COLOUR INDEX FOR DETERMINING THE NATURE OF ANAEMIA CAUSED BY HELMINTHIASIS IN POULTRY

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COLOUR index indicates the proportion of haemoglobin present in each red cell with respect to the normal. The ideal value of colour index is unity, although a slightly lower value of 0.85 is normally found in healthy human population[1]. When the colour index is higher than this normal value, the associated anaemia is called "hyperchromic", and when it is lower than the normal value, the resultant anaemia is termed "hypochromic". However, it is interesting to find a paucity of reference to colour index in the pertinent literature on avian haematology. Hence the present investigation was conducted on plausible alteration of colour index in domestic fowl due to helminthiasis.

The total erythrocyte counts and the total blood haemoglobin levels were estimated following the methods of Nambiar[1] and Cartwright[2] respectively in 400 birds, which included both healthy (uninfected) and helminthic infected (field cases) fowls. The colour index was calculated from these data, using the following formula:

$$\text{Colour index} = \frac{\%\ \text{haemoglobin}}{\%\ \text{red blood corpuscles}}$$

The haemoglobin percentage was calculated taking 15 g of haemoglobin per 100 ml of blood as 100%. Similarly the percentage of red cell count of the sample blood was determined[3] by taking a count of 5 million red cells per cubic millimeter as 100%.

The visceral samples collected were thoroughly examined for the presence of helminthic worms. The different types of gastro-intestinal helminths that were observed to infect the fowl are *Raillietina tetragona*, *Raillietina echinobothrida*, *Raillietina cesticillus*, *Cotugnia diagonopora*, *Choanotaenia infundibulum*, *Hymenolepis carioca* and *Ascaridia galli*. The worm infestation was divided into 4 types i.e. single, double, triple and quadruple infections after taking into account the number of different species of worms involved in infecting the same fowl. Further each of these multiple infections i.e. double, triple and quadruple infections, constitute Group-I and II. Group I comprises all worm combinations which result in leucopenia whereas group II comprises the remaining worm combinations which result in leucocytosis 4, 5. The data have been shown in table 1.

(a) *In healthy fowls:*

The values of colour index obtained in healthy cockerels and pullets of 3–4 months age were 1.02 ± 0.09 and 1.17 ± 0.53 respectively, indicating that the females have slightly higher colour index than males. This is to be expected because the mean corpuscular volume of red blood cells in pullets ($146.41 \pm 44.84\ \mu^3$) is higher than that in cockerels ($115.32 \pm 28.38\ \mu^3$), indicating that red blood corpuscles are bigger in size in pullets than in cockerels[6]. Hence the amount of corpuscular haemoglobin is greater (35.36 ± 15.68 picograms) in pullets than that (30.84 ± 8.96 picograms) of cockerels[7].

(b) *In deseased fowls:*

(i) *In cockerels*: Infections with *R. cesticilus* and *C. infundibulum* resulted in hypochromic anaemia, whereas in all other single infections hyperchromic anaemia was seen. Group I of double and triple infections and group II of quadruple infection resulted in hyperchromic anaemia, whereas in all other multiple infections normochromic condition prevailed.

(ii) *In pullets*: In all types of single infections, the colour index was less than normal suggesting hypochromia. In *Ascaridia galli* infection, although the colour index was equal to the normal, the standard deviation (± 1.04) was exceptionally high indicating a high range of variation within the individual samples. In all multiple infections, the colour index was less, suggesting hypochromia.

Obviously, helminthiasis caused mostly hyperchromic anaemia in cockerels, while in pullets it resulted in hypochromic anaemia.

1 March 1986; Revised 29 May 1986

1. Nambiar, K. T. K. M.V.Sc., Dissertation, submitted to Madras University, Madras, 1961, p. 68.
2. Cartwright, G. E., *Diagnostic laboratory haematology*, 1958, p. 34.

Table 1 *Colour index values obtained in healthy (uninfected) and helminth infected domestic fowl, Gallus domesticus, of 3–4 months age*

Cockerels			Pullets		
Infection		Colour index	Infection		Colour index
None-healthy (8)		1.02±0.09	None-healthy (7)		1.17±0.53
Single infection[1]:			Single infection[1]:		
RT (17)		1.08±0.30	RT (10)		1.00±0.50
RE (7)		1.10±0.33	RE (8)		0.93±0.36
*RC (3)		0.60±0.10	RC (2)		1.00±0.17
CI (2)		0.78±0.08	CI		—
HC (3)		1.22±0.07	HC		—
AG (12)		1.16±0.28	AG (15)		1.14±1.04
Double infection[2]:			Double infection[2]:		
	RT+RE (6)	1.20		RC+CD (2)	2.14
	RE+AG (9)	1.18		RT+HC (4)	0.76
Group I	RT+CD (4)	1.26	Group I	RE+AG (7)	0.70
	RT+HC (3)	1.14		RT+CD (3)	0.89
	Group mean	1.20±0.04		Group mean	1.12±0.59
	RT+AG (20)	1.18		RT+RE (8)	0.78
	AG+HC (6)	1.14		RT+AG (14)	0.94
	CD+AG (4)	0.99		RT+RC (2)	0.80
Group II	RE+HC (2)	0.95	Group II	RE+RC (1)	0.93
	RC+AG (1)	0.77		CD+HC (2)	0.72
	RE+RC (2)	1.04		AG+HC (4)	0.85
	Group mean	1.01±0.14		CD+AG (3)	0.73
				Group mean	0.82±0.10
Triple infection[3]:			Triple infection[3]:		
	RE+CD+HC (1)	1.18		RT+RC+CD (2)	0.80
	RC+CD+AG (1)	1.26		RT+RE+AG (11)	0.83
Group I	RT+RE+HC (3)	1.09	Group I	RT+RC+AG (6)	0.85
	RE+AG+HC (3)	2.62		RT+AG+HC (5)	0.85
	RT+CD+HC (1)	1.03			
	Group mean	1.44±0.60		Group mean	0.83±0.02
	RT+CD+AG (11)	1.17		RT+CD+AG (6)	0.84
	RT+RE+AG (5)	0.92		RE+CD+AG (1)	0.64
	RC+AG+HC (2)	1.02		RT+CD+HC (1)	0.71
	CD+AG+HC (1)	1.23		RC+CD+AG (1)	0.82
Group II	RE+RC+AG (1)	0.97	Group II	RT+RE+RC (3)	0.78
	RE+CD+AG (2)	1.07		RE+CD+HC (1)	0.71
	RT+AG+HC (6)	0.94			
	RT+RC+AG (5)	0.96		Group mean	0.75±0.06
	Group mean	1.04±0.10			
Quadruple infection[4]:			Quadruple infection[4]:		
	RT+CD+AG+HC (3)	1.13		RT+RE+RC+AG (2)	1.36
	RT+RE+CD+HC (1)	0.94		RT+RE+RC+CD (1)	1.04
Group I	RT+RC+CD+HC (1)	0.80		RE+RC+AG+HC (1)	0.78
	RT+RC+AG+HC (1)	0.99		RT+RC+AG+HC (1)	0.28
	Group mean	0.97±0.14		RT+CD+AG+HC (5)	0.68
			Group I	RT+RE+AG+HC (5)	0.69
	RT+RE+RC+AG (1)	1.50		RC+CD+AG+HC (1)	0.93
Group II	RT+RE+CD+AG (2)	0.92		RT+RC+CD+AG (1)	0.76
	RT+RE+AG+HC (6)	0.92		RT+RE+CD+AG (1)	1.11
	Group mean	1.11±0.28		Group mean	0.85±0.30

Figures in parentheses indicate the number of fowls possessing such infection. Infection with 1. one 2. two. 3. three 4. four different species of worms affecting the same fowl.
RT = *Raillietina tetragona*; RE = *Raillietina echinobothrida*; RC = *Raillietina cesticillus*; CD = *Cotugnia diagonopora*; AG = *Ascaridia galli*; HC = *Hymenolepis carioca*; CI = *Choanotaenia infundibulum*
Group I = Infections resulting in leucopenia. Group II = Infections resulting in leucocytosis.

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GLYCOLYTIC OXIDATION OF GLUCOSE IN GASTROCNEMIUS MUSCLE OF TOAD, *BUFO MELANOSTICTUS* DURING PROGRESSIVE DENERVATION

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THE muscle metabolism is intriguingly regulated by the trophic influence of the innervating nerve to a large extent[1]. Elimination experiments of denervation[2] have shown a high metabolic interdependence between muscle and nerve cells. They have also supplied convincing evidence for nervous regulation of the physiological, metabolic and structural properties of the muscle. Several reports have been made on metabolic changes in hypertrophy of gastrocnemius muscle[6–9], but very few reports are available on changes during progressive denervation of muscle. It is a well-established fact that the muscle utilizes energy for its specific function and also for the maintenance and renewal of structure and chemical composition and for adaptation to new stimuli, in other words for trophic processes[1]. Carbohydrates are considered to be the main source of energy during muscle activity. The present study deals with the changes taking place in some of the substrates and glycolytic enzymes during progressive denervation atrophy.

The toads, *Bufo melanostictus* of medium size (30±5 g) were collected in and around Tirupati and maintained in glass aquarium tanks with sand as bed. The toads were fed with earthworms *ad libitum*. The toads were acclimatized to the laboratory conditions for a week before they were subjected to denervation process. The sciatic nerve section on one leg was performed as suggested by Swami and Satyanarayana[10]. The toads were double-pithed on 7th, 14th, 21st and 28th day postoperatively and both the denervated and contralateral gastrocnemius muscles were quickly excised in cold conditions. Five per cent tissue homogenates were prepared in a suitable medium and centrifuged at 1000 g for 10 min to remove the cell debris. The supernatants were assayed for phosphorylase[11], aldolase[12], lactate dehydrogenase (LDH)[13] and metabolites such as glycogen[14], lactate[15] and pyruvate[16]. The protein content was determined by the method of Lowry *et al*[17]. The data was analyzed by student *t* test to assess the difference between control and experiment.

The data showed that glycogen levels decreased significantly one week after denervation suggesting its reduced synthesis. The specific activity of phosphorylase, a glycogen-cleaving enzyme, was decreased during the first and the second week and an increase in the third and fourth week after denervation. Though phosphorylase activity was decreased during the first and second week after denervation, the glycogen was depleted indicating its reduced synthesis in the early stages of denervation. Similar results were reported by several investigators in various animals during denervation[7,18,19]. The enhanced phosphorylase activity and the decreased glycogen levels in the later weeks of denervation could be due to both the increased glycogenolysis and the decreased glycogenesis. In support of this, Ramachandra Rao[20] reported diminished uptake of ^{14}C glucose into glycogen in the denervated muscle.

In order to assess the extent of mobilization of glycogen into glycolytic pathway, aldolase activity was determined. Aldolase showed maximum decrease in activity in the first week and the extent of decrease declined with progressive denervation. This suggests reduced mobilization of glucose (glycogen) in the later period of denervation through glycolysis and increased oxidation through HMP shunt during prolonged periods of denervation. This is in consonance with earlier reports[21,22].

Except in the second week of denervation, the pyruvate content was significantly reduced whereas lactate level was elevated in the first week and thereafter no appreciable change was observed in comparison with contralateral muscle. The decreased pyruvate formation in the atrophied muscle could be attributed to the reduced glycolytic oxidation of glucose as evidenced by the decreased