BIOAVAILABILITY OF IRON IN GROWING RABBITS FED EXCESS LEVELS OF DIETARY IRON, UNDER EGYPTIAN CONDITIONS.

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Abstract - Five levels of dietary iron (Fe), 215, 290, 365, 415 and 515mg/kg were fed for 56 days to five groups of growing New Zealand White rabbits (average initial body weight 728g) each of 14 rabbits (7 males and 7 females) to investigate Fe bioavailability and determine the maximum safe level of dietary iron, under Egyptian conditions. Ferrous sulfate ($FeSO_4$ $2H_2O$) was used as the supplemental Fe source. The results of the present study indicated that viability of rabbits (%) daily feed intake, NFE digestibility, haemoglobin and blood serum contents of total lipids, calcium, magnesium and iron were not affected significantly by the dietary iron treatments. Increasing the dietary iron above 290mg/kg decreased ($P<0.01$ or 0.05) the final live body weight, daily body gain, feed conversion efficiency, DM, OM, CP, CF, EE digestibilities, N-utilization and blood serum concentrations of phosphorus, glucose and total protein. Levels of serum GOT and GTP enzymes were increased ($P<0.05$ and 0.01, respectively) with increasing the dietary iron level. No clinical signs of iron toxicity were observed in rabbits of any dietary group throughout the experimental period. Gross and microscopical examination did not reveal any significant alteration in liver, spleen, kidneys and lungs of rabbits given the dietary iron levels up to 365mg/kg. Mild to moderate degenerative hyperplastic, emphysema and proliferating changes were observed in the indicated organs of rabbits fed the dietary iron levels 415 or 515mg/kg.

INTRODUCTION

Iron is one of the most important mineral elements in human and animal nutrition. It is a component of haemoglobin and myoglobin, and of the enzymes, cytochromes, catalases, peroxidases and ribonucleotide reductase.

Therefore, iron serves important functions in oxygen transport and metabolism and synthesis of neurotransmitters and DNA (YOUDIN and GREEN, 1977).

Limited information is available on the deleterious effects of excess dietary iron for rabbits that might result from excessive use of mineral supplements in the manufactured diets.

The present work was conducted to :

1 - Study the bioavailability of iron in growing rabbits fed excess levels of dietary iron. Growth performance, nutrients digestibility, nitrogen utilization, blood composition and organs histopathology were used as indices for the ion bioavailability,

2 - Determine the maximum level of dietary iron which the rabbits can consume without adverse effects on their growth performance.

MATERIAL AND METHODS

The experimental work of the present study was carried out at Rabbit Research Unit, Department of Animal Wealth, Institute of Efficient Productivity, Zagazig University, Zagazig, Egypt. Blood biochemical analysis was performed at the laboratories of Biochemistry Department, Faculty of Pharmacy, Zagazig University. The experiment lasted for 56 days, initiated on December, 1994.

A total number of 70 growing NZW rabbits with an average initial body weight of 728 ± 17g were assigned to five dietary treatments. The dietary treatments included addition of iron (Fe) at levels of 0, 75, 150, 200 and 300 mg/kg to a basal commercial diet (contained total Fe level of 215 mg/kg) to provide a total dietary iron levels of 215, 290, 365, 415 and 515 mg/kg for the five treatments, respectively. Ferrous sulfate ($FeSO_4$ $2H_2O$) was used as the supplemental iron source (contains 29.8% Fe). The ingredients and chemical composition of the basal diet are shown in Table 1. Rabbits were housed each of 3 together in wire cages (60x55x40 cm) provided withe feeders and stainless nipples. Feeds and water were offered to rabbits in *ad libitum* amounts throughout the experimental period. Individual live body weights and feed intake data were biweekly recorded. A digestibility trial was conducted at the 7th week of the experiment by using 4 male rabbits from each dietary treatment. The rabbits were individually housed in metabolic cages that permit to collect faeces and urine separately. The trial lasted for 10 days, 4 days as a preliminary period followed by 6 days to quantify the consumed feed and faeces and urine output. Routine chemical analysis of samples of

Table 1 : Ingredients and chemical composition of basal diet (Pelleted commercial diet) fed to rabbits during the experimental period.

Item	% of total diet
Ingredients :	
Wheat bran	30.20
Yellow corn	15.00
Barley grain	15.00
Clover hay	13.00
Sunflower meal	10.00
Decorticated cottonseed meal	6.55
Soybean meal	5.00
Molasses	3.00
Limestone	1.59
Vitamins and minerals premix (1)	0.30
Common salt	0.30
DL-methionine	0.06
Total	100.00
Chemical composition, % (as fed basis) :	
Dry matter	89.72
Crude protein	18.55
Ether extract	2.27
Crude fibre	12.86
Nitrogen free extract	49.18
Methionine + cystine (2)	0.57
Ash	6.86
Calcium	0.86
Phosphorus	0.67
Magnesium	0.38
Iron (mg/kg)(3)	215
Manganese (mg/kg)	70
Copper (mg/kg)	23
Zinc (mg/kg)	113
Digestible energy (Kcal/kg) (4)	2600

(1) Each 3 kilograms of premix contained : Vit. A 12,000,000 IU ; Vit. D 15,000,000 IU ; Vit. E 50g ; Vit. K_3 2g ; Vit. B_1 2g ; Vit. B_2 6g ; Vit. B_6 2g ; Vit. B_{12} 0.01g ; Niacine 50g ; Pantothenic acid 20g ; Biotine 0.2g ; Folic acid 5g ; Choline chloride 1200g ; Zn 70g ; Mn 30g ; Fe 75g ; Cu 5g ; I 0.75g ; Se 0.1g ; Mg 400g

(2,4) The value was calculated according to NRC (1977).

(3) Total dietary iron 215mg/kg = 140mg in natural feed ingredients + 75mg added as premix.

feed and faeces and N in urine was performed according to A.O.A.C. (1990). Calcium, magnesium, iron, zinc, copper, manganese contents in the basal diet were chemically determined by using atomic absorption spectrophotometry (Perkin Elmer, Model 2380) at different wave lengths. Total phosphorus content in feed samples was assayed by using spectrophotometer according to BLACK (1965). The values of total digestible nutrients (TND) were calculated according to the classic formula of CHEEKE *et al.* (1982).

At the end of the digestibility trial all males (n=4) were slaughtered and blood, liver, spleen, kidney, heart and lungs were obtained. Two blood samples were taken from each rabbit, one for haemoglobin assay and the other to serum preparation. Samples of the internal organs were washed with tap water, dehydrated with filter papers and placed in glass bottles containing 10% formol saline, then imbedded in paraffin wax and sectioned at 5 microns thickness. Sections were stained with hematoxylin and eosine according to BANCROFT *et al.* (1990).

Blood haemoglobin was measured in the fresh whole blood directly at the time of collection according to SCHALM *et al.* (1975). Blood serum was separated by blood centrifugation at 3000 r.p.m. for 15 minutes. Serum was stored frozen (-20°C) in plastic vials until biochemical analysis for serum total protein, total lipids, glucose, urea-N, calcium, phosphorus, magnesium and iron concentrations, serum transaminase enzymes (GOT and GTP) activities by using commercial kits (Bio-Merieux, Laboratory Reagent and Products, France).

Data of the experiment were statistically analyzed by analysis of variance as a completely randomized design, and differences between means were determined by Duncan's New Multiple Range Test (STEEL and TORRIE, 1980). All data percentages were transformed to their arc-sin values before analysis. Viability percentages were statistically analyzed by using Chi-Square.

RESULTS AND DISCUSSION

Effects of feeding excess or iron on :

1) Growth performance - Data presented in Table 2 show that dietary iron treatments did not affect feed intake and viability of rabbits (%). Increasing the dietary iron level above 290 mg/kg significantly ($P<0.01$) reduced the daily body gain and feed conversion efficiency. STANDISH *et al.* (1969) observed a reduction in feed intake, daily body gain and feed conversion efficiency of steer calves with increasing the level of dietary iron from 0 to 1600 ppm. The researchers attributed the negative effects of excess iron on growth of animals to that excess iron can interfere with the utilization of some minerals which play important roles in body composition such as phosphorus, copper, zinc and manganese (KOONG *et al.*, 1970 ; HUMPHRIES *et al.*, 1983 ; PRABOWO *et al.*, 1988 ; SCHMIDT *et al.*, 1992).

2) Apparent digestibility of nutrients and nitrogen utilization - Table 2 shows that the apparent digestibility of CP, CF and EE was significantly decreased ($P<0.01$ or 0.05) with increasing the dietary Fe level. The reduction in digestibility of such nutrients was accompanied by a reduction in digestibility of DM ($P<0.05$) and OM ($P<0.01$). The data in Table 2 indicate that rabbits had less ability to digest the nutrients of the diet with

Table 2 : Growth performance, nutrients digestibility and nitrogen utilization ($\bar{X} \pm SE$)[1] in growing NZW rabbits fed excess levels of dietary iron.

Items	Level of dietary iron (mg/kg)					Signi-
	215 (control)	290	365	415	515	ficance
Initial rabbit number	14	14	14	14	14	
Initial live body weight (g)	727 ± 31	723 ± 31	729 ± 42	724 ± 39	725 ± 34	NS
Final live body weight [1](g)	2219^{b} ± 73	2432^{a} ± 51	2212^{b} ± 70	2122^{bc} ± 60	2016^{c} ± 49	**
Daily body gain (g)	26.6^{b} ± 1.3	30.5^{a} ± 1.3	26.5^{b} ± 1.3	25.0^{bc} ± 1.1	23.0^{c} ± 0.9	**
Daily feed intake (g)[2]	103.8 ± 4.8	110.0 ± 3.6	106.3 ± 4.3	108.0 ± 4.5	107.3 ± 4.6	NS
Feed conversion (kg feed/kg gain)[3]	3.90^{ab} ± 0.22	3.61^{b} ± 0.23	4.01^{ab} ± 0.29	4.32^{ab} ± 0.22	4.67^{a} ± 0.26	*
Viability (%)	92.9	92.9	100	92.9	85.7	NS
Apparent digestibility (%) [4]						
DM	61.5^{ab} ± 1.6	64.36^{a} ± 1.4	59.86^{bc} ± 0.9	60.0^{bc} ± 0.5	56.4^{c} ± 2.2	*
OM	64.3^{ab} ± 0.7	67.1^{a} ± 1.3	62.6^{bc} ± 0.8	63.3^{bc} ± 0.7	60.5^{c} ± 1.40	**
CP	74.9^{b} ± 0.3	80.5^{a} ± 0.9	75.1^{b} ± 0.9	76.7^{b} ± 0.5	71.5^{c} ± 1.3	**
EE	70.5^{ab} ± 1.9	76.8^{a} ± 2.2	67.9^{b} ± 2.2	67.8^{b} ± 3.7	65.7^{b} ± 0.3	*
CF	26.8^{ab} ± 1.5	30.0^{bc} ± 2.2	23.4^{bc} ± 0.9	22.3^{c} ± 0.8	23.4^{bc} ± 0.3	**
NFE	69.8 ± 1.2	71.4 ± 1.2	67.9 ± 1.2	68.5 ± 0.8	66.3 ± 1.4	NS
Nitrogen utilization						
Feed intake [5]	95.0^{b} ± 8.6	148.3^{a} ± 1.6	149.3^{a} ± 6.2	138.3^{a} ± 10.1	112.3^{b} ± 6.9	**
N-intake (g/day)	2.82^{b} ± 0.25	4.40^{a} ± 0.05	4.43^{a} ± 0.19	4.10^{a} ± 0.30	3.33^{b} ± 0.21	**
Faecal-N (g/day)	0.71^{c} ± 0.06	0.87^{b} ± 0.03	1.08^{a} ± 0.05	0.96^{ab} ± 0.09	0.97^{ab} ± 0.01	**
Urinary-N (g/day)	1.29^{b} ± 0.15	2.50^{a} ± 0.06	2.60^{a} ± 0.09	2.38^{a} ± 0.17	1.72^{b} ± 0.18	**
N-retained (g/head/day)	0.82^{ab} ± 0.09	1.03^{a} ± 0.14	0.75^{b} ± 0.05	0.76^{b} ± 0.04	0.64^{b} ± 0.03	*
(% of intake)	29.0^{a} ± 2.1	23.4^{b} ± 2.8	16.9^{c} ± 0.5	18.5^{c} ± 0.4	19.2^{bc} ± 0.5	**

Means in the same row having different letters differ significantly (P<0.05) ; NS : not significant ; * : P<0.05 ; ** : P<0.01.
[1] The experiment lasted for 56 days ; [2] Calculated during the whole experimental period (56 days) ; [2,3] Statistically analyzed by unweight means method. ; [4] Average values of 4 animals in each dietary treatment ; [5] Calculated during the period of digestibility trial (6 days).

increasing the dietary Fe level above 290 mg/kg. HARRISON *et al.* (1992) found that addition of Fe in the form of ferrous sulfate or ferrous ammonium sulfate decreased (P<0.05) *in vitro* DM digestion compared with control cultures (without added Fe). STANDISH and AMMERMAN (1971) attributed the negative effects of ferrous sulfate on the activity of microflora to iron and not to sulfur. HARRISON *et al.* (1992) interpreted the reduction in DM digestibility to the decrease of microbial activity with increasing the level of iron supplementation in the rumen. On the basis of such explanation, the depression occurred in the digestion of crude fibre (CF) in our experiment may be due to the negative effect of excess iron on the activity of microflora in the caecum of rabbit. Decreasing the digestibility of CP and EE can attributable to the decrease of activity of the enzymes responsible for th digestion of such nutrients in gastrointestinal tract.
Data in Table 2 indicated that N-retention was negatively effected (P<0.05) by increasing the dietary Fe. Rabbits fed the dietary Fe level 290 mg/kg retained more nitrogen (as grams) than the other groups.
This result is in agreement with the results obtained for the live body weight and daily body gain.

3) Haemoglobin and blood serum constituents - Data of blood an serum analysis are shown in Table 3. Blood haemoglobin concentration was not affected by increasing the dietary iron level. STANDISH *et al.* (1969) reported that elevating the dietary iron up to 1600ppm did not affect blood haemoglobin content of steer calves. Serum total iron also was not influenced significantly by the dietary Fe treatments. The lack response of blood serum Fe to dietary Fe level can be explained through the theory of mucosal block mechanism (McDONALD *et al.*, 1982). According to this theory excessive amounts of Fe are prevented from entering the body by a regulating mechanism mediated by the mucosal cells of the gastrointestinal tract and that Fe is therefore largely controlled by the body requirements. Since, haemoglobin is a pigment containing Fe, therefore it is expected to find that the haemoglobin level is related to the level of Fe in blood.

Table 3 : Haemoglobin and some constituents of blood serum ($\bar{X} \pm SE$)[1] of growing NZW rabbits fed excess levels of dietary iron.

Items	Level of dietary iron (mg/kg)					Signi-
	215 (control)	290	365	415	515	ficance
Haemoglobin (g/dl)	8.73 ± 0.25	8.25 ± 0.44	8.60 ± 0.09	8.90 ± 0.37	8.92 ± 0.22	NS
Total protein (g/dl)	8.44^{b} ± 0.47	11.88^{a} ± 0.61	7.29^{b} ± 0.45	7.5b ± 1.47	6.31^{b} ± 0.31	**
Total lipids (mg/dl)	488 ± 50	473 ± 108	483 ± 55	599 ± 87	681 ± 88	NS
Glucose (mg/dl)	117.7^{a} ± 2.8	125.5^{a} ± 3.2	124.3^{a} ± 2.7	117.8^{a} ± 4.4	103.8^{b} ± 5.0	**
GOT (U/L)	18.30^{c} ± 0.92	24.40^{b} ± 0.10	25.85^{b} ± 1.49	27.78^{b} ± 0.14	33.38^{a} ± 2.10	*
GPT (U/L)	7.00^{c} ± 0.16	10.30^{b} ± 1.22	8.13^{bc} ± 0.67	9.73^{bc} ± 0.46	13.78^{a} ± 1.36	**
Urea-N (mg/dl)	17.44^{a} ± 0.36	12.01^{b} ± 1.11	15.07^{a} ± 1.17	16.17^{a} ± 0.77	16.14^{a} ± 1.31	*
Calcium (mg/dl)	11.58 ± 0.77	10.22 ± 0.27	10.00 ± 0.56	10.17 ± 0.49	10.39 ± 0.36	NS
Phosphorus[2] (mg/dl)	5.60^{a} ± 0.47	4.30^{b} ± 0.41	4.10^{bc} ± 0.53	3.85^{bc} ± 0.26	3.08^{c} ± 0.15	**
Magnesium (mg/dl)	2.69 ± 0.07	2.27 ± 0.12	2.23 ± 0.21	2.74 ± 0.16	2.43 ± 0.16	NS
Iron (mg/dl)	1.59 ± 0.10	1.81 ± 0.10	1.74 ± 0.29	2.00 ± 0.37	2.12 ± 0.27	NS

Means in the same row having different letters differ significantly (P<0.05) ; NS : not significant ; * : P<0.05 ; ** : P<0.01 ; [1] Average of 4 animals for each dietary treatment ; [2] Inorganic phosphorus.

Serum inorganic phosphorus level was linearly depressed (P<0.01) by increasing the dietary iron from 215 to 515 mg/kg. PRABOWO *et al.* (1988) observed a reduction in plasma phosphorus in wether lambs fed high levels of dietary Fe. STANDISH *et al.* (1971) attributed the previous finding to that excess of Fe may combine with phosphorus in gastrointestinal tract, decreasing the toxic effect of iron, but rendering the phosphorus unavailable, i.e. decreasing its concentration in blood.

Serum calcium and magnesium concentrations were not influenced significantly by increasing the dietary Fe level. Serum total protein was higher (P<0.01) while serum urea-N was lower (P<0.05) in group of rabbits fed the dietary iron level 290 mg/kg than in the other groups. This result indicates that this group of rabbits had a greater utilization of amino acids for protein synthesis.
Although levels of serum GOT and GPT enzymes were within the normal physiological range, it were in somewhat higher (P<0.05) in group fo rabbit fed the iron level 515 mg/kg than in the other groups.

Serum glucose concentration recorded the lowest value (P<0.01) with rabbits fed the dietary Fe level 515 mg/kg compared to rabbits of other dietary treatments. Level of serum total lipids showed non significant increase by elevating the dietary Fe level from 215 to 515 mg/kg.

4) Clinical toxicity symptoms of iron and histopathological findings - No clinical signs of iron toxicity were observed on rabbits of any dietary group throughout the experimental period which lasted for 56 days.

Gross and microscopical examination showed that liver, spleen, kidneys and lungs of rabbits fed the dietary Fe levels up to 365 mg/kg were normal in size and cellular structure. However, rabbits given the dietary iron 415 or 515 mg/kg revealed mild to moderate histopathological changes in these organs. Liver showed vacuolar and hydropic degeneration in the hepatic cells (Plate 1). Spleen cleared hyperplasia in the white pulp which appeared enlarged with proliferation in its lymphocytes (Plate 2). Kidneys of these rabbits were congested and renal tubules showed degenerative changes (Plate 3). Lungs suffered from emphysema in which the air sacs appeared distended with air and ruptured in its wall (Plate 4).

Results of growth performance, supported by the results of blood and serum analysis and by the histopathological examinations, indicate that rabbits can tolerate iron in the diet up to 365 mg/kg as a maximum safe level. The highest bioavailability of iron was achieved when Fe was fed in the diet at level of 290 mg/kg.

Plate 1 : Cross section in liver of rabbit fed the dietary iron level 515 mg/kg, showing vacuolar and hydropic degeneration in the hepatic cells (H&E x 150)

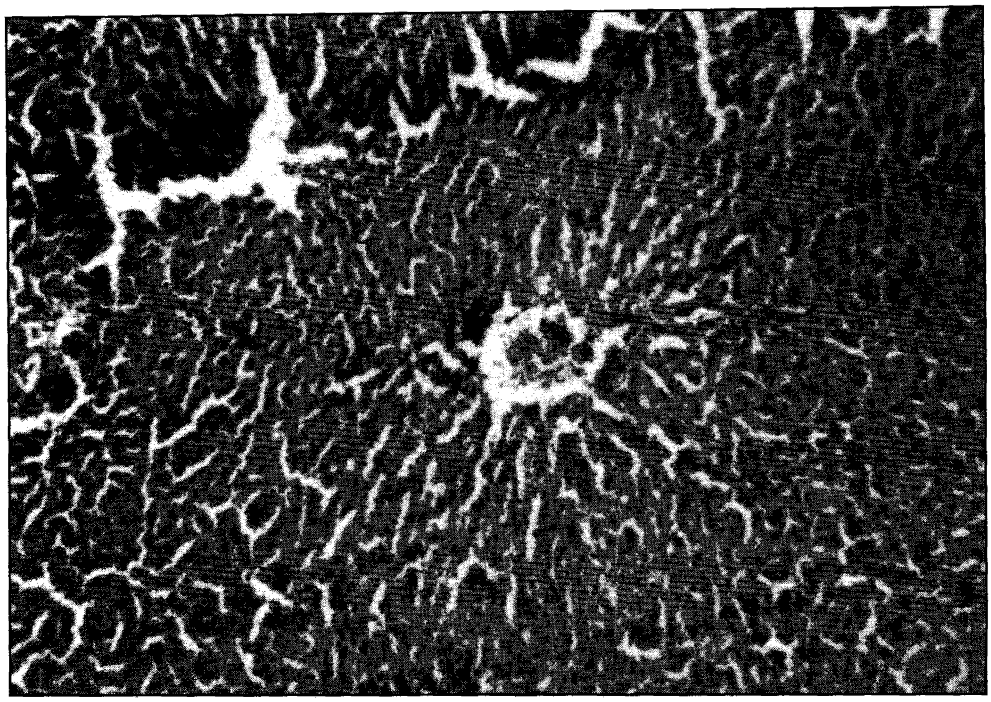


Plate 2 : Section in spleen of rabbit fed the dietary iron level 515 mg/kg, showing hyperplasia of whit pulp which appeared enlarges with proliferation in its lymphocytes (H&E x 150)

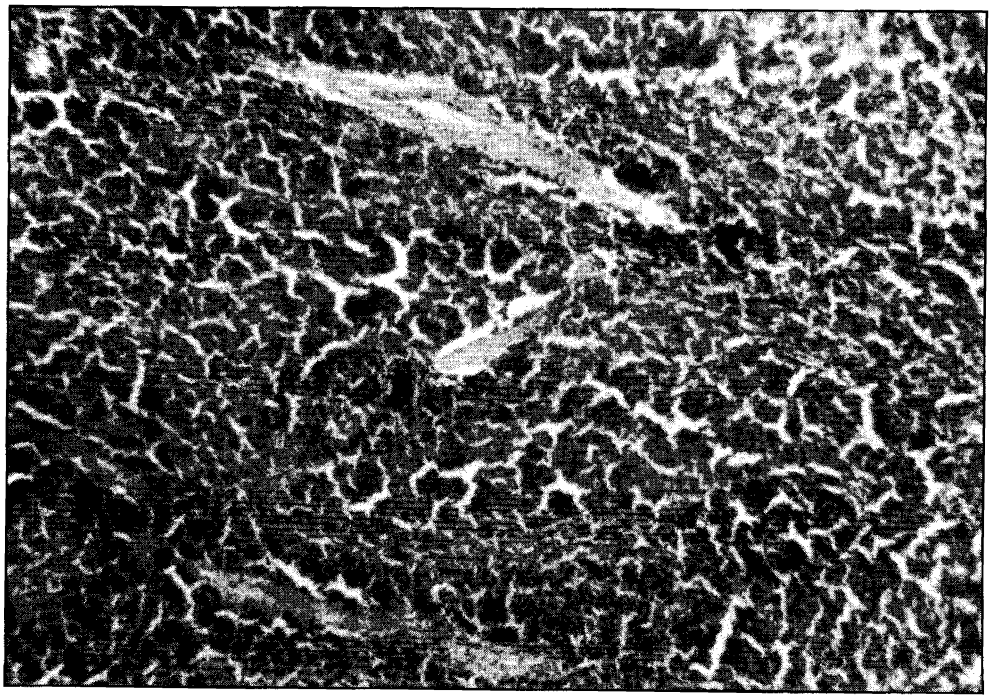


Plate 3 : Section in kidney of rabbit fed the dietary iron level 515 mg/kg, showing congestion of internal tubular of blood vessels and degenerative changes in the renal tubules (H&E x 150)

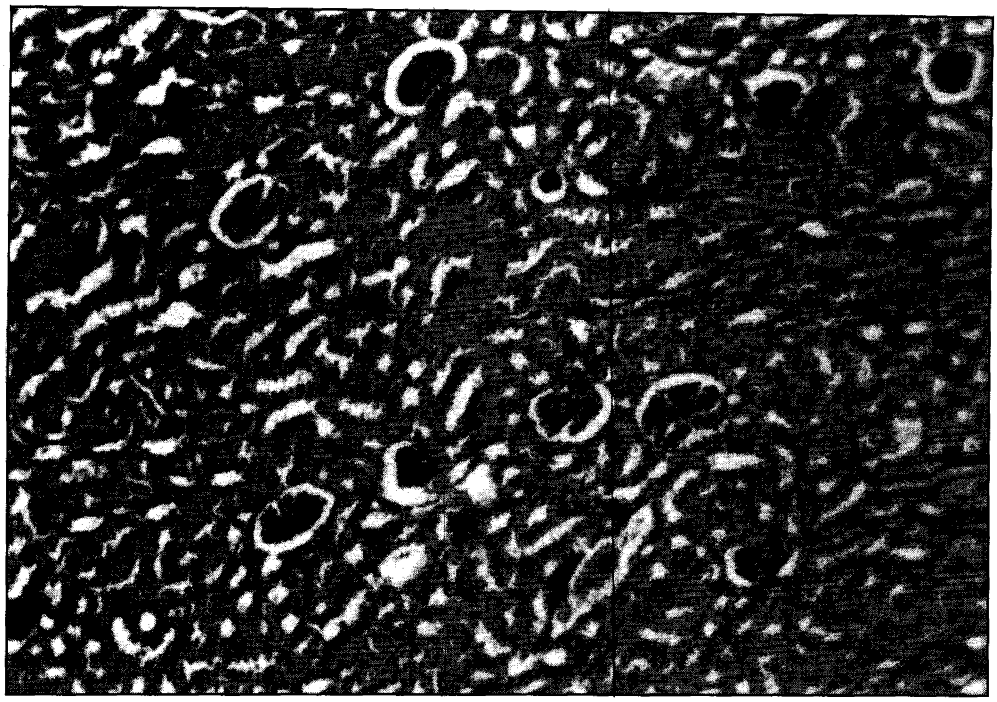
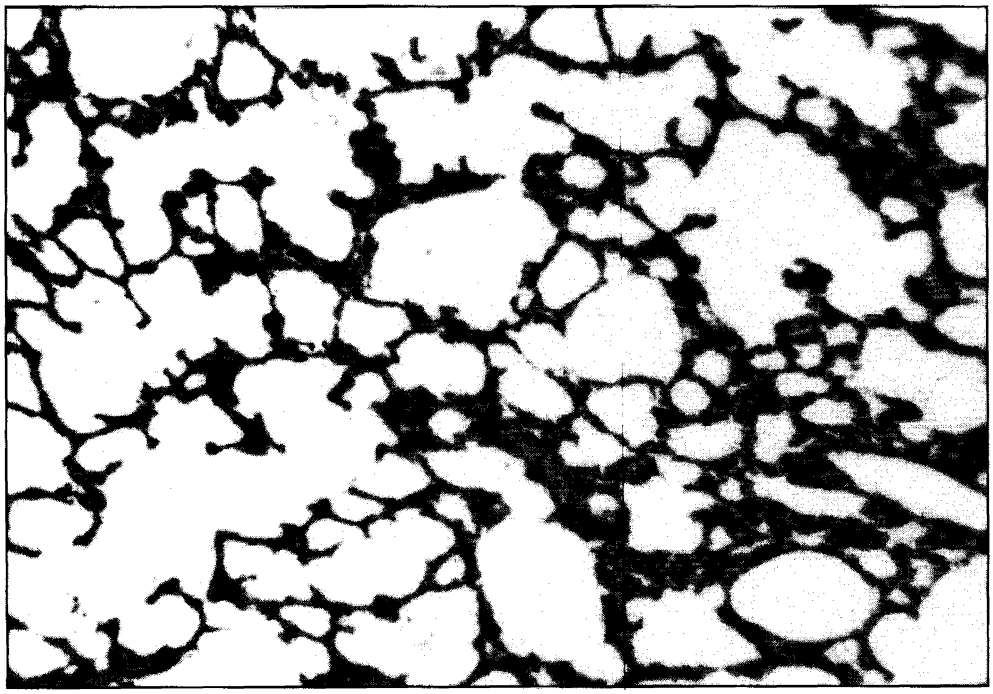


Plate 4 : Section in lung of rabbit fed the dietary iron level 515 mg/kh showing emphysema in which the air sacs appeared distended with the air in its wall (H&E x 150)



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