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BLOOD BIOCHEMICAL REFERENCE RANGES FOR SOWS UNDER MODERN MANAGEMENT CONDITIONS

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SUMMARY

Published reference ranges for blood biochemistry in swine generally do not relate to sows in modern breeding units, and results were often obtained by methods that are now outdated. The ranges widely used in clinical practice reflect these inappropriate sources. The data presented here were obtained using modern methods of analysis on blood samples from healthy, conventionally managed sows from six breeding herds of known disease status in eastern England, and thus represent appropriate ranges for this class of swine. The values differ from earlier reports principally in higher values for total bilirubin, creatine kinase, and more particularly of total plasma and serum proteins. The latter are shown to be due to higher immunoglobulin concentrations than those previously reported.

INTRODUCTION

Recently published reference ranges for blood biochemistry in swine generally relate to animals in experimental herds, and frequently only to animals less than 1 year old (e.g. Doornenbal *et al.*, 1983; James *et al.*, 1987). In these studies the herds were either specific pathogen free or of inadequately specified disease status. Much earlier work (reviewed by Tumbleson *et al.*, 1986), involved husbandry practices that are no longer current, results obtained by methods that are now outdated, or both. The ranges widely used in clinical practice (e.g. Blood *et al.*, 1983) reflect these inappropriate sources.

We present here reference ranges obtained by analysis of blood samples from a well defined population of commercial breeding sows using modern laboratory methods. The animals sampled were clinically healthy, conventionally managed sows at varying ages and differing stages of the breeding cycle, drawn from six breeding herds of known disease status on farms in eastern England. The values obtained are considered to represent valid reference ranges for healthy sows under modern husbandry conditions.

MATERIALS AND METHODS

Pigs sampled

Plasma samples were obtained in March, June, November and December 1986 from a total of 77 sows from five herds (herds 1-5 in Table I). Herd 1 was sampled on two

occasions, different sows being used on each occasion. Sera were obtained from 11 sows in herd 6 (Table I) in November 1987. The herds had Meat and Livestock Commission classification of Nucleus and/or Multiplier. Approximately 10% of the adult sows were sampled on each occasion. All pigs sampled were clinically normal, and were at varying stages of the reproductive cycle. The breeds involved are indicated in Table I.

The herds were conventionally managed. Sows were loose-housed and bedded on straw during the dry period, in groups of 10–20. Movement was only restricted by crating in the farrowing house (for approximately 4 weeks) and in stalls during the service period (approximately 5 weeks). Sows were routinely vaccinated for erysipelas and treated twice yearly with anthelmintic in the feed.

Feed was compounded on-farm and nutrient intake was adequate according to the current recommendations (Anon., 1982). The rations were based on cereals, primarily wheat or barley.

Table I
Breeding performance

	<i>Herd</i>						<i>National* average</i>
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	
Meat and Livestock Commission Classification	Nucleus H & LR	Nucleus W & D	Nucleus LW	Nucleus LW & LW×W	Multiplier LW×LR	Nucleus LW	
Breed of sows†							
Average number of sows	300	207.5	105.7	391.5	195.5	79.5	144
<i>Breeding performance</i>							
Litters per sow in herd per year	2.4	2.2	1.8	2.3	2.3	2.0	2.2
Live pigs born per litter	10.0	10.4	10.1	11.1	10.9	10.8	10.4
Percentage born dead	6.8	5.0	5.9	5.1	6.3	2.4	NR
Age at weaning (days)	18	21	21	25	20	42	28
Weaners per litter	9.1	8.9	8.8	9.6	9.3	9.5	9.1
Weaners per sow in herd per year	21.5	19.7	16.2	22.3	19.3	19.4	20.3
Sow mortality per year (%)	0.6	1.0	0.9	4.1	4.1	3.1	NR

*For breeding and fattening herds (Ridgeon, 1988).

†Breeds: H, Hampshire; LR, Landrace; W, Welsh; D, Duroc; LW, Large White.
NR, not recorded.

Sample collection

The sows were restrained by snaring the upper jaw and samples of blood were taken by jugular venepuncture into evacuated glass containers (Vacutainers, Becton

Dickinson) using 19 gauge needles. The containers were either without anticoagulant for serum, or with lithium heparin as anticoagulant for the preparation of plasma. Samples of blood were also taken into containers with potassium EDTA as anticoagulant, for the determination of haematological values. Those results will be described in a separate paper.

Analytical methods

Plasma was used for all assays, except where indicated in Table II, which lists the assay methods employed. Wherever possible, internal and external (UKEQAS) quality control schemes were used.

Table II
Analytical methods

Methods using dedicated apparatus	
Instrumentation Laboratory IL-919	
Glucose	Kinetic glucose oxidase-peroxidase
Urea	Kinetic urease-glutamate dehydrogenase
Creatinine	Kinetic Jaffé (alkaline picrate)
Corning 902 Na/K Analyzer	
Sodium	Direct ion-selective electrode
Potassium	Direct ion-selective electrode
Wescor 5100C	
Osmolality	Vapour pressure osmometer
Gelman System	
Electrophoresis*	Cellulose acetate, barbitone buffer pH 8.6, stained with Ponceau S
Methods using commercial kits on a Baker Encore II centrifugal fast analyser	
Baker kits	
Albumin*	Bromocresol green-equine calibrant
Total protein†	Biuret
Phosphate	UV phosphomolybdate
BCL kits	
Alkaline phosphatase	4-Nitrophenyl phosphate, 37°C
Aspartate aminotransferase	NAD-linked, 37°C
Creatine kinase	<i>N</i> -Acetylcysteine-activated, NADP-linked, 37°C
Gamma-glutamyl transferase	γ -Glutamyl-5-carboxy-4-nitroanilide, 37°C
Calcium	<i>o</i> -Cresolphthalein complexone
Cholesterol	Cholesterol esterase-cholesterol oxidase-peroxidase
Sigma kit	
Chloride	Mercuric thiocyanate
Gilford kit	
Magnesium	Magon complex
Other methods	
Bilirubin, total/direct	van den Bergh, with/without urea-benzoate accelerator (Powell, 1944), on Baker Encore
Glutathione peroxidase‡	Cumene hydroperoxide, NADP-linked, 37°C (BCL Product Information Sheet), on Baker Encore
Sorbitol dehydrogenase	Fructose-NAD, 37°C (Gerlach & Schürmeyer, 1960), on Clinicon 4010 photometer

*Serum as sample.

†Serum or plasma as sample.

‡Erythrocyte lysate as sample (heparinized blood 1:20 H₂O).

Statistical analysis

Where analytical results for more than 20 animals are available, the reference range for an analyte is given as the 2.5th to 97.5th intercentile range. If fewer than 20 results are available, the full range of measured values is given. Analysis of age-related changes is initially by product moment correlation, a probability level of $P \leq 0.01$ being taken to indicate a significant correlation. All significant correlations are then confirmed by rank correlation, to reduce the effects of outliers. Comparison of age and gestation distributions is by unpaired Student's *t*-test, using the appropriate approximation whenever a significant variance ratio was determined (Snedecor & Cochran, 1967).

Table III
Disease status

	<i>Herd</i>					
	1	2	3	4	5	6
<i>Infectious disease status of adult pigs</i>						
Atrophic rhinitis	—	—	+	—	—	—
Brucellosis	—	—	—	—	—	—
<i>Clostridium novyi</i> infection	0	0	0	0	0	0
Cystitis/pyelonephritis	0	0	0	0	+	0
Enzootic pneumonia	+	+	+	+	+	+
Epidemic diarrhoea	—	—	—	—	—	—
Erysipelas	0	0	0	+	+	0
Glasser's disease	0	0	0	0	0	0
<i>Haemophilus</i> pneumonia	0	0	0	+	0	0
Influenza	—	—	+	+	0	0
Intestinal adenomatosis complex	0	0	0	0	0	0
Leptospirosis	0	0	0	0	0	0
Listeriosis	0	0	0	0	0	0
Louse infestation	—	+	—	—	—	—
Mycobacterial infection	0	0	0	0	0	0
Mycoplasmal arthritis	+	+	+	+	+	+
Nematode infestation	0	0	0	0	0	0
Pasteurellosis	0	0	0	0	0	0
Parvovirus infection	+	+	+	+	+	+
Pox	0	0	0	0	0	0
Respiratory coronavirus	+	+	+	+	+	0
Salmonellosis	0	0	0	0	0	0
Sarcoptic mange	+	+	+	+	+	+
Streptococcal meningitis	0	0	0	0	0	0
Swine dysentery	—	—	—	—	—	—
Transmissible gastroenteritis	—	—	—	—	—	+
Talfan	0	0	0	0	0	0

+, presence of infection recorded; —, absence of infection recorded; 0, neither presence nor absence of infection recorded.

The following pig diseases were notifiable to the Ministry of Agriculture, Fisheries and Food under UK legislation at the time of sampling and none was present on these farms: anthrax, Aujeszky's disease, foot and mouth disease, rabies, swine fever (classical and African), swine vesicular disease, Teschen disease.

RESULTS

Characteristics of the reference population

Sow condition was generally good in all herds. Thin sow syndrome was not observed, but several sows were over-fat. Breeding performance figures for each herd at the time of sampling are shown in Table I, and the disease status for each herd at this time is indicated in Table III, which shows the presence or absence of named diseases.

Ages and stages of gestation of the pigs sampled

The distribution of ages for the 63 sows for which age was known is shown in Fig. 1. The age range was 11-74 months, with a median of 24 months. There were no significant age differences between breed groups (Large Whites and crosses ($n=24$) versus the others ($n=39$), $P=0.7$), nor was there any correlation of age with stage of gestation ($r=0.03$, $P=0.8$).

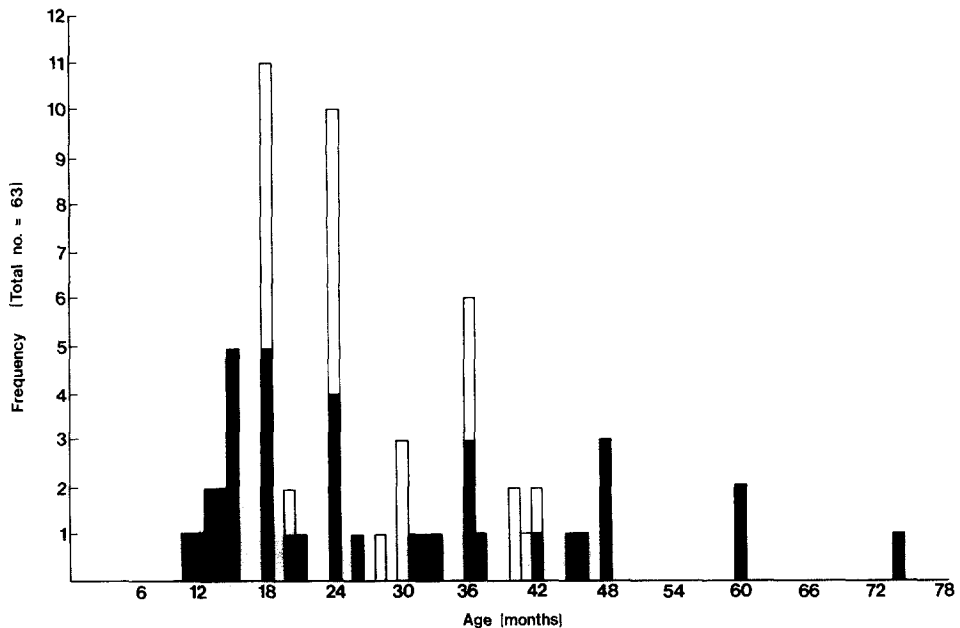


Fig. 1. Distribution of age for sows of known age ($n=63$). Large Whites and Large White crosses □, other breeds ■.

The distribution of stage of gestation is shown in Fig. 2. The range was 0-14 weeks (median 5 weeks). It is clear from Fig. 2 that gestational stage was markedly different for the Large Whites and crosses sampled ($n=38$) when compared to the other breeds ($n=39$) ($P<0.0001$).

The 11 sows sampled for serum were Large White crosses, aged 20-49 months, and at 1-15 weeks of gestation.

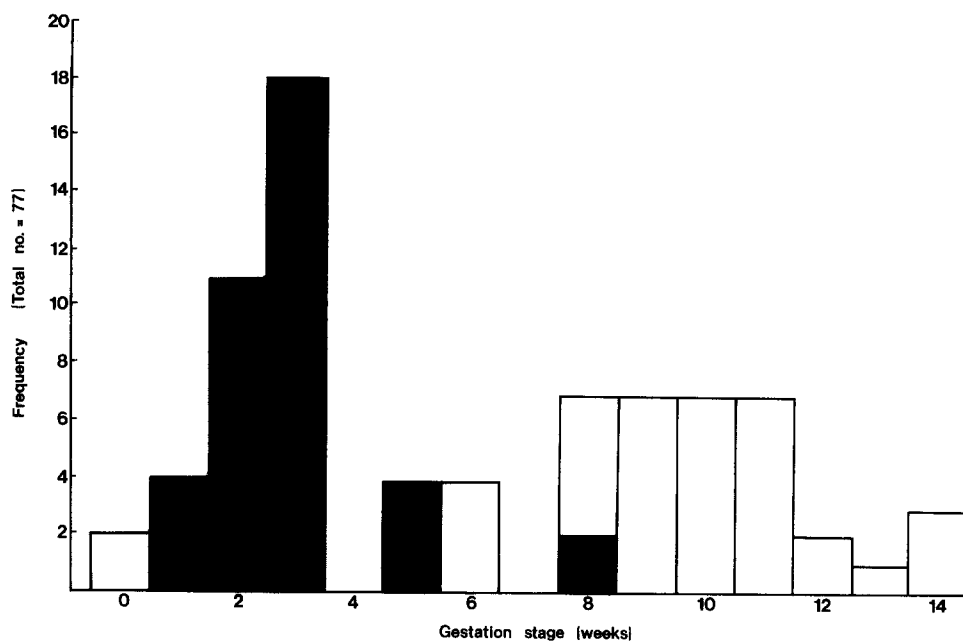


Fig. 2. Distribution of gestational stage for sows ($n=77$). Large Whites and Large White crosses □, other breeds ■.

Reference ranges

The reference ranges for all plasma, serum and erythrocyte analytes measured are given in Table IV. The median value for each analyte is also shown, as an appropriate 'average' value for the population.

Serum and plasma proteins

The appearance of a typical serum protein electrophoretogram is shown in Fig. 3. In each of the globulin bands (alpha, beta and gamma), only one protein peak is discernible. The concentrations of the globulin bands, derived from the electrophoretogram by densitometry, are given in Table IV. Protein concentrations were not significantly correlated with age (plasma total protein: $r=0.14$, $P=0.3$; serum total protein: $r=-0.23$, $P=0.5$; beta- and gamma-globulins: $r=0.30$, $P=0.4$).

Trends related to age and gestational stage

As correlation with age was performed for all 23 analytes, a more stringent definition of significance than that conventionally used was required, and was set at $P \leq 0.01$. On this basis, the plasma concentrations of three analytes were significantly correlated with age of the sow in the age range sampled. These were cholesterol ($r=-0.47$, $P=0.0004$, $n=53$), phosphate ($r=-0.52$, $P < 0.0001$, $n=63$) and alkaline phosphatase ($r=-0.63$, $P < 0.0001$, $n=62$).

Valid correlation with stage of gestation could not be performed, since this variable was not randomly distributed over breed groups (Fig. 2).

Table IV
Reference ranges

<i>Analyte</i>	<i>Median</i>	<i>Units</i>	<i>Range*</i>	<i>n †</i>
Bilirubin, direct	4	$\mu\text{mol/l}$	1-13	38
Bilirubin, total	5	$\mu\text{mol/l}$	2-16	38
Calcium	2.72	mmol/l	2.49-2.95	75
Chloride	106	mmol/l	98-111	77
Cholesterol	1.8	mmol/l	1.4-2.3	67
Creatinine	150	$\mu\text{mol/l}$	53-229	77
Glucose	3.9	mmol/l	(3.2-4.7)	15
Magnesium	0.87	mmol/l	0.72-1.45	64
Osmolality	298	mOsmol/kg	289-363	57
Phosphate (inorganic)	1.91	mmol/l	1.49-2.55	77
Potassium	4.66	mmol/l	3.57-6.03	77
Sodium	144.0	mmol/l	139.2-148.0	77
Urea	5.4	mmol/l	4.0-7.6	77
Alkaline phosphatase	88	iu/l	33-175	75
Aspartate aminotransferase	39	iu/l	22-88	77
Creatine kinase	859	iu/l	273-4127	75
Gamma-glutamyl transferase	40	iu/l	13-74	77
Sorbitol dehydrogenase	0.5	iu/l	(0-2.6)	10
Glutathione peroxidase	57.1	iu/ml RBC	33.7-74.4	67
Plasma total protein	82.4	g/l	73.9-99.8	77
Serum total protein	88.0	g/l	(82.5-97.2)	11
Albumin	36.5	g/l	(31.5-41.2)	11
Globulins				
alpha band	11.6	g/l	(10.7-13.8)	11
beta band	15.4	g/l	(13.6-19.6)	11
gamma band	19.8	g/l	(16.2-28.8)	11
Serum albumin :globulin ratio	0.73		(0.54-0.89)	11

*Range indicates 2.5th to 97.5th intercentile range, except where shown in parentheses, when it indicates full range of values obtained.

†*n* indicates the number of sows.

DISCUSSION

Performance figures for each herd may be compared with the national average for 1986 (Ridgeon, 1988) (Table I). There is general agreement of the performance in each herd with the averages for all parameters reported, although there is some variation between the herds in age at weaning, and herd 6 shows a low percentage born dead. The detailed descriptions (Tables I and III) of the performance and disease status of the population from which our samples were drawn provide unique confirmation of the validity of our reference ranges in the investigation of conventionally managed sows.

The reference ranges in Table IV are broadly in agreement with earlier publications that deal with adult sows (Rico *et al.*, 1977; Blood *et al.*, 1983; Friendship *et al.*, 1984; Reese *et al.*, 1984; Tumbleson *et al.*, 1986) with the exception of those for total bilirubin, creatine kinase, and more notably for total plasma and serum proteins.

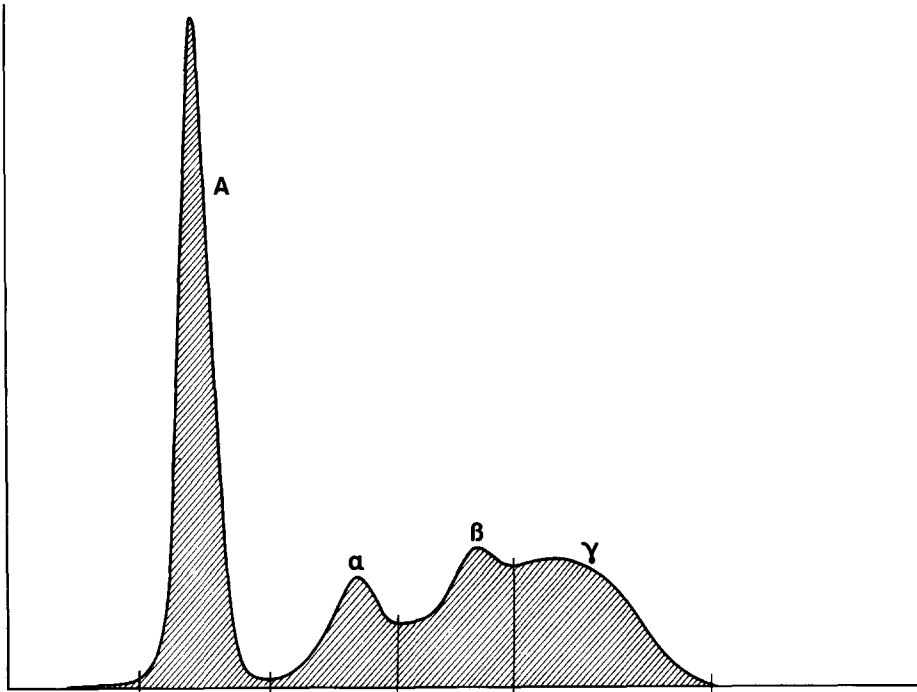


Fig. 3. Typical electrophoretogram of serum proteins in the sow. Albumin (A), and α -, β -, and γ -globulin bands are marked. This individual had an albumin : globulin ratio of 0.77.

The upper limit of the bilirubin range is four times that given by Blood *et al.* (1983), by Friendship *et al.* (1984), and by Doornenbal *et al.* (1983) for piglets up to 4 months of age. Doornenbal *et al.* (1983) found higher values in pigs over 4 months of age. We consider that our findings may thus represent a more appropriate reference range for the adult sow.

The distribution of creatine kinase values was non-Gaussian, with a tail to high values. The top of the reference range was higher than those given by Blood *et al.* (1983) or by Reese *et al.* (1984), but similar to the findings of Friendship *et al.* (1984). The high values are presumed to result from muscle damage, a consequence of the problems of capture and restraint of farmed sows. Comparably high values have been recorded in periparturient sows (reported in Tumbleson *et al.*, 1986), in experimental young pigs subjected to constraint in slings (James *et al.*, 1987), and in pigs after experimental exercise (Doizé *et al.*, 1989). Our values thus appear to constitute a more appropriate reference range than would be obtained from bleeding a group of animals more accustomed to handling.

The values for total plasma or serum protein were unexpectedly high when compared with published ranges (Blood *et al.*, 1983; Reese *et al.*, 1984; Tumbleson *et al.*, 1986), while the albumin levels were, on average, rather similar. This suggests higher immunoglobulin concentrations, which was confirmed by serum protein electrophoresis (Fig. 3, Table IV). The gamma-globulin concentrations were higher than those previously reported (Blood *et al.*, 1983; Tumbleson *et al.*, 1986), but showed no age dependency in the age range sampled.

Data reviewed in Tumbleson *et al.* (1986) supports the suggestion that swine 1 year old or more show higher serum total protein than younger animals. The 1-year-old stock described by Reese *et al.* (1984) have lower protein levels, but were specific pathogen free. Friendship *et al.* (1984), studying pigs from weaning to maturity, observed serum protein values that increased with age, adult sows showing ranges similar to those that we describe. Thus, although our protein reference ranges differ markedly from those in current use, they may be considered as more appropriate values for conventionally managed sows.

The age-related changes in plasma phosphate and alkaline phosphatase are expected and are well recognized physiological trends, supported by other work (Doornenbal *et al.*, 1983; Friendship *et al.*, 1984; Tumbleson *et al.*, 1986; James *et al.*, 1987). The fall in cholesterol concentration with age is consistent with the higher values found in younger animals as reported by others (Friendship *et al.*, 1984; Tumbleson *et al.*, 1986; James *et al.*, 1987).

We consider that the ranges we have given represent valid reference ranges for blood biochemistry in breeding sows of average productivity and disease status under modern husbandry conditions. Interpretation of abnormal values has been discussed by Wilson *et al.* (1972), and is described in standard texts (e.g. Blood *et al.*, 1983).

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