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EFFECT OF ASCORBIC ACID, VITAMIN E, AND MELATONIN ON PERFORMANCE AND IMMUNE RESPONSE OF BROILERS

(With 6 Tables and 8 Figures)

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تأثير فيتامين ج و هـ والميلاتونين على كفاءة الأداء والأستجابة المناعية في بدارى التسمين

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أجريت هذه التجربة لدراسة تأثير تغذية كميات عالية من فيتامين ج ، هـ وإضافة الميلاتونين كمضادات للأكسدة وكذلك مخلوط منهم على كفاءة الأداء والإستجابة المناعية في بدارى التسمين لمدة ٦ أسابيع. كان عدد الكتاكيت ١٥٠ كتكوت في عمر يوم موزعة بالتساوي على ٥ مجموعات غذيت على العليقة الاساسية خلال فترة التجربة (بادي ونامي- ناهي). في المجموعة الأولى تم إضافة ١٠ مجم فيتامين هـ و٢٥٠ مجم فيتامين ج و٥٢, مجم فيتامين أ /كجم عليقة أساسية وأعتبرت كعليقه ضابطة. تم إضافة مستويين عاليين من فيتامين ج ، هـ (٥ جم ، ٢٠٠ مجم/كجم عليقه ضابطة) للكتاكيت في المجموعة الثانية والثالثة بالترتيب. أضيف الميلاتونين إلى المجموعة الرابعة بمعدل ١٠ مجم/لتر ماء وفي المجموعة الخامسة أضيف خليط من فيتامين ج ، هـ(٢,٥جم، ١٠٠مجم/كجم عليقه ضابطة) بالإضافة إلى الميلاتونين (٥ مجم/لتر ماء). قدرت المقاييس الخاصة بتقييم أداء النمو ومعدل إستهلاك الطعام والزيادة في وزن الجسم وكفاءة التحويل الغذائي ووجد زيادة معنوية في معدل النمو والتحويل الغذائي في المجموعات التي غذيت على علائق مضاف إليها فيتامين هـ وخليط من فيتامين ج ، هـ، الميلاتونين وكذلك مجموعة الميلاتونين بالمقارنة بالمجموعة الضابطة في حين لا توجد فروق معنويه في المجموعة التي غذيت على عليقه مضافاً إليها فيتامين ج. تم الحصول على المقاييس المناعية بتقدير كل من قيمة الأجسام المناعية لفيروس مرض النيوكاسل باستخدام إختبار تثبيط التخثر الدموي وصورة الدم بالإضافه إلى المناعة الباثولوجية وأظهرت النتائج إرتفاع نسبة الأجسام المناعية في إختبار تثبيط التخثر الدموي وأيضاً زيادة في أعداد الخلايا الليمفاوية من نوعي T , B في كل من البرسا والطحال والغده التيموسيه في كل المجموعات المعالجة وخصوصاً في المجموعة الخامسة التي غذيت على عليقه مضاف إليها خليط من فيتامين ج ، هـ بالإضافة إلى الميلاتونين. من هذه الدراسة نستخلص أن إضافة مستوى عالى من فيتامين ج أو خليط من فيتامين ج و هـ والميلاتونين أدى إلى تحسن كفاءة الأداء والاستجابة المناعية فى بدارى التسمين.

SUMMARY

The effect of high dietary vitamin C, vitamin E (α-tocopheryl–acetate) and melatonin as antioxidants and their combinations on performance and immune response was determined in broiler chicks for six weeks. One hundred fifty, one-day old Arbor acre chicks were equally distributed into five experimental groups. Chicks of all experimental groups were fed basal starter and grower-finisher diets. The diet of group 1 supplemented with 10 mg vitamin E, 250 mg ascorbic acid, and 0.52 mg vitamin A/kg diet to cover the requirements from these nutrients according to the *NRC* and considered as control. Groups 2 and 3 fed on the same diet of group 1 but fortified with 5 g vitamin C/kg diet for group 2 and 200 mg vitamin E/kg diet for group 3 (20 times that of optimal requirements of each). Melatonin was added at the rate of 10 mg /liter water to group 4 which fed on the same diet of group 1, while chicks of group 5 fed the same control diet supplemented with 2.5 g vitamin C and 100 mg vitamin E/kg diet (10 folds that optimal requirements), in addition to 5mg melatonin/liter drinking water. The growth performance, feed intake, body weight gain and feed conversion efficiency were assessed. The supplementation of the basal diets with vitamin E (group 3), mixture of vitamins and melatonin (group 5) improved weight gain and feed conversion efficiency in comparison with control. The same result was also achieved by group 4. Vitamin C supplementation had no significant effect on weight gain and feed conversion efficiency. The immunological assays were achieved by determining the antibody titers to Newcastle disease virus (NDV) using haemagglutination inhibition test (HI), hematological picture of blood samples, in addition to the immunohistochemical studies of the spleen, bursa of fibracious and thymus to detect B and T lymphocytes proliferation. The results indicated that enhancement of immune functions reflected by high HI titers, marked increase in the lymphocytes percentage, and an increase of B and T cell reaction in the lymphoid organs of the different treated groups specially group 5. The results of this study suggest that higher supplemental levels of vitamin E and the combination of vitamin E, C and melatonin would improve performance of chicks and positively affect immune response.

Key words: Ascorbic acid, Vitamin E, Melatonin, Broiler performance, Immune response.

INTRODUCTION

Nutrition plays a significant role in the development and function of the immune system. Essential nutrients such as vitamins, may affect not only humoral and cell-mediated immune responses, but also several nonspecific humoral factors, such as lysosomes or hormones, which regulate the immune response. The most important small molecular antioxidants in vivo probably are vitamin C, vitamin E and GSH which represent the classical antioxidants. However, melatonin may also represent an important novel class of antioxidants in terms of its obviously different mechanisms from the other antioxidants described.

L-ascorbic acid (AA) has an important metabolic role as a result of its reducing properties as an electron carrier. Ascorbic acid is not an essential nutrient for poultry species because they possess the enzyme gulonolactone oxidase that is a part of the biosynthetic pathway. However, there has been considerable interest in a possible nutritional role for AA on the basis that: (a) endogenous synthesis may not be adequate to meet the physiological needs for optimal performance (b) requirements for AA may be increased under circumstances "stressful conditions" (Pardue & Thaxton, 1986 and Whitehead & Keller, 2003).

Vitamin E has been reported as an excellent biological antioxidant that protect cells and tissues from lipoperoxidative damage induced by free radicals (Halliwell & Gutteridge, 1989 and Yu, 1994). Recent research has indicated that relatively high concentrations of vitamin E added to the feed enhance the humoral immune response, increased the titer of hemagglutinating antibodies or disease resistance in poultry (Xu *et al.*, 1989; El-Boushy, 1990; Cook, 1991 and Boren & Bond, 1996). Some studies have shown that vitamin E tend to increase performance in broilers (Hidiroglou *et al.*, 1992; Swain *et al.*, 2000; Villar-Patino *et al.*, 2002).

Vitamin C has been demonstrated to enhance antioxidant activity of vitamin E by reducing the tocepheroxyl radicals back to their active form of vitamin E (Jacob, 1995) or by sparing available vitamin E (Retsky & Frei, 1995). Regarding antioxidant property, there is a positive synergistic effect of vitamin E and C on the immune response and antibody levels of broiler chickens against Newcastle live and dead virus vaccines (Gonzalez-Vega-Aquire *et al.*, 1995). Sahin & Kucuk (2001a) concluded that a combination of higher dietary levels of ascorbic acid and vitamin E provided the greatest performance in Japanese quails.

Melatonin, a derivative of tryptophan, is a chief secretory product of the pineal gland and its structure was identified four decades ago (Reiter, 1980). Melatonin is a potent endogenous free radical scavenger, which prevents oxidative damage at the level of cells, tissues, organs and organisms (Dun-Xian *et al.*, 2000). Understanding the role of melatonin in affecting different physiological functions, specially immune response is becoming increasingly important in the basic and applied science. Melatonin enhances the immune response and therefore, improves production efficiency (Kliger *et al.*, 2000 and Brennan *et al.*, 2002).

The present study was initiated to determine whether dietary supplemental vitamin C, vitamin E or melatonin and their combinations affect performance and immune response of broiler chicks.

MATERIALS and METHODS

Chicks and feeding

A total of 150 one day old broiler chicks (Arbor acre), obtained from a local commercial source, were used in this study. The chicks were nearly of a uniform weight, averaging 40g, and randomly distributed into five equal experimental groups (30 chick each). The chicks were reared on the floor in an experimental room, of five compartments, bedded with a layer of wood shavings and provided with clean feeders and waterers.

Chicks of all experimental groups were fed basal starter and grower-finisher diets (Table 1). The diet of group 1 supplemented with 10 mg vitamin E (DL-α-tocopheryl–acetate), 250 mg ascorbic acid, and 0.52 mg vitamin A/kg diet to cover the requirements from these nutrients according to the NRC (1994) and considered as control. Groups 2 and 3 fed on the same diets as control but fortified with 5 g vitamin C/kg diet for group 2 and 200 mg vitamin E/kg diet for group 3 (20 times that of optimal requirements of each). Melatonin was added at the rate of 10 mg /liter water to group 4 which fed on the same diet of group 1, while chicks of group 5 fed the same control diet supplemented with 2.5 g vitamin C and 100 mg vitamin E/kg diet (10 folds that optimal requirements), in addition to 5mg melatonin/liter drinking water.

GROUP	EXPERIMENTAL DIETS
1	Basal diet supplemented with 10 mg vitamin E, 250 mg ascorbic acid and 0.52 mg vitamin A/kg diet (control).
2	Control diet fortified with 5 g ascorbic acid/kg diet.
3	Control diet supplemented with 200 mg vitamin E/kg diet.
4	Control diet in addition to10 mg melatonin /liter water.
5	Control diet supplemented with 2.5 g vitamin C and 100 mg vitamin E/kg diet in addition to 5 mg melatonin /liter water.

Birds in all groups were fed on the starter diet for the first three weeks and on the grower-finisher diet for the last three weeks of age. The diets were fed ad-libitum and a fresh clean water was continuously available throughout the experimental period which extended for 6 weeks.

Feed consumption and body weight of the chicks were recorded on a weekly basis and feed conversion efficiency was determined. Five randomly selected birds from each group were slaughtered at the end of the experiment for immunological studies. The birds of the six experimental groups were vaccinated with Newcastle Disease Virus vaccine every other week to induce an immune response.

Immunological Assays:

A) Haematological examination:

Blood samples were collected from the slaughtered birds at the end of the experiment. The samples were used for the detection of:

1- Total erythrocytic count/mm^3 blood.
2- Total white blood cells count/mm^3 blood.
3- Differential leucocytic count on blood film stained with Wright's stain.

B) Serum:

A blood specimen was collected from each of the slaughtered birds, in the six groups, at the end of the experiment. The blood samples were allowed to clot at ambient temperature, centrifuged for 10 minutes at 3000 rpm, and serum from each sample was collected. The serum samples (1 ml/vial) were kept frozen at –20 C° until haemagglutination inhibition test was done.

Haemagglutination inhibition test (HI):

It was applied as a rapid mean for measuring the immune response of the birds to Newcastle vaccination. The antibody titers in response to NDV was assessed by the ten-two fold serial dilution

technique for antisera against Lassota strain of NDV propagated in chicken embryo as described by Beard & Wilkes (1973).

C) Spleen, Thymus and bursa evaluation:

Specimens were taken from spleen, thymus and bursa, then fixed in cold acetone, processed and paraffin infiltrated. The paraffin blocks were sectioned at 7μ. The prepared sections were used for the immunological studies using the following histochemical indices: a- Alkaline phosphatase reaction, for detection of activated B-lymphocytes (Gomeri method, 1952). b- Non specific esterase activity, for detection of T-lymphocytes (Lodja *et al.*, 1976).

Statistical Analysis:

All data obtained were statistically analyzed according to Snedecor & Cochran (1989) in order to test the significancy of the differences between groups and evaluate the calculated means.

RESULTS and DISCUSSION

Broiler performance:

The performance of the chicks in the different groups as reacting to the vitamin supplementation was evaluated during the experimental period by body weight development, feed intake, and efficiency of feed conversion. The data are summarized in Tables 2 – 4.

Calculating the amount of food consumed in the different groups in comparison to control (group 1), it was found that the supplementation of high levels of ascorbic acid and vitamin E (groups 2 and 3) did not affect the rate of food consumption. These results agreed with the findings of Bratov & Frigg (1992) and Batrov (1998) who found that, the feed intake, weight gain and feed efficiency were not significantly affected by vitamin E supplementation of the broiler chicks up to 7 weeks of age. Compared to control group, feed intake per bird for the whole growing period was decreased by 3.34% and 2.25% in groups 4 and 5 respectively.

The growth data revealed that, the supplementation of vitamin E, melatonin separately or when combined together with vitamin C improved weight gain by 4.92%, 3.16% and 11.35% in groups 3, 4, and 5 respectively in comparison to those of control. The improvement in the weight gain achieved by chicks fed on the diet supplemented with vitamin E confirms the findings of Hidiroglou *et al.* (1992); Swain *et al.* (2000) and Villar-Patino *et al.* (2002) who reported that vitamin E supplemented diets resulted in better growth performance and lower rates of feed conversion. The better performance of the chicks fed diets

supplemented with high doses of dietary vitamin E may be due to the increased nutrient digestibility as reported in Japanese quails by Sahin & Kucuk (2001b). Clark & Classen, (1995) and Brennan *et al.* (2002) found that, melatonin fed either continuously or in a diurnal pattern had a minor effect on the performance of broiler chickens. From table 4, there were no significant differences in both body weight gain and feed conversion between the group fed on diet supplemented with high level of vitamin C and control. These results are in agreement with that previously found by Fletcher & Cason (1991); Takahashi *et al.* (1991); Kultu & Forbes (1994); Puron *et al.* (1994); Fratzer *et al.* (1996) and Marron *et al.* (2001) under non-stressful conditions. A number of experimental studies have shown that supplementation of AA improves performance in heat-stressed broilers (Pardue *et al.* 1985; Kassim & Norziha, 1995; Jafar & Blaha, 1996; Sayed & Shoeib, 1996; Raja & Qureshi, 2000; Kutlu, 2001 and Celik & Ozturkan, 2003).

Taking the consideration for the average initial weight of the chickens in each of the five groups, it was found that the groups 3, 4 and 5 surpassed the control one in times of initial weight duplication, while chicks in group 2 showed the lowest. It could be concluded that the vitamin C supplementation has no beneficial effect on the body weight and feed conversion. The efficiency of feed utilization as gm of feed per gm of gain for different treatments are shown in Table 4. Favorable effect of higher level of vitamin E, and mixture of melatonin, vitamins E and C on broiler weight gain was parallel to the improvement in the feed conversion. Similar results were reported by Sahin & Kucuk (2001a) in Japanese quail.

Immunological Assays:

Immune response to NDV: According to Beard & Wilkes (1973), the HI test is generally considered as a reliable, economical and rapid mean of measuring the immune response of poultry to Newcastle vaccination. From this experiment we were able to detect positive significant differences ($P< 0.05$) at HI titers in response to vitamin E and C and mixture of vitamin C and E, in addition to melatonin (Table 5). The immune enhancing effect of vitamins and melatonin was confirmed by this test, especially the chicks fed on high level of vitamins E and C (200 mg and 5 g/kg diet respectively), which recorded the highest values of HI titers, followed by chickens in groups 4 and 5.

Several studies (Franchini *et al.*, 1986; El-Boushy, 1990; Cook, 1991; Boren & Bond, 1996 and Swain *et al.*, 2000) reported that, vitamin E supplementation increased the antibody titers of the broiler

chicks. Broiler chicks, supplemented with vitamin C and vaccinated with NDV showed a better antibody response to the viral antigen (Franchini, *et al.*, 1994 and Abou-Zeid, *et al.*, 1999). Gonzalez-Vega-Aquire *et al.* (1995) demonstrated that the supplementation of vitamin C and E combination improved antibody levels of broiler chickens against Newcastle modified live and dead virus vaccine. Many parameters of the immune system, including resistance to infection, specific antibody production and in vitro mitogenic responses of lymphocytes, are altered by supplementing diets that are deficient or marginal in vitamin E (marsh *et al.*, 1981; Cook, 1991; Meydani & Blumberg, 1993 and Boren & Bond, 1996).

Haematological examination: The haematological picture (Table 6) showed an increase in the total erythrocytic and leucocytic cells count with a marked increase in the percentage of lymphocytes in the different supplemented groups, especially in the group fed on diets supplemented with vitamin E and C, in addition to melatonin (group 5) in comparison with control. Abou-Zeid *et al.* (1999) reported that dietary ascorbic acid supplementation gave higher bursa and thymus indices, white blood cell counts and HI titers in broilers. In contrast, in the absence of stress, AA supplementation up to 800 mg/kg did not increase the humoral or cell-mediated immune response up to 28 days in broilers vaccinated with NDV vaccine alone or in combination with 80 mg/kg vitamin E (Wen *et al.*, 1997).

Immuno-histochemical study: The alkaline phosphatase reaction revealed few B cell reaction (which took blackish colour) in both spleen and bursa of fibracious (Fig. 1 &2). In the different supplemented groups, the B-cell reaction was increased, where the best results were observed in the group fed on mixture of vitamin C and E, in addition to melatonin (group 5). In this group, there was marked increase of the B-cells reaction, especially in spleen, in which B-cells were observed in both white and red pulp of the spleen (Fig. 3). In bursa of fibracious, B-cell reaction was of moderate pattern (Fig. 4). This may be attributed to a positive synergistic effect of vitamin E, C and melatonin on the immune response (bendich *et al.*, 1984). Non specific esterase reaction which is a marker of T-cells revealed few T-cell reaction (which took brownish colour) in both spleen and thymus of the control birds (Fig. 5&6). T-cell reaction increased in the other supplemented groups, especially in group 5, where the T-cell reaction became more prominent in both spleen (Fig. 7) and thymus (Fig.8).

The positive effect of vitamin E supplementation on lymphocyte response was reported by Kramer *et al.* (1991); Boren & Bond (1996); Swain *et al.* (2000) and Putnpongsiriporn *et al.* (2001). Anti-oxidation property of vitamin E has been considered to have a role in the development of immune response in the chickens. Vitamin E has been reported to protect cells involved in immune response, such as lymphocytes, macrophages, and plasma cells, against oxidative damage and to enhance the function of proliferation of these cells (Franchini *et al.*, 1991 and Meydani & Blumberg, 1993). Melatonin enhanced T and B lymphocyte proliferation in spleen of birds (Kliger *et al.*, 2000 and Brennan *et al.*, 2002). Melatonin is a broad-spectrum antioxidant, also synergizes with vitamin C, vitamin E and glutathione in the scavenging of free radicals, which prevents oxidative damage at the level of cells, tissues and organs (Dreher *et al.*, 1998 and Dun-Xian *et al.*, 2000).

The results of this study suggest that higher supplemental levels of vitamin E and the combination of vitamin E, C and melatonin would improve performance of chicks and positively affect immune response.

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LEGEND OF FIGURES

Figure 1: Lymph node from control group showing few B cell reaction, which took the blackish discolouration. Alkaline phosphatase. 10X 25.

Figure 2: Bursa from control group showing few B reaction, which took the blackish colour. Alkaline phosphatase.10X 10.

Figure 3: lymph node from birds of the group supplemented with AA, vitamin E and melatonin showing an increase in the number of activated B lymphocytes. Alkaline phosphatase.10X 25.

Figure 4: Bursa from birds of the group supplemented with AA, vitamin E and melatonin showing moderate increase in the number of activated B lymphocytes. Alkaline phosphatase.10X 10.

Figure 5: Spleen from control group showing few T cell reaction, which took the brown colour. Non specific esterase.10X 25.

Figure 6: Thymus from control group showing few T cell reaction, which took the brown colour. Non specific esterase.10X 25.

Figure 7: Spleen from birds of the group supplemented with AA, vitamin E and melatonin showing an increase in the number of activated T cells. Non specific esterase.10X 40.

Figure 8: Thymus from birds of the group supplemented with AA, vitamin E and melatonin showing an increase in the number of T cells. Non specific esterase.10X 40.

Table 1: The composition of the diets fed during the experimental period

INGREDIENT	DIET	
	Starter (0-3 weeks)	Grower-finisher (3-6 weeks)
Physical composition (%):		
White corn, ground	45.62	58.80
Soybean meal (44%)	36.96	27.55
Fish meal, herring (72%)	4.00	4.00
Dried fat	9.99	6.78
Dicalcium phosphate	1.42	0.87
Limestone, ground	1.20	1.31
Common salt	0.41	0.37
Mineral mixture*	0.10	0.10
Vitamin B+K_3+choline*	0.10	0.10
Muv D_3 **	0.10	0.10
L-methionine	0.10	0.02
Calculated chemical Composition (%) and energy value #:		
Crude protein	23.02	20.0
Calorie/protein ratio	139.1	160
Methionine	0.50	0.37
Lysine	1.33	1.10
Calcium	1.01	0.88
Total phosphorous	0.69	0.58
Available phosphorous	0.47	0.35
ME (Kcal/kg diet)	3200	3200

* MUVCO mineral mixture, Vitamin B+K_3+choline used at the described rate for broilers (1 kg / ton).

** MUVCO D_3: Each kg contains 5,000,000 IU, used at the rate of 1 kg / ton diet.

\# The chemical composition was calculated on "as-fed basis".

Table 2: Feed intake (g) of chicks during the experimental period

AGE IN WEEKS	GROUP				
	1	2	3	4	5
0-1	123	106	87	93	83
1-2	313	284	246	252	313
2-3	536	459	532	509	552
3-4	742	791	748	714	770
4-5	937	868	885	843	838
5-6	945	1100	1083	1065	959
Total	3596	3608	3581	3476	3515

Table 3: Body weight development (g) of chicks during the experimental period

AGE IN WEEKS	GROUP				
	1	2	3	4	5
0	41.o	42.0	40.0	39.0	41.0
	±4.0	±3.5	±3.9	±4.2	±3.8
1	120.0	114.0	113.0	120.0	121.0
	±7.71	±7.22	±8.02	±7.32	±6.85
2	332.0	308.0	295.0	306.0	326.0
	±10.25	±11.85	±10.17	±11.32	±9.81
3	702.0	651.0	637.0	633.0	678.0
	±13.32	±18.63	±13.96	±14.87	±11.76
4	1124.0	1084.0	1054.0	1084.0	1134.0
	±26.12	±27.02	±24.87	±24.48	±24.95
5	1356.0	1236.0	1396.0	1216.0	1336.0
	±28.33	±29.11	±28.53	±27.93	±28.22
6*	1688.0	1680.0	1768.0	1715.0	1875.0
	±30.67[c]	±30.71[c]	±29.98[b]	±30.18[b]	±32.56[a]
Times the initial	41.2	40.0	44.2	44.0	45.7

* Values (mean ± SE) which are not significantly different are followed by same superscript (P<0.05).

Table 4: Chick performance of the six groups

AGE IN WEEKS	GROUP				
	1	2	3	4	5
Feed intake (g)					
0 – 3	992	849	865	854	948
3 – 6	2624	2759	2716	2622	2567
0 – 6	3596	3608	3581	3476	3515
Weight gain (g)					
0 – 3	661±	609±	597±	594±	637±
	12.35	11.88	10.94	12.76	14.24
3 – 6*	986±	1029±	1131±	1082±	1197±
	24.31[c]	23.12[b]	25.28[a]	19.86[b]	22.58[a]
0 – 6	1647±	1638±	1728±	1699±	1834±
	29.72[c]	33.63[c]	29.89[b]	28.42[b]	35.16[a]
Feed conversion efficiency					
0 – 3	1.47	1.39	1.45	1.44	1.49
3 – 6	2.66	2.70	2.40	2.42	2.14
0 – 6	2.18[a]	2.20[a]	2.07[b]	2.05[b]	1.92[c]

* Values (mean ± SE) in the same row which are not significantly different are followed by same superscript (P<0.05).

Table 5: Antibody titers of the chicks against NDV in the six experimental groups

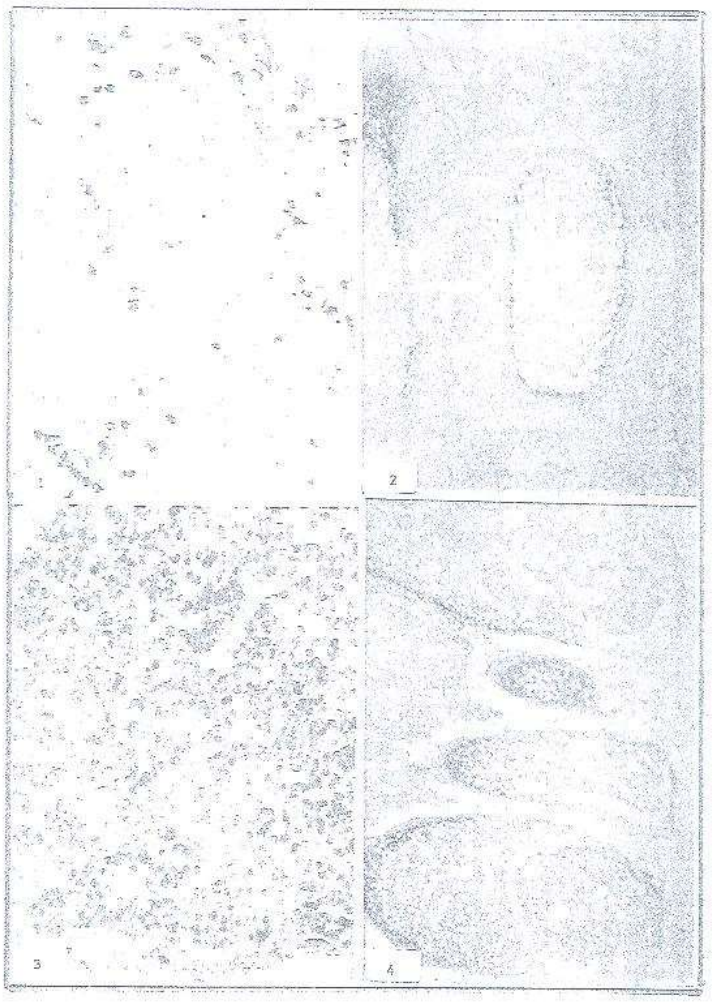
	GROUP				
	1	2	3	4	5
Log_2 titers*	3.75± 0.41 [c]	5.25± 0.25 [a]	5.50± 0.29 [a]	4.50± 0.29 [b]	4.25± 0.48 [b]

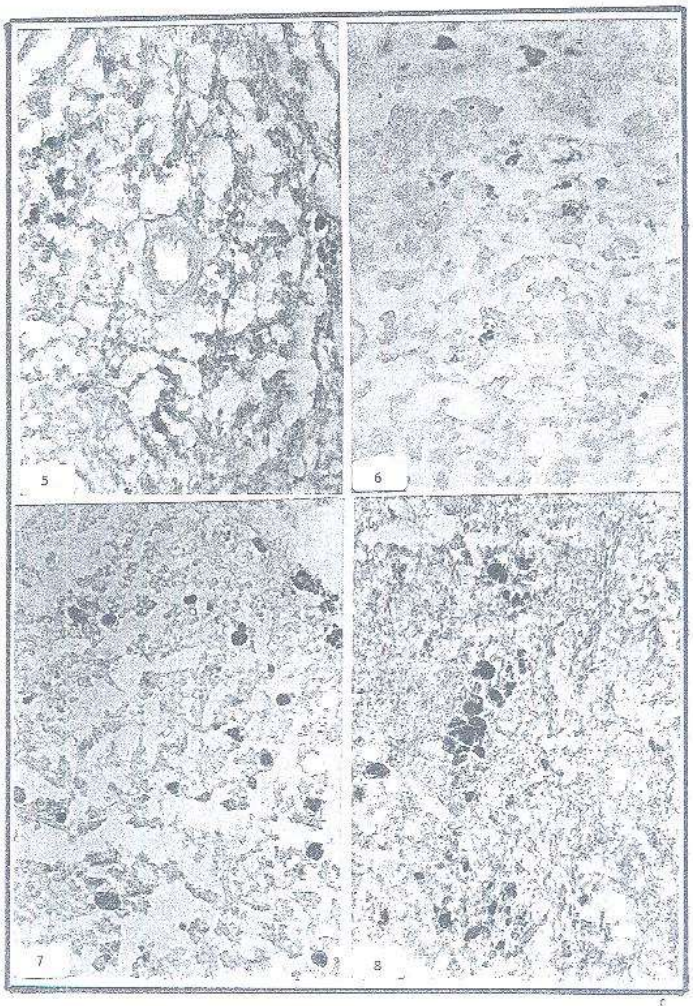
* Values (mean ± SE) in the same row which are not significantly different are followed by same superscript (P<0.05).

Table 6: Haematological picture in the six experimental groups

Blood parameter	GROUP				
	1	2	3	4	5
Total erythrocytes ($_x10^6$ µl)*	3.81± 0.09[b]	3.20± 0.09[c]	3.40± 0.12[c]	3.9± 0.015[b]	4.50± 0.02[a]
Total leucocytes ($_x10^3$ µl)	11.1± 0.11 [b]	11.1± 0.21 [b]	10.9± 0.11 [b]	11.5± 0.022 [b]	12.6± 0.09 [a]
Hetrophils (%)	30.0± 0.13 [b]	31.0± 0.35 [b]	29.0± 0.30 [b]	32.0± 0.039 [b]	24.0± 0.041[c]
Lymphocytes(%)	52.0± 0.30[c]	57.0± 0.63[b]	58.0± 0.51[b]	56.0± 0.45[b]	61.0± 0.32[a]
Monocytes (%)	12.0± 0.11 [b]	13.0± 0.15 [b]	15.0± 0.18[a]	13.0± 0.11 [b]	12.0± 0.20 [b]
Eosinophils (%)	3.0± 0.01	2.0± 0.03	2.0± 0.06	4.0± 0.07	3.0± 0.09
Basophils (%)	1.0± 0.009	2.0± 0.007	1.0± 0.001	2.0± 0.01	1.0± 0.009

* Values (mean ± SE) in the same row which are not significantly different are followed by same superscript (P<0.05).

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