The respective mean values for geldings and mares/fillies were 69 and 67 g/l for total protein, 303 and 346 U/l for LD and 404 and 537 U/l for AST.

Effect of age of horses

Age-related influences significantly altered five of the plasma constituents (Table 2), when the sex-related and location-related influences were excluded. Table 4 gives the mean values of these constituents for each of the five age groups considered. The mean values for the five-year-olds and horses older than five years (aged) are approximate since only

TABLE 3. Effect of location of horses on plasma constituents.

Constituent	Mean values*	
	City	Country
Potassium (mmol/l)	3.5	3.3
Chloride (mmol/l)	101	99
Bicarbonate (mmol/l)	29	30
Urea (mmol/l)	4.2	4.9
Phosphorus (mmol/l)	1.14	1.21
Bilirubin (μ mol/l)	43	37
ALP (U/l)	187	215
LD (U/l)	304	343
AST (U/l)	401	531

*Mean values of 27 city horses and of 36 country horses.

four horses in each group were considered (Table 1). It is apparent from Table 4 that phosphorus, AST and LD decreased with age whereas bilirubin and total protein increased with age.

TABLE 4. Effect of age of horses on plasma constituents*.

Constituent	Age in years				
	2	3	4	5	Aged
Protein (g/l)	66	68	68	69	71
Phosphorus (mmol/l)	1.29	1.18	1.12	1.05	1.01
Bilirubin (μ mol/l)	34	40	45	47	38
ALP (U/l)	245	189	187	154	195
LD (U/l)	358	330	302	258	302

*Mean values of 4 to 26 horses (see Table 1).

Effect of stage of training

It is apparent from Table 1 that stage of training significantly altered a number of plasma constituents. The mean values of these constituents for each of the sampling occasions is given in Table 5. The least significant difference value (LSD) at $p = 0.05$ for each of the constituents is also given in Table 5. Trends were apparent for a few of these constituents with increases in creatinine and bilirubin values and decreases in urea and LD values during the period under study. Transient changes were also apparent during the training period with an increase in chloride and a decrease in total protein during the first two months of training and a decrease in phosphorus during the last two months of the training period when horses were racing.

TABLE 5. Effect of stage of training on plasma constituents.

Constituent	Weeks of training*					LSD
	1	5	9	13	17	(p = 0.05)
Sodium (mmol/l)	140	142	142	142	142	0.9
Chloride (mmol/l)	99	100	101	101	101	0.8
Urea (mmol/l)	4.8	4.8	4.6	4.3	4.4	0.23
Glucose (mmol/l)	5.4	5.0	5.2	4.9	5.1	0.35
Protein (g/l)	69	68	67	67	67	1.2
Phosphorus (mmol/l)	1.18	1.23	1.19	1.17	1.13	0.049
Cholesterol (mmol/l)	2.9	2.9	3.1	3.0	3.1	0.12
Creatinine (mmol/l)	0.13	0.14	0.14	0.15	0.15	0.004
Bilirubin (μmol/l)	36	37	41	42	43	2.5
ALP (U/l)	219	192	195	205	204	10.1
LD (U/l)	2.559	2.520	2.508	2.488	2.488	0.0304

*Mean values of 63 horses, with LD values given as log values.

Discussion

It is evident from our study that about one-half of the horses were 'lost' during the first three months of training. Premature withdrawal from training was due to the transfer of horses to interstate stables, particularly from the country stable, inability of the horse to perform to expectations, 'competitive' injuries and inability to train, usually with the two-year-old horses. Other less common reasons were pregnancy and retirement of horses. Most horses surveyed were less than five years old.

With the horses in training for at least three to four months, it was found that biochemical constituents in the blood of these horses were affected by factors related to the stable, the gender of the horse, age and the stage of training. A number of biochemical constituents in horses were found to differ between stables. The city stables were too small to consider separately and hence horses in these stables were grouped together and compared with horses located in a large country stable. Despite the variability in environmental conditions for horses among the city stables, differences in blood biochemical constituents emerged between the city group and the country horses. A possible explanation for the differences observed between the two groups for a number of the plasma constituents is due to the disparity in the ages of the two groups of horses; the country horses had a higher proportion of young horses. The lower value for bilirubin and the higher values for phosphorus, ALP and LD in the country horses as compared to the city horses are consistent with the effect of age on these constituents. Similar trends with age have been observed in Thoroughbred horses for serum phosphorus (Simesen 1972) and serum ALP (Blackmore and Elton, 1975).

Potassium, chloride, bicarbonate, urea and AST values were found to be altered by location but not by age of the horse (see Table 2) and were thus more difficult to interpret. It was anticipated that the unconditioned two-year-olds entering the training programme in the country stable would have had higher plasma muscle enzyme activities than older horses entering training after a short spell. However, AAT, unlike LD, was not altered by the age of the horse or by stage of training. The decrease in LD values as training progressed in the present study agrees with similar trends for serum AAT activities in Thoroughbreds in training observed by others (Cardinet *et al.*, 1963; Cornelius *et al.*, 1963; Mullen *et al.*, 1979). It is possible that in the present study the greater variability of the AST values as compared to the LD values within and between horses may have masked any differences in AST values due to age and stage of training. An indication of this variability was the wide range of AST values (U/l), calculated as the mean ± 2 standard deviations (with the corresponding LD ranges in brackets), for the horses completing the training programme, which were: 189–1110 (198–641) for week 1, 174–1328 (195–545) for week 5, 141–1630 (195–531) for week 9, 164–1430 (188–520) for week 13 and 168–1279 (181–544) for week 17 of the programme.

Plasma LD and AST values were found to be higher in mares and fillies than in geldings. The higher activities of LD in mares and fillies and the lower concentrations of total protein in mares and fillies than in geldings may in part be due to the effect of age of the horse. The increase in total plasma protein with age was probably due to an increase in the globulin fraction of the total protein as shown for English shire horses (Blackmore and Kent, 1977).

Clear trends were apparent with increases in bilirubin and creatinine and decreases in urea as well as LD, as training progressed. The rise in creatinine as training progressed is probably due to an increased production of creatinine as a result of an increased utilization of phosphocreatine by the working muscle. An increased utilization of such phosphate-containing substrates by the working muscle may further account for the fall in plasma phosphorus concentrations observed when horses were racing: a period when the phosphate intake of horses was probably raised as a result of increased grain content in the diet.

Mullen *et al.* (1979) observed a decrease in serum albumin concentrations in two-year-old Thoroughbreds after the start of training and suggested that the blood albumin reservoir was required for the growth of muscle. Such an explanation is consistent with the observations in the present study of a decrease in plasma protein during the early stages of training and the fall in plasma urea concentrations as training progressed.

The suitability of various blood constituents as indicators of fitness of horses in training has received attention. If small deviations from optimum fitness are to be detected by measuring blood biochemical variables, then narrow limits must be placed on these variables. The establishment of such narrow limits may be difficult since the present study has demonstrated that a number of factors, particularly age and stage of training, can influence the biochemical composition of blood plasma of horses in training.

Acknowledgements

We are grateful to Mr B. Hall, Division of Mathematics and Statistics, CSIRO, Adelaide, for the analysis of the data and to Mr C. S. Hayes and other trainers for providing horses and facilities. We wish to acknowledge the financial support of the Australian Equine Research Foundation.

References

Aitken, M. M., Anderson, M. G., Mackenzie, G. and Sanford, J. (1974). Correlations between physiological and biochemical parameters used to assess fitness in the horse. *J. S. Afr. Vet. Ass.* **45**, 361–370.

Blackmore, D. J. and Elton, D. (1975). Enzyme activity in the serum of thoroughbred horses in the United Kingdom. *Equine Vet. J.* **7**, 34–39.

Blackmore, D. J. and Kent, J. E. (1977). Serum enzyme and protein concentrations in English shire horses. *Vet. Rec.* **100**, 91–92.

Cardinet, G. H., Fowler, M. E. and Tyler, W. S. (1963). The effects of training exercise, and tying-up on serum transaminase activities in the horse. *Am. J. Vet. Res.* **24**, 980–984.

Cornelius, C. E., Burnham, L. G. and Hill, H. E. (1963). Serum transaminase activities of thoroughbred horses in training. *J. Am. Med. Ass.* **142**, 639–642.

Judson, G. J., Frauenfelder, H. C. and Mooney, G. J. (1983). Plasma biochemical changes in Thoroughbred racehorses following submaximal and maximal exercise. In: *Equine Exercise Physiology*. Snow, D. H., Persson, S. G. B. and Rose, R. J. (eds.) Granta Editions, Cambridge. pp. 408–415.

Milne, D. W., Skarda, R. T., Gabel, A. A., Smith, L. G. and Ault, K. (1976). Effects of training on biochemical values in standardbred horses. *Am. J. Vet. Res.* **37**, 285–290.

Mullen, P. A., Hopes, R. and Sewell, J. (1979). The biochemistry, haematology, nutrition and racing performance of two-year-old thoroughbreds throughout their training and racing season. *Vet. Rec.* **104**, 90–95.

Simesen, M. G. (1972). Some clinical-chemical values in normal thoroughbreds and trotters. *Nord. Vet.-Med.* **24**, 85–90.

Snow, D. H. and Mackenzie, G. (1977). Some metabolic effects of maximal exercise in the horse and adaptations with training. *Equine Vet. J.* **9**, 134–140.

Williamson, H. M. (1974). Normal and abnormal electrolyte levels in the racing horse and their effect on performance. *J. S. Afr. Vet. Ass.* **45**, 335–340.

The Influence of Racing on Selected Serum Enzymes, Electrolytes and Other Constituents in Thoroughbred Horses

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Summary

Blood samples were collected from 26 Thoroughbred horses the day before and on the first, second, third, fourth and fifth days after racing. A number of plasma constituents were measured. Alterations of these constituents after racing varied between individuals. There was no correlation between alterations in serum biochemistry and racing performance.

Introduction

The effects of exercise on serum and plasma biochemistry have been widely investigated in Standardbred trotters and pacers and in endurance horses (Snow and Mackenzie, 1977; Rose *et al.*, 1979). However, due to difficulties in obtaining blood samples immediately after racing, fewer parameters have been determined in Thoroughbred race-horses. Keenan (1979) reported changes in plasma biochemical values in horses after racing and during a recovery period and found that most values had returned to their pre-race levels by one hour after racing.

The purpose of this study was to examine the reaction of some serum biochemical values for up to five days after racing.

Materials and Methods

This study was performed during the 1978 racing season, and a total of 26 Thoroughbred horses was studied. All horses were in the same racing stable. Jugular venous blood samples were collected into plain tubes for later serum removal, the day before and on the first, second, third, fourth and fifth days after racing. All samples were collected at the same time each day.

Serum concentrations of aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (AP), gamma glutamyl transferase