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ROLE OF INORGANIC CHROMIUM IN MODULATING PERFORMANCE AND IMMUNITY IN BROILERS

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SUMMARY

A 6-weeks study was conducted to investigate the effect of dietary supplementation of inorganic chromium as chromium chloride on performance and immunity of broilers. A total of 270, day-old Hubbard broiler chicks were divided into three dietary groups in a 3X3 factorial design. The first group was fed on a basal control diet (Control group). The second group was fed on the basal diet supplemented with 20 ppm chromium chloride. While the third group was fed on the basal control diet supplemented with 40 ppm chromium chloride. Results indicated that there was a highly significant (P<0.01) improvement in all chromium supplemented groups (20 and 40 ppm) throughout the experimental period regarding body weight development. However, statistical analysis revealed non-significant differences between 20 and 40 ppm chromium supplementation. Chromium also improved weight gain, reduced feed intake and improved feed conversion. Results of lymphocyte blastogenesis in chickens revealed that 20 ppm chromium supplementation significantly (P<0.05) increased the stimulation index 21, 28 and 35 days from the beginning of the study while the stimulating effect of 40 ppm chromium supplementation significantly (P<0.05) increased the stimulation index earlier at 14 days. Moreover, chromium supplementation at a level of 20 and 40 ppm significantly (P<0.05) increased the haemagglutinin (HA) antibody titre 21 and 28 days post immunization with sheep red blood cells (SRBCs) but birds received 40 ppm chromium showed earlier higher HA titre. There is a general agreement between the results of the immuno-assays, histopathological examination as well as the indices of the lymphoid organs in

terms of enhancing the immune response. Results of the serum chemistry profile indicated that chromium supplementation had no significant effect on serum glucose, total protein or albumin. Inorganic chromium significantly ($P< 0.05$) reduced serum total cholesterol, serum triglycerides as well as LDL cholesterol and increased the serum HDL cholesterol. The chromium dietary supplementation had no effect on serum alkaline phosphatase, serum ALT, serum AST or serum uric acid indicating that inorganic dietary chromium had no deleterious effect on the liver or kidney functions.

Key Words: Inorganic chromium, Immunity, performance, Broilers, blood lipids.

INTRODUCTION

The role of trivalent chromium as an essential trace element in human and laboratory animal nutrition is well documented (Anderson, 1987; Offenbacher and Pi-Sunyer, 1988; National Research Council (NRC) of human, 1989; Lien et al., 1993). Although an appropriate recommendation on the chromium requirement of poultry has been not made (NRC, 1994), there is an increasing body of evidence which suggests that chromium may also be an essential trace element for poultry.

Chromium is involved in carbohydrate metabolism and recognized as glucose tolerance factor (GTF) (Schwarz and Mertz, 1959, Rosebrough and Steele, 1981). It functions primarily by potentiating the action of insulin which is a master hormone of metabolism; it not only helps controls blood sugar levels, but also helps regulate the metabolism of fats, proteins, and energy (Steele and Rosebrough, 1981; Okada et al., 1984; Ohba et al., 1986; Press et al., 1990; McCarty, 1991 and Page, 1991). It was reported early that chromium might have a role in nucleic acid metabolism because of a significant increase in stimulation of amino acids incorporation into liver protein in vitro (Weser and Koolman 1969). Inconsistent responses of circulating lipid and lipoprotein concentrations due to chromium supplementation were reported (Lukaski, 1999). Chromium also is involved in modulating the immune response in animals (Chang and Mowat 1992; Moonsie-Shageer and Mowat, 1993; Chang et al., 1994, and Cerulli et al., 1998).

Two general forms of chromium have been used in supplementation trials, the inorganic form ($CrCl_3.6H_20$) and organic forms that seem to have greater availability (Lukaski, 1999). However, because small concentrations are required, chromium chloride and not organic forms may be an economical source in many diets. Plant products contain a low content of chromium, implying that broiler may have a deficiency of chromium because their diets consist either of all or a large proportion of plant ingredients (Schroeder, 1971

and Gibson, 1989). In the present study, the effect of dietary supplementation of 20 and 40 ppm inorganic chromium ($CrCl_3.6H_20$) on different performance parameters, immune response, serum chemistry profile was investigated.

MATERIAL AND METHODS:

Chickens

A total of 270, day-old Hubbard broiler chicks were divided into three dietary groups in a 3X3 factorial design. The first group was fed on a basal control diet (Control group). The second group was fed on the basal diet supplemented with 20 ppm chromium chloride. While the third group was fed on the basal control diet supplemented with 40 ppm chromium chloride. The chicks were floor reared in an electrically heated room provided with clean feeders and waterers and kept under standard hygienic and managemental conditions. The birds were fed ad-libitum with constant access to fresh water. The birds were vaccinated against Newcastle disease using Hitchner B1 and La Sota vaccines at 5 and 18 days of age respectively.

Experimental diets: The birds were fed ad-libitum on unmedicated starter and finisher diets (Table 1) to serve as basal diets to which chromium chloride ($CrCl_3.6H_2O$ - Merck-Germany) was added.

Blood Samples: Blood samples were obtained by heart puncture at 3, 7, 14, 21 and 28 days post inoculation. Samples included serum for HI assay and chemistry profile and heparinized blood for lymphocyte blastogenesis assay.

Measurements:

Body weight development: Birds were weighed individually every week. Individual weight gain was calculated. Feed consumption and conversion were calculated weekly.

Lymphoid organs weight Index: was calculated for thymus, bursa and spleen according to Montogomery et al., (1985) as follows:

organ weight/body weight X10000.

Hemagglutination Test: Was performed according to the standard procedure described by Anon, (1971). Birds were immunized intramuscularly at 14 days old with SRBCs in a dose of 10 mg/bird.

Lymphocyte Blastogenesis Test: The test was performed according to the method described by Charles et al., (1978) as well as Luci (1984). The separation of lymphocytes was adopted after Boyum (1968) while the determination of viable cell number was conducted according to Hanks and Wallace (1958). The lymphocytes culturing was carried out as described by Confer et al., (1981) using phytohemagglutinin-P as a mitogen at a

concentration of 10ug/well. The evaluation of the lymphocyte blastogenesis response was based on the modified MTT dye uptake assay which was conducted according to the method described by Garn et al., (1994). The lymphocyte response was expressed in terms of Stimulation Index according to Carpenter et al., (1978).

Histopathological Examination: It was performed on thymus, bursa and spleen. Specimens were kept in 10 % formol saline.

Serum Chemistry Profile: Total protein and albumin were determined using commercial kits (bioMerieux Vitek, Inc. ref. 61 602 and ref. 61 051 respectively). The serum glucose was determined by the enzymatic method using a commercial kit (bioMerieux Vitek, Inc. ref. 61 272). Serum triglycerides, total cholesterol, LDL cholesterol as well as HDL cholesterol were performed using commercial kits (bioMerieux Vitek, Inc. ref. 61 236, ref. 61 224, 61 226 and 61 227 respectively). Serum alkaline phosphatase and transaminases (ALT and AST) were determined using commercial kits (bioMerieux Vitek, Inc. ref.61 511 and ref. 61 691 and ref. 61 692 respectively) Uric acid was measured in the serum by specific diagnostic kits (Eli Tech Diagnostics. Cat. # 304 910 015)

Statistical Analysis: The obtained data were analyzed by One Way Analysis of Variance with Newman-Keuls post test using GraphPad Prism Software (1999).

RESULTS AND DISCUSSION

The effect of dietary supplementation of two levels (20 and 40 ppm) of inorganic chromium on body weights of chickens is shown in table (2). Dietary supplementation of chromium in broiler diets markedly enhanced final body weights (Lien et al., 1999). In the present study, results indicated that there were a highly significant ($P<0.01$) improvement in all chromium supplemented groups (20 and 40 ppm) throughout the experimental period. However, statistical analysis revealed non-significant differences between 20 and 40 ppm chromium supplementation. The data of the effect of dietary chromium on overall performance of chickens is shown in table (3). Chromium supplementation (20 and 40 ppm) improved weight gain, reduced feed intake and improved feed conversion. The obtained results nearly coincided with those obtained with Steele and Rosebrough, (1979) and Rosebrough and Steele, (1981) in turkey poults as well as Lien et al., (1999) in broilers. However. Cupo and Donaldson, (1987) reported that supplemental chromium had no effect on body weights of chicks. Similar results were reported by Kim et al., (1996). In other species, chromium supplementation significantly im-

proved average daily weights gain of feeder calves by 30 % and feed efficiency by 27 % (Chang and Mowat, 1992) and significantly increased weight gain but not feed efficiency in pigs (Page et al. 1993).

The enhancing effect of chromium supplementation could be attributed to its involvement in stimulating the biological activity of insulin by increasing the insulin-sensitive cell receptors or binding activity (Mertz et al., 1974; Anderson et al., 1987, 1991; McCarty, 1991; Morris et al., 1993; Ward et al., 1994 as well as Lien et al., 1999). Insulin can also stimulate anabolism and inhibit catabolism (Lien et al., 1999). In addition, chromium is involved in protein metabolism (Lukaski, 1999). Chromium may have a role in nucleic acid metabolism because of a significant increase in stimulation of amino acid incorporation into liver protein in vitro (Weser and Koolman 1969 and Lukaski, 1999). Moreover, Okada et al., (1983) reported an evidence of a direct interaction of chromium with DNA templates that resulted in a significant stimulation of RNA synthesis in vitro (RNA is responsible for protein synthesis during growth and regeneration) and subsequently identified a unique protein containing 5-6 atoms of chromium to which the anabolic function was ascribed (Okada and Tsukada 1985, Okada et al., 1989 and Lukaski, 1999).

The effect of dietary chromium on Lymphocyte Blastogenesis in chickens as judged with stimulation index using MTT assay is shown in table (4). Results indicated that 20 ppm chromium supplementation significantly ($P<0.05$) increased the stimulation index 21, 28 and 35 days from the beginning of the study while 40 ppm chromium supplementation significantly ($P<0.05$) increased the stimulation index earlier at 14 days. The effect of dietary chromium on the cellular immune system has not been investigated in poultry. Chromium may enhance some aspects of cell-mediated immunity (Lukaski, 1999). Chromium supplementation increased proliferation of peripheral blood lymphocytes in terms of increased blastogenic activity of peripheral blood lymphocytes incubated with a mitogen (Chang et al., 1994, and Cerulli et al., 1998).

The effect of dietary chromium on haemagglutinin antibody titre in chickens immunized with sheep RBCs.is shown in table (5). Results indicated that in comparison to the control non-supplemented group, chromium supplementation at a level of 20 ppm significantly ($P<0.05$) increased the haemagglutinin antibody titre 21 and 28 days post immunization with SRBCs. On the other side, chromium supplementation at a level of 40 ppm significantly ($P<0.05$) increased the haemagglutinin antibody titre at 2 weeks earlier (7 and 14 days post immunization with SRBCs)

compared to 20 ppm chromium supplemented group. There is no available literature regarding the impact of chromium supplementation on the humoral immune response of poultry. However, Moonsie-Shageer and Mowat, (1993) pointed out that antibody titers of stressed feeder calves (to human RBCs) fed chromium were higher during the primary response to the challenge. Animals and birds mounted a response to a fairly complex antigen (SRBCs) and, therefore, might be expected to mount a nearly similar response to both bacterial and viral challenge. The poor responsiveness to immunization could be attributed to the development of immuno-tolerance as a result of stress (Carlson et al., 1980). Chromium may improve the effectiveness of vaccination by improving the immune function through reduced cortisol or through other mediators released by the cells of the immune system. Additional evidence on the enhancing effect of chromium on humoral immunity was reported by Chang and Mowat (1992) who reported an improvement of IgM and total immunoglobulin levels and by Moonsie-Shageer and Mowat, (1993) in terms of increased IgG1 by

Table (1): Composition and Calculated Analysis of Experimental Diets:

	(%)	
Ingredient	Starter	Finisher
Ground yellow corn	58.0840	63.9609
Soybean meal (44 % CP)	32.7280	28.6718
Fish meal (72.3 % CP)	2.5000	-
Meat Meal (52 % CP)	1.1330	2.3116
Dicalcium phosphate	1.2140	1.1533
Limestone	1.3470	0.8336
NaCl	0.3500	0.3500
Vitamin and Mineral Premix *	0.2000	0.2000
Synthetic methionine	0.1340	0.1732
Vegetable oil	2.3100	2.3456
Calculated analysis:		
CP %	22.5	20.0
TME Kcal/kg	3050	3100
Caloric/protein ratio	135.55	155
Crude fat %	5.0	5.0
Crude fiber %	2.91	2.92
Calcium %	1.06	0.85
Available Phosphorus %	0.45	0.42
Sodium %	0.22	0.22
Lysine %	1.34	1.13
Methionine %	0.53	0.51
Methionine + Cystine %	0.9	0.85
Arginine %	1.61	1.43
Linoleic acid %	2.61	2.71

* Supplied per kg of diet: Vitamin A: 6600 IU; Vitamin D_3: 2200 IU; B_2: 4.4 mg; Pantothenic acid: 13.2 mg; Niacin, 39.6 mg; Choline chloride: 500 mg; B_{12}: 0.022 mg; Mn: 0.55 mg; Fe: 50 mg; Cu: 4 mg; Zn: 40 mg

Table (2): Effect of dietary chromium on body weight development

Age in days	0 ppm chromium (Control)	20 ppm chromium	40 ppm chromium
0	42.5 ±0.65	42.5 ±0.65	42.5 ±0.65
7	83.92^{a} ±1.196	94.29^{b} ±1.321	93.06^{b} ±1.441
14	214.7^{a} ±8.86	284.7^{b} ±4.56	294.1^{b} ±6.96
21	390.3^{a} ±13.77	472.5^{b} ±11.15	448.5^{b} ±6.65
28	630.3^{a} ±11.03	737.0^{b} ±11.72	729.0^{b} ±8.77
35	924.8^{a} ±14.97	1012.0^{b} ±18.1	1035.0^{b} ±21.58
42	1205.0^{a} ±35.21	1362.0^{b} ±27.82	1387.0^{b} ±18.16

Values are means ± SEM
Values in the same row with different superscripts vary significantly at $P \leq 0.01$

Table (3): Effect of dietary chromium on overall performance

Age in days	0 ppm chromium (Control)	20 ppm chromium	40 ppm chromium
Initial Wt. (g.)	42.5	42.5	42.5
Final Wt. (g.)	1205	1362	1387
Total Gain (g.)	1162.5	1319.5	1344.5
Total Feed Consumed (g.)	2557.5	2586.22	2608.33
Feed: Gain Ratio	2.2	1.96	1.94

Table (4): Effect of dietary chromium on Lymphocyte Blastogenesis in chickens as judged with stimulation index using MTT assay.

Age in days	Lymphocytes Stimulation Index					
	0 ppm chromium (Control)		20 ppm chromium		40 ppm chromium	
	Injected with RBCs	Without RBCs	Injected with RBCs	Without RBCs	Injected with RBCs	Without RBCs
Day old	1.7 ±0.3	1.7 ±0.3	1.7 ±0.3	1.7 ±0.3	1.7 ±0.3	1.7 ±0.3
7	1.89 ±0.17	1.89 ±0.17	1.9 ±0.09	1.9 ±0.09	1.98 ±0.1	1.98 ±0.1
14	1.95 ±0.3	1.95 ±0.3	2.04 ±2.1	2.04 ±2.1	2.14* ±0.8	2.14 ±0.8
17[A]	2.1 ±0.8	1.96 ±0.5	2.13 ±1.7	2.09 ±1.1	2.16* ±0.9	2.15* ±0.7
21[B]	2.18 ±0.7	2.0 ±0.7	2.73* ±1.2	2.11 ±1.4	2.29* ±1.3	2.19* ±1.1
28[C]	2.2 ±1.3	2.1 ±0.1	2.43* ±1.0	2.2 ±1.2	2.61* ±0.7	2.31* ±0.8
35[D]	2.32 ±1.7	2.29 ±1.3	2.47* ±0.3	2.39* ±1.0	2.6* ±0.2	2.39* ±0.7
42[E]	2.39 ±1.9	2.3 ±0.6	2.41 ±0.7	2.4 ±1.1	2.43 ±1.2	2.38 ±0.9

Values are means ± SD.
* Significantly different at P≤ 0.05 compared to control.
[A] 3 days post immunization with SRBCs
[B] 7 days post immunization with SRBCs.
[C] 14 days post immunization with SRBCs.
[D] 21 days post immunization with SRBCs.
[E] 28 days post immunization with SRBCs.

Table (5): Effect of dietary chromium on haemagglutinin antibody titre in chickens immunized with sheep RBCs.

Age in days	Haemagglutinin antibody titre					
	0 ppm chromium (Control)		20 ppm chromium		40 ppm chromium	
	Non-immunized	Immunized with SRBs	Non-immunized	Immunized with SRBs	Non-immunized	Immunized with SRBs
14[A]	0	0	0	0	0	0
21[B]	0	2.0	0	2.1	0	2.5*
28[C]	0	3.1	0	3.5	0	3.8*
35[D]	0	3.8	0	5.1*	0	5.8*
42[E]	0	3.5	0	4.6*	0	4.9*

* Significantly different at P≤ 0.05 compared to control.
A zero time of immunization with SRBCs.
B 7 days post immunization with SRBCs.
C 14 days post immunization with SRBCs.
D 21 days post immunization with SRBCs.
E 28 days post immunization with SRBCs.

Table (6): Effect of dietary chromium on weight indices of lymphoid organs in chickens

Age in weeks	Bursa (B), Spleen (S), Thymus (T) weights/ body weight ratio								
	0 ppm chromium (Control)			20 ppm chromium			40 ppm chromium		
	B/BW	S/BW	T/BW	B/BW	S/BW	T/BW	B/BW	S/BW	T/BW
4	1.17	1.59	1.745	1.6	0.83	1.48	1.23*	0.83	2.22*
5	0.923	1.31	2.22	2.15*	0.79	2.25	1.32*	1.027	2.009
6	1.73	1.61	2.06	1.55	0.69	3.16*	1.76	1.79*	3.11*

* Significantly different at P≤ 0.05 compared to control.

Table (7): Effect of dietary chromium on serum chemistry profile:

	0 ppm chromium (Control)	20 ppm chromium	40 ppm chromium
Total protein g/100 ml	4.204 ±0.236	4.311 ±0.261	3.973 ±0.295
Albumin g/100ml	1.972 ±0.077	2.253 ±0.062	2.125 ±0.127
Glucose mg/ml	183.4 ±6.932	189.1 ±5.781	201.0 ±13.42
Total Cholesterol mg/100 ml	74.33^{a} ±2.176	67.01^{b} ±2.190	64.3^{b} ±1.182
HDL cholesterol mg/100ml	20.91^{a} ±0.714	27.43^{b} ±0.467	25.36^{b} ±1.08
LDL cholesterol mg/100ml	36.09^{a} ±2.273	23.98^{b} ±2.199	26.39^{b} ±1.993
Triglycerides mg/ 100ml	83.44^{a} ±2.206	74.22^{b} ±2.223	75.37^{b} 2.344
Alkaline phosphatase (U/I)	147.3 ±5.6	136.9 ±3.1	141.3 ±3.0
AST (U/ml)	38 ±1.34	41 ±0.63	41.67 ±1.2
ALT (U/ml)	40 ±0.81	39 ±1.0	36.44 ±1.16
Uric acid (mg/dL)	7.74 ±0.75	8.23 ±0.61	10.0 ±0.54

Values are means ± SEM
Values in the same row with different superscripts vary significantly at P≤ 0.05

(Figures 1-9): Histopathological Examination of bursa, spleen and thymus

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

supplemental chromium.

The results of the histopathological examination of the bursa, spleen and thymus are presented in (Figures 1-9). In the group fed on 20 ppm dietary chromium, there was diffuse hyperplastic activation allover the mucosal lymphoid follicles in the bursa (Fig.1). Focal circumscribed round areas of lymphoid cells aggregations were observed (Fig.2) in the spleen with hyperemic red pulps. In the thymus, the cortical portion showed hyperplastic activation of the lymphocytes in association with focal extravasation of red blood cells in the corticomedullary junction (Fig.3). In the group fed on 40 ppm dietary chromium, there was hyperplastic activation in the lymphoid follicles of the mucosal layer of the bursa (Fig.4). Focal aggregations of lymphocytes were observed in diffuse manner allover the splenic tissue (Fig.5). Lymphoid hyperplasia was noticed in the cortical portion with focal areas of haemorrhages in the corticomedullary junction of the thymus (Fig.6). In the control non supplemented group, the normal histological structure was observed in the bursa, spleen and thymus in Figures 7, 8 and 9 respectively.

The impact of dietary chromium on weight indices of lymphoid organs in chickens is shown in table (6). Compared to the control group, the bursa weight indices were significantly higher ($P<0.05$) in the 20 and 40 ppm supplemented groups 4 and 5 weeks following the beginning of the study. In addition, no significant effect was observed on the spleen weight indices except in the 40 ppm supplemented group at the age of 6 weeks. Moreover, the thymus weight indices were significantly higher ($P<0.05$) in the 20 ppm supplemented group 6 weeks following the beginning of the study as well as 40 ppm supplemented group 4 and 6 weeks following the beginning of the study. The higher weight indices of bursa support the enhancing effect of chromium on humoral immunity as measured by haemagglutinin antibody titre in chickens immunized with sheep RBCs (Table 5). On the other side, the higher weight indices of thymus confirm the enhancing effect of chromium on cellular immunity as measured by Lymphocyte Blastogenesis (Table 4). It is of note that there is a general agreement between the results of the immuno-assays (Tables 4 and 5), histopathological examination (Figures 1-9) as well as the indices of the lymphoid organs (Table 6).

The impact of dietary supplementation of chromium on serum chemistry profile is shown in table (7). Chromium supplementation had no significant effect on total protein or albumin. Unlike the findings of Lien et al., (1999) who reported a significant reduction in serum glucose as a result of chromium supplementation, results in the present study indicated that chromium supplementation

had no significant effect on serum glucose. These results coincided with those obtained in turkey poults (Rosebrough and Steele, 1981). In vitro studies on the uptake and oxidation of glucose by liver slices from chicks revealed that chromium increased the rate of glucose utilization 16% over control (Cupo and Donaldson, 1987).

Chromium supplementation results in equivocal responses in circulating lipids and lipoprotein concentrations (Lukaski 1999). The dietary supplementation of two levels (20 and 40 ppm) of inorganic chromium significantly ($P< 0.05$) reduced serum total cholesterol, serum triglycerides as well as LDL cholesterol. However, statistical analysis revealed non-significant differences between 20 and 40 ppm chromium supplementation. On the other side, chromium supplementation significantly ($P< 0.05$) increased the serum HDL cholesterol. Similar data were reported by Lien et al., (1999) in broiler and by (Lukaski 1999) in human subjects. A previous study indicated that insulin with its stimulated biological activity due to dietary chromium, can increase the lipoprotein lipase activity and eventually decrease the contents of triglycerides rich lipoproteins (Garfinkel et al., 1976 and Howard et al., 1993). It also can increase liver LDL receptors, thereby reducing the LDL content and concomitantly the HDL proportion is increased (Brindley and Salter, 1991 and Lien et al., 1999).

The chromium dietary supplementation had no effect on serum alkaline phosphatase, serum ALT, serum AST or serum uric acid indicating that inorganic dietary chromium had no deleterious effect on the liver or kidney functions.

Conclusion: The data obtained in the present study indicated that dietary supplementation of inorganic chromium in the form of chromium chloride was beneficial in improving performance, humoral and cellular immune responses and in reducing cholesterol, LDL cholesterol as well as triglycerides and increasing HDL cholesterol. The use of chromium chloride had no adverse effect on the liver or the kidney.

Histopathological Examination

Fig.1: Bursa of chickens (20 ppm chromium supplementation) showing hyperplastic activation of the lymphoid follicles (H&E x40)

Fig.2: Spleen of chickens (20 ppm chromium supplementation) showing focal lymphocytic aggregation. (H&E x40)

Fig.3: Thymus of chickens (20 ppm chromium supplementation) showing lymphoid cells proliferation in the cortical portion with focal extravasation of the blood cells in the corticomedullary junction. (H&E x40)

Fig.4: Bursa of chickens (40 ppm chromium supplementation) showing hyperplasia of the lymphocytes in the mucosal follicles. (H&E

x40)

Fig.5: Spleen of chickens (40 ppm chromium supplementation) showing focal lymphoid cells aggregations. (H&E x40)

Fig.6: Thymus of chickens (40 ppm chromium supplementation) showing lymphoid cells hyperplasia in the cortical portion with focal haemorrhages in the corticomedullary junction. (H&E x40).

Fig.7: Bursa of chickens (control group) showing the normal histological structure of the mucosal epithelium and lymphoid follicles. (H&Ex40).

Fig.8: Spleen of chickens (control group) showing the normal histological structure of the white and red pulps. (H&E x40).

Fig.9: Thymus of chickens (control group) showing the normal histological structure of the cortical and medullary portions. (H&E x40).

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REFERENCES

Anderson, R. A.; Polansky, M. M.; Bryden, N. A. . and Canary, J. J. (1991): Supplemental chromium effects on glucose, insulin, glucagon and urinary chromiumlosses in subjects consuming controlled low-chromium diets. Am. J. Clin. Nutr. 54:909-916.

Anderson, R. A.; Polansky, M. M.; Bryden, N. A. Bathena, S. J. and Canary, J. J. (1987): Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. Metabolism. 36:351-355.

Anderson, R.A. (1987): Chromium. In: W. Mertz (Ed.) Trace elements in Human and Animal Nutrition. Vol. I p. 225. Academic Press, New York.

Anon, (1971): Methods for examining poultry biologics and for identifying avian pathogens. Nat. Acad. Sciences, Washington, D.C.

Boyum, A. (1968): Isolation of mononuclear cells and granulocytes from human blood. Scan. J. Clin. Lab. Invest., 21:77-89

Brindley, D. N. and Salter,A. M. (1991): Hormonal regulation of the hepatic low density lipoprotein . J. Lipid Res. 30:349-360.

Carlson, J. H. Horton, D. P. Braddy, P. M. (1980): Antibody responses to IBR vaccination in feedlot cattle. Bovine Pract. 15:84

Carpenter, C. R.; Arnold, S. R. and Boss Jr, H. R. (1978): Suppression of mitogen-stimulated blastogenic response during reticuloendotheliosis virus-induced tumorigenesis: Investigation into the mechanism of action of the suppressor. J. Immunol, 120 :1313-1320.

Cerulli, J.; Grabe, D. W.; Gauthier, I; Malone, M and McGoldrick, M. D. (1998): Chromium picolinate toxicity. Ann. Pharmacother. 32:428-431.

Chang, X. G.; Mallard, B. A. and Mowat, D. N. (1994): Proliferation of peripheral blood lymphocytes of feeder calves in response to chromium. Nutr. Res. 14:851-864.

Chang, X., and Mowat, D.N. (1992): Supplemental chromium for stressed and growing feeder calves. J. Anim. Sci. 70:559-565

Charles, R.; Carpenter, A. B.; Henry, R. and Bos, J. R. (1978): Suppression of the mitogen-stimulated blastogenic response during reticuloendotheliosis virus induced tumorigenesis. J. Immunol., 120 (4): 1313-1320.

Confer, A. W.; Springer, W. T.; Shane, S. M. and Donovan, J. F. (1981): Sequential mitogen stimulation of peripheral blood lymphocytes from chickens inoculated with infectious bursal disease virus. Am. J. Veterinary Res., 42:2109-2113.

Cupo, M. A. and Donaldson, W. E. (1987): Chromium and Vanadium effect on glucose metabolism and lipids synthesis in the chick. Poul. Sci. 66:120-126.

Garfinkel, A. S.; Nilsson-Ehale, P. and Schotz, M. C. (1976): Regulation of lipoprotein lipase induction by insulin. Biochemica and Biophysica Acta. 424:264-269.

Garn, H.; Krause, H.; Enzymann, V. and DroBler, K. (1994): An Improved MTT assay using electron coupling agent menadione. J. Immunol. Methods., 168: 253-256.

Gibson, R. S. (1989): Assessment of trace element status in humans. Progress in food and Nutrition Science, 13:67:111

GraphPad Prism. (1999): Analyzing Data with GraphPad Prism. GraphPad Software, Inc. San Diego, California, USA

Hanks, J. H. and Wallace, , J. H. (1958): Determination of cell viability. Proc. Soc. Exp. Biol. Med., 98: 183-192.

Howard, B. V.; Schneiderman, N.; Falkner, B.; Haffner, S. M. and Laws, A. (1993): Insulin, health behaviors and lipid metabolism. Metabolism. 42 (9,51): 25-35.

Kim, Y. H.; Han, In. K.; Choi, Y. J. Shin, I. S. ; Chae, B. J. and Kang, T. H. (1996): Effects of dietary levels of chromium picolinate on growth performance, carcass quality and serum traits in broiler chicks. A.J.A.S. , 9:341-347.

Lien, T. E.; Chen, S. Y.; Chen, C. L. and Wu, C. P. (1993): The effects of various levels of chromium picolinate on growth performances and serum traits of pigs. J. Chinese Soc.. Anim. Sci. 22:349-357

Lien, T. E.; Horng, Y. M. and Yang, K. H. (1999): Performance, serum characteristics, carcase traits and lipid metabolism of broilers as affected by supplement of chromium picolinate. British Poul. Sci. 40:357-363.

Luci, F. L. (1984): Proliferation response of chicken T and B lymphocytes to mitogens. Chemical regulation of immunity in veterinary medicine., 15: 44-52

Lukaski, H. C. (1999): Chromium as a supplement. Annu. Rev. Nutr.19: 279-302

McCarty, M. E. (1991): The case for supplemental chromiuman survey of clinical studies with chromium picolinate. Fourna Apply. Nutrition.43:58-66

Mertz, W.; Toepfer, E.W.; Roginski, E.E. and Polansky, M.M. (1974): Present knowledge of the role of chromium. Fed. Proc. 33:2275.

Monnsie-Shageer, S., and Mowat, D.N. (1993): Effect of level of supplemental chromium on performance, serum constituents, and immune status of stressed feeder calves. J. Anim. Sci. 71:232-238.

Montogomery, R. D.; Villegas, R.; Dawe, D. L. and Brown, J. (1985): Effect of Reovirus on lymphoid organ weights and antibody response in chickens. Avian Dis., 29: 552-

Morris, B. W.; Gray, T. A. and MacNeil, S. (1993): Glucose-dependent uptake of chromium in human and rat insulin-sensitive tissue. Clin. Sci., 84 :477-482.

National Research Council (1989): Recommended dietary allowances. Washington, DC. National Academy Press.

National Research Council (1994): Nutrient requirements of poultry. Washington, DC. National Academy Press.

Offenbacher, E.G. and Pi-Sunyer, G.X. (1988): Chromium in human nutrition. Ann. RE. Nutr. 8:543.

Ohba, H.; Suzuki, Y. and Ohba, H (1986): Enhancement of ribonucleic acid synthesis by chromium III-bound chromatin. J. Inorg. Biochem.27:179-188

Okada, S. and Tsukada, H. (1985): Nucleolar chromiumñbound protein enhancing nucleolar RNA synthesis in regenerating rat liver. In Trace Elements in Man and Animals. No.5 Ed. Mills, Bremner and Chesters. Commonwealth Agricultural Bureau, London, England.

Okada, S. and Tsukada, H. and Ohba, H (1984): Enhancement of nuclear RNA synthesis by chromium (III) in regenerating rat liver. J. Inorg. Biochem.21:113-119

Okada, S.; Suzuki, M. and Ohba, H. (1983): Enhancement of ribonucleic acid synthesis by chromium (III) in mouse liver. J. Inorg. Biochem., 19:95-103

Okada, S.; Tsukada, H. and Tezuka, M. (1989): Effect of chromium (III) on nuclear RNA synthesis. Biol. Trace Elem.,21:35-39.

Page, T.G. (1991): Chromium, tryptophane and picolinate in diets for pigs and poultry. A dissertation. Louisiana State University. U.S.A.

Page, T.G.; Southern, L.L.; Ward, T.L. and Thompson, Jr. D.L.(1993):. Effect of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. J. Anim. Sci. 71:656-662.

Press, R. I.; Geller, J. and Evans, G. W. (1990): The effect of chromium picolinate on serum cholesterol and apolipoprotein fractions in human subjects. West. J. Med. 152:41-45

Rosebrough, R.W. and Steele, N.C. (1981): Effect of supplemental chromium or nicotinic acid on carbohydrate metabolism during basal, starvation, and refeeding periods in poults. Poultry. Sci. 60:407.

Schroeder, H. A.(1971): Losses of vitamins and trace minerals resulting from processing and preservation of foods, Am. J. Clin. Nutr. 24:562-569.

Schwarz, K. and Mertz, W. (1959): Chromium (III) and the glucose tolerance factor. Arch. Biochem. Biophys. 85:292.

Steele, N. C. and Rosebrough, R. W. (1979): Trivalent chromium and nicotinic acid supplementation for the turkey poults. Poultry Sci. 58:983-984.

Steele, N. C. and Rosebrough, R. W. (1981): Effect of trivalent chromium on hepatic lipogenesis by the turkey poults. Poultry Sci. 60:617-622.

Ward, T. L.; Berrio, L. F.; Southern, L:. L.; Fernandez, J. M. and Thompson, D. L. (1994): In-vivo and in-vitro evaluation of chromium tripicolinate on insulin binding in pig liver cell plasma membranes. FASEB J., 8:A194

Weser, U. and Koolman, J. (1969): Untersuchungen zur proteinbiosynthese in rattenieber-zellerkernen. Hoppe Seylerís Z. Physiol. Chem. 350:1273-1278.