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DETERMINATION OF HAEMATOLOGY AND BLOOD CHEMISTRY VALUES IN HEALTHY SIX-WEEK OLD BROILER HYBRIDS

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SUMMARY

Fourteen "comparison values" were determined for 6-week-old broilers. A total of 980 birds were sampled from 21 flocks to provide "comparison values" for body weight and blood characteristics. Production and management details are described and sources of variation in the "comparison values" within and between flocks are discussed. The presence of correlations between "comparison values" and between performance and "comparison values", both within and between flocks, is investigated.

INTRODUCTION

The diagnostic application of haematology and biochemistry in human and veterinary medicine is a well established procedure. The present organisation within the poultry industry, where large numbers of individuals of low genetic variance are maintained in controlled environments, presents an ideal situation for the use of clinical pathology. The circumstances are unique in that clinical examinations and clinical pathology estimates can readily be combined with post mortem examinations on individuals sacrificed to supplement disease investigations. Earlier demands for large size samples to determine clinical pathology estimates and limited veterinary involvement in poultry disease were major obstacles to a parallel development of diagnostic clinical pathology in poultry. The introduction of rapid micro methods and automated line analysis to haematology and biochemistry have removed many of these obstacles to the development of clinical pathology and "profile" studies in poultry.

The application of clinical pathology to diagnosis in human medicine is dependent upon an extensive knowledge of "reference values" obtained from clearly defined "normal populations" and a theory of their usage has been considered in detail and

recently reviewed by Dybker and Grasbeck (1973). Acceptable "reference values" currently used in human medicine have been developed over many years and are the consequence of numerous analyses by many laboratories of many populations. Similar avian "reference values" will only result from extensive data collections and the present investigations were directed to determining provisional values which would be useful in current clinical pathology studies. Limitations of data preclude the use of the term "reference values" and we propose to apply the term "comparison values" to the flock means and limits obtained in the current investigations of healthy broiler hybrids.

MATERIALS AND METHODS

Flocks

A total of 21 different flocks were sampled from three commercial broiler concerns, A, B and C. The birds sampled were commercial broiler hybrids and the flocks were considered healthy in that the owner reported satisfactory progress of the birds, there was or had been no discernible disease outbreak, mortalities were in the low commercially accepted level, and the birds finished and were marketed satisfactorily. The details of performance and mortalities are shown in Table 1. All flocks were vaccinated against Marek's disease and those from producers A and B were vaccinated against infectious bronchitis and Newcastle disease. Some of the flocks were known to be free of *M. gallisepticum* and rapid plate agglutination tests on all sera collected failed to reveal any positive reactions against this organism.

Table 1. Food analyses and flock production data for 21 flocks

	Mean	Standard deviation	Range
Ash	6.263%	0.8240	4.5 to 7.1
Dry matter	87.8182%	0.7950	87.2 to 89.5
Ether extract	3.6333%	0.2712	3.3 to 4.4
Crude protein	18.8273%	0.9597	17.5 to 20.4
Available carbohydrate	52.1125%	2.3283	48.8 to 54.9
Calcium	0.9809%	0.2353	0.80 to 1.53
Inorganic phosphate	0.5891%	0.0966	0.49 to 0.78
Mortality	3.321%	1.0676	1.98 to 6.75
FCR	2.4672%	0.1877	2.16 to 2.77
EPEF	122.6842%	12.601	101 to 149

All flocks were sampled when the birds were around 6 weeks of age (40 to 47 days). Birds were weighed at the time of sampling and age and weight at marketing were recorded later. The European Production Efficiency Factor (EPEF) (British Oil and Cake Mills Limited Quarterly Poultry Bulletin 1967, No.56) was calculated from these data for each flock and data on Food Conversion Ratio were obtained from flock owners (Table 1).

$$\text{EPEF} = \frac{\text{Average Live Weight} \times \frac{\text{Total Live Weight sold (lb)}}{\text{No. of chicks started (incl. extras)}}}{\text{Average Age (days)} \times \frac{\text{Total Feed Used (lb)}}{\text{No. of Broilers Sold}}} \times \frac{10,000}{2.2}$$

A sample of food was taken from each flock and was analysed by the Poultry Research Centre (Table 1). All birds were on the deep litter system and stocking density was approximately 0.58 ft² per bird. The pen size varied considerably between flocks and averaged just over 2,000 birds per pen. All flocks examined from producers A and C were from pens with mixed sexes and from producer B 7 flocks contained all-female pens and 6 all-male pens.

Sampling

With the pen lights dimmed the required number of birds were quietly picked at random, crated and removed to just outside the pen, where they were weighed individually. One hundred birds were sampled from the first 5 flocks but thereafter, as the analysis had shown that a smaller sample would be adequate, only 30 birds were sampled in the remaining flocks. After weighing the birds were bled from the wing vein directly into 2 tubes, one ml of blood into a tube containing anticoagulant (EDTA), 6 ml of blood into a chemically clean glass tube for serum separation. Subsequently 0.1 ml of blood from each EDTA tube was transferred into a tube containing 1.9 ml of 0.3 N perchloric acid for glucose estimation.

Estimations

A total of one haematological and 12 biochemical estimates were made on the samples from each bird, except for the first 5 flocks, for which the biochemistry was performed on 20 bulks of 5 samples per flock. The packed cell volume percentage was estimated from the EDTA sample using a Hawksley Micro-Haematocrit.

Glucose levels were estimated on the perchloric acid samples by using the Boehringer Corporation (London) Ltd kit based on the GOD/Perod method of Werner *et al.* (1970). Total serum protein was estimated using a biuret reagent supplied by British Drug Houses and albumin estimated using the Bromocresol green dye binding method of Bartholomew and Delaney (1966). Serum globulin was calculated by subtraction. Cholesterol levels were estimated using the Liebermann Buchard reaction according to the principle of Huang *et al.* (1961) and modified by Ness *et al.* (1964). Inorganic phosphate was determined by the method of Delsal and Manhoury (1958) and uric acid by the method of Caraway (1963). The optical densities of the above reactions were read on a Gilford 300 N rapid-sampling spectrophotometer at appropriate wavelengths.

Serum calcium, sodium, potassium and magnesium estimates were determined using a Perkin Elmer 290 B atomic absorption spectrophotometer with a sample presentation system. Serum calcium determinations were made at a dilution of 1:25 in 0.5% lanthanum chloride in distilled water; sodium, potassium and magnesium at 1:250, 1:100 and 1:50 dilutions respectively in distilled water.

RESULTS

All "comparison values" had a significantly greater variance between flocks than within flocks, with the possible exceptions of total protein, albumin and globulin. Most variables were satisfactorily normal within flocks, as judged by skewness (Fisher's g_1), with the exception of cholesterol, potassium and perhaps glucose and calcium. Flock means were not seriously skew for any variate. The overall means (unweighted means of flock means) and 95% confidence limits for flock means are shown in Table 2. An analysis of variance of flock means, between and within producers and sex groups of flocks of similar age, showed that significant differences were present between groups in body weight ($P < 0.01$), glucose ($P < 0.05$) and globulin and magnesium ($P < 0.1$). As expected, body weight differed significantly with producer and sex. Glucose was the only other variate which showed formally significant differences between producers. The reality of the differences of globulin and magnesium are more doubtful, but the suggestion seems again to be of a difference between producers. No sex differences have been detected in blood reference values. The glucose level in normal flocks varied with producer but all blood characteristics varied, even between flocks of the same sex and producer.

Table 2 Flock mean "comparison values" for 6-week-old broilers

		No. of flocks	Mean	Standard deviation	95% confidence limits
Weight kg	(Kg)	21	1.176	0.127	0.926 to 1.426
Packed cell volume %	(PCV)	21	28.799	0.860	27.113 to 30.484
Total protein g/100 ml	(TP)	21	3.811	0.205	3.408 to 4.213
Albumin g/100 ml	(A)	21	1.347	0.136	1.080 to 1.614
Globulin g/100 ml	(G)	21	2.460	0.242	1.985 to 2.935
Albumin/globulin ratio	(A/G)	21	0.55	0.10	0.35 to 0.75
Cholesterol mg/100 ml	(Chol)	21	100.1	14.0	70.0 to 129.0
Blood glucose mg/100 ml	(G)	17	173.4	28.0	117.0 to 229.0
Inorganic phosphate mg/100 ml	(Pi)	21	7.468	1.563	4.403 to 10.534
Uric acid mg/100 ml	(UA)	21	7.78	1.683	4.41 to 11.15
Magnesium mg/100 ml	(Mg)	21	2.300	0.246	1.818 to 2.782
Calcium mg/100 ml	(Ca)	21	10.501	0.751	9.028 to 11.974
Sodium mEq/l	(Na)	20	152.9	7.0	138.0 to 166.0
Potassium mEq/l	(K)	19	9.08	1.505	6.07 to 12.09

A comparison of the overall flock means for glucose, cholesterol, albumin and globulin from producers A, B and C is shown in Table 3. Significant differences are present between the glucose, albumin and globulin values of the flocks of producer C (which were not vaccinated) and those of producers A and B (which were). The cholesterol value in the flocks of producer A is highly significantly different from producer C but not from producer B.

Examination for comparison value correlations between flock means showed the following significant correlations: positive, globulin with total protein, glucose with albumin, calcium with cholesterol and calcium with magnesium; negative, bodyweight

Table 3. Comparison of flock mean glucose, cholesterol and serum protein values from 3 producers.

	Producer	Mean	Standard deviation
Glucose mg/100 ml	A	149.95	23.44
	B	174.24	24.01
	C	215.77	4.28
Cholesterol mg/100 ml	A	96.60	9.21
	B	99.92	16.86
	C	111.92	15.01
Albumin g/100 ml	A	1.296	0.0745
	B	1.324	0.1320
	C	1.567	0.0616
Globulin g/100 ml	A	2.572	0.2112
	B	2.474	0.2006
	C	2.040	0.1780

with potassium, globulin with albumin, total protein with inorganic phosphorus and glucose with globulin (Table 4).

DISCUSSION

"Comparison values" obtained in the present investigation are compared with values published previously (Table 5). The majority of previous estimates are unfortunately based on small numbers, they often refer to only a few values and are frequently determined by techniques not now used. A comparison is therefore difficult and earlier estimates may be more useful when subsequent work on age and breed variation is completed.

While the presence of certain correlations (Table 4) were to be expected, the reasons for others are less clear. The relationships between total protein, albumin and globulin were predictable and perhaps the positive correlation between glucose and albumin may be explained on a nutritional basis. The negative correlation of body weight and potassium, while intriguing, was unexpected. A further examination was made of this relationship within flocks and a negative correlation was not present.

Broiler hybrid differences between the 3 producers may have contributed to between producer variance but this cannot at present be other than speculative. The comparison of the flock groups from the 3 producers are of particular interest and encourage further studies to investigate the application of these data to profile studies. The mean serum albumin value of the flocks of the 2 producers who vaccinated, A and B, was lower, and the serum globulin values higher, than the mean in the non-vaccinated flocks of producer C. The blood glucose values were significantly lower in flocks of A and B compared to flocks of C, while the serum cholesterol values were significantly lower in flocks of A compared to those of C. Although the flocks of producer A were healthy at the time of sampling, some months later fatty liver and kidney syndrome (FLKS) occurred and the comparatively low mean blood glucose recorded in these flocks, which is typical of FLKS (Bannister *et al.*, 1975), and perhaps the lower mean serum cholesterol, indicate the development of this condition and encourage further

Table 4. Flock mean correlation matrix for 13 observations.

	Kg	PCV	TP	A	Gl	Chol	G	Pi	UA	Mg	Ca	Na	K
Kg	1.0000												
PCV	0.0074	1.0000											
TP	-0.1123	0.0716	1.0000										
A	0.3064	0.1254	0.1237	1.0000									
Gl	-0.2731	-0.0228	0.8159	-0.4713	1.0000								
Chol	0.3106	-0.1766	0.1631	0.3228	-0.0502	1.0000							
G	0.4018	-0.3068	-0.4996	0.5595	-0.7661	0.3506	1.0000						
Pi	-0.2157	-0.1961	-0.5228	-0.0112	-0.4561	0.0459	0.3900	1.0000					
UA	0.0598	0.3731	0.0947	0.0882	0.0202	0.2225	0.2216	0.4269	1.0000				
Mg	-0.1970	-0.2928	-0.0346	0.2845	-0.1915	0.1120	0.1226	0.0591	0.0767	1.0000			
Ca	-0.1081	-0.2214	-0.2874	0.2706	-0.4323	0.5912	0.4224	0.2111	0.1174	0.4419	1.0000		
Na	-0.4009	-0.0969	0.1574	0.0990	0.0765	0.2486	0.1026	-0.0335	0.2624	-0.0328	0.3205	1.0000	
K	-0.5573	-0.2130	-0.1313	0.1310	-0.1977	0.2104	0.0134	0.3170	0.1556	0.3066	0.4796	0.7598	1.0000

10 % Significance level 0.4409

5 % Significance level 0.5139

1 % Significance level 0.6411

0.1% Significance level 0.7603

Table 5. Proposed "comparison values" and previously published values.

	"Comparison Value"	Literature Value	Breed	Age	No.	Reference
PCV	28.8	27% 31.9%	New Hampshire White Leghorn	5 weeks 7 weeks	10 7	Pudelkiewicz, W.J. <i>et al.</i> (1959) Tapper, D.N. and Kare, M.R. (1960)
Total protein	3.8g/100 ml	3.91g/100 ml	New Hampshire	6 weeks	—	Morgan, G. and Glick, B. (1972)
Albumin	1.35g/100 ml	1.61g/100 ml	New Hampshire	6 weeks	—	Morgan, G. and Glick, B. (1972)
Cholesterol	100.1mg/100 ml	113mg/100 ml	White Leghorn	4-5 weeks	24	Caldwell, C.T. and Suydam, D.E. (1960)
		145mg/100 ml	New Hampshire	5 weeks	10	Pudelkiewicz, W.J. <i>et al.</i> (1959)
Blood glucose	173.4mg/100 ml	156mg/100 ml	White Leghorn	7 weeks	7	Tapper, D.N. and Kare, M.R. (1960)
Inorganic phosphate	7.47mg/100 ml	2.5mg/100 ml	White Leghorn	4-6 months	14	Beljan, J. <i>et al.</i> (1971)
Uric acid	7.78mg/100 ml	1.8mg/100 ml	New Hampshire	5 weeks	10	Pudelkiewicz, W.J. <i>et al.</i> (1959)
Magnesium	2.3mg/100 ml	1.05mg/100 ml	Barred Plymouth Rock	6 months	40	Sapre, V.A. and Mehta, M.L. (1970)
Calcium	10.50mg/100 ml	16.97mg/100 ml	Leghorn	14 weeks	12	Taylor, T.G. and Hertelendy, F. (1961)
Sodium	152.9mEq/l	131.6mEq/l	White Leghorn	6 weeks	4	Kravis, E. and Kare, M. (1960)
Potassium	9.08mEq/l	4.0mEq/l	White Leghorn	6 weeks	4	Kravis, E. and Kare, M. (1960)

investigation of the use of clinical pathology reference values in profile studies to determine sub-clinical disease.

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RESUME

Détermination de valeurs hématologiques et chimiques du sang chez des broilers hybrides en bonne santé âgés de six semaines

Quatorze valeurs biométriques de comparaison ont été déterminées sur des broilers âgés de six semaines. Au total 980 oiseaux de 21 troupeaux furent étudiés individuellement pour le poids du corps et les caractéristiques sanguines. Les détails de production et de gestion sont décrits et les causes des variations des valeurs hématologiques ont été comparées entre les troupeaux et dans les troupeaux. La présence des corrélations entre les valeurs de comparaison individuelles et entre les performances et les valeurs de comparaison est recherchée entre les troupeaux et dans les troupeaux.

ZUSAMMENFASSUNG

Die Bestimmung von zytologischen und chemischen Blutwerten bei gesunden 6 Wochen alten Masthybriden

Vierzehn Parameter wurden bei 16 Wochen alten Broilerküken gemessen. Bei insgesamt 980 Tieren aus 21 Herden wurden Körpergewicht und Blutwerte gemessen. Die Produktions- und Managementfaktoren werden beschrieben und die Ursachen der Variabilität der verschiedenen Parameter innerhalb und zwischen den Herden diskutiert. Das Vorhandensein von Korrelationen zwischen den einzelnen Parametern und zwischen Blutwerten und Leistungskriterien innerhalb und zwischen den Herden wurde untersucht.