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EFFECT OF ECHINACEA EXTRACT SUPPLEMENTATION ON GROWTH PERFORMANCE AND HEMO-BIOCHEMICAL TRAITS OF GROWING RABBITS

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ABSTRACT: *The effect of Echinacea purpurea extract supplementation on growth performance and hemo-biochemical traits of growing rabbits has been studied on forty eight growing New Zealand White rabbit. The experimental rabbits divided into four equal groups. The first group was served as the control, while the second, third and fourth groups were treated orally with 2.5, 5.0 and 7.5 mg Echinacea purpurea extract/kg body weight daily. Results showed that final body weight, average daily body weight gain and average feed conversion were significantly enhanced in Echinacea extract treatment groups compared with control. Thus, the treatment with Echinacea caused a significant increase in hemoglobin (Hb), Packed cell volume (PCV), white blood cell count (WBC) and red blood cell count (RBC) and indices of erythopoietic status. Echinacea extract treatment showed significant higher in lymphocytes and eosinophils percentage compared with control, while neutrophils, monocytes and basophils showed significant decreases. Also, there was significant increase in plasma total protein (TP) and globulin (G) and significant decreases in urea concentration in Echinacea treated group compared to the control. Similar results were found in plasma AST and ALT especially in medium dose. These results indicate that treatment of growing rabbits with Echinacea extract in different doses improved growth performance, stimulates immune functions and protein metabolism without harmful effects on liver and kidney functions.*

INTRODUCTION

Echinacea is a hardy perennial plant indigenous to North America, which belongs to the Asteraceae or Compositae plant family and includes nine different species (McGregor, 1968). Of these species Echinacea purpurea, Echinacea angustifolia, and Echinacea pallida have medical properties (Schulthess, 1991), and are commercially traded as medicinal plants.

Echinacea is one of the most important medical herbs. It is widely used around the world to treat common cold and other infectious disorders with the claim to have paramunity-inducing and non-specific immune responses stimulating effects. Numerous studies attest the health-promoting properties of extracts derived from plants of the genus Echinacea (Bauer and Wagner, 1991). Echinacea treatment results in an increase of various cytokines, lymphocytes, and phagocytosis activity (Sasagawa et al., 2006). Generally, Echinacea is thought to create activity in the immune system by stimulating T-cell production, phagocytosis, lymphocytic activity, cellular respiration, activity against tumour cell (thought its application is debatable), and inhibiting hyaluronidase enzyme secretion (Brauning, et al., 1992). Especially polysaccharides and glycoproteins containing high amounts of arabinose and galactose are often considered to be important (Wagner *et al.*, 1989).

The chemical analysis of the plants in the genus Echinacea has identified seven groups of medically important components including polysaccharides, flavonoids, caffeic acid derivatives, essential oils, polyacetylenes, alkylamides, and miscellaneous chemicals. Some experts believe that the polysaccharides are primary active ingredients for immune modulating effects (Wagner, et al., 1988). It appears that the immune-stimulating effects of Echinacea result from polysaccharides surrounding tissue cells and thereby providing protection from bacterial and pathogenic invasion (Newall, et al., 1996). The polysaccharide components have also been shown to promote tissue regeneration by stimulating fibroblasts and inhibiting the enzyme hyaluronidase, which breaks down the intracellular cement called hyaluronic acid (Enbergs & Woestmann, 1986).

Studies on the use of Echinacea to improve animals welfare are rare. Kuhn et al. (2005) reported an immune stimulating effect in sows by a repeated application of Echinacea every 5 days. Also, treatment of fish with Echinacea induce increases in growth, immunostimulant and reduce diseases (Mashally *et al.*, 2008). In contrast to their study Maass et al. (2005) did not find significant effects during a continuous application.

Therefore, the present study aimed to investigate the effect of Echinacea on the growth performance and blood hematology and biochemistry of growing rabbits. Mortality rate and the cost-benefits ratio were evaluated.

MATERIALS AND METHODS

This study was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agricultural Research Center. Forty eight weaned New Zealand White (NZW) rabbits aged 35 days with an average initial weight of 570±8.30 g the animals were allotted to four experimental groups (12 each). The animals were given drinking water supplemented with Echinacea purpurea extract from weaning up to 91 d of age (fattening period). First group was served as control (Con). Second, third and fourth groups were given water supplemented with 2.5, 5.0 and 7.5mg Echinacea purpurea extract/kg of body weight/day. The dosage of the Echinacea extract for growing rabbits was adjusted on the basis of human medical recommendations due to a lack of data for rabbits. The rabbits were individually housed in galvanized wire cages and all rabbits were fed *ad libitum* with a commercial pelleted ration containing 18 % crude protein, 13 % crude fiber and 2670 kcal / kg diet. All the experimental animals were reared under the same environmental and management conditions during experimental period. Ambient air temperature ranged between 20-30 °C. Live body weight (BW), feed intake (FI) and mortality rate (MR) were weekly recorded. Daily weight gain (DBWG) and feed conversion (FC) were calculated.

Blood samples were withdrawn from ear vein of all animals in heparinzed tubes from each treated group every month. Plasma was obtained by centrifugation of blood samples at 3500 rpm for 20 min, and stored at –20 °C. Noncoagulated blood was used, shortly after collection, for hemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), white blood cell count (WBC) and white blood cell types using the conventional methods (Hepler, 1966). Indices of erythropoietic status were calculated.

Blood plasma of glucose, total protein, albumin, cholesterol, total lipids, urea, creatinine, the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were measured according to commercial kits (Vitro Scient company). The economic efficiency of the treatment was done according to Debertin (1986).

Data were analyzed as a completely randomized design (Steel and Torrie, 1980) using the General Linear Model procedure of SAS (1986).

Means were compared by Least Significant Difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of Echinacea purpurea extract on growth performance:

Data in Table (1) and figure (1) summarized the effect of treatment with low, medium and high doses of Echinacea extract on body weight, daily body weight gain, feed intake, feed efficiency and mortality rate of growing rabbit during fattening period (from 5 weeks to 12 weeks). The values of final body weight was significantly higher ($P< 0.01$) in Echinacea extract treatment groups compared with control. Similar trend was found in average daily body weight gain. On the other hand, feed intake showed no significant difference between groups. Therefore, the average feed efficiency was significantly improved as a result of treatment with Echinacea extract compared with control. Transformation of these results as a percentage of control group showed linearly increases in body weight, daily body weight gain, feed intake and feed efficiency during experimental period especially at end of treatment period (Figure 1). These results agree with the findings of Nasir and Grashorn (2008) who reported improving of feed efficiency of broiler treated with Echinacea than control when the Echinacea fermented juice supplementation was given through drinking water.

Also, Roth-Maier et al., (2008) found that treatment with Echinacea purpurea as a feed additive for broilers and layers improved feed efficiency. Thus, the improvement of FC in the Echinacea purpurea treated group in week 1-4 was 4% was better, but depressed in week 5-8 by 11%. They found that no influence of the various dosages of Echinacea on body weight, while feed intake was depressed by 4%. They concluded, that Echinacea purpurea as a feed additive for broilers and layers is not beneficial for growth or layer performance. Similar results were found by Hermann et al., (2003) who found that no differences ($P > 0.10$) were observed in ADG, ADFI, or G:F ratio. for pigs receiving diets supplemented with Echinacea at 2 or 4% of the total diet. Also, Böhmer, et al., (2008) reported that performance of the hens and pigs were not affected by Echinacea purpurea juice as feed additive. Moreover, Maass, et al., (2005) did not find any significant differences for growth performance of the dried herb Echinacea purpurea as feed additive in diets of sows, piglets, and grower/finisher pigs.

The significantly increased total WBC count in Echinacea treatment groups was associated with the increase in lymphocytes and eosinophils (Data shown in Table 3). This may explain the efficacy of Echinacea in

terms of the health status and non-specific immune response that lowered the mortality rate of Echinacea treatment groups compared with control (Table 1). Similar result was found by Mesalhy et al., (2008) who found that mortality rate was reduced in Nile tilapia treated with Echinacea extract.

Effect of Echinacea extract on Hematological parameters:

Hematological changes in growing rabbit supplemented with Echinacea extract was shown in Table (2). The treatment with Echinacea caused significant increase in hemoglobin (Hb), where the values 10.56, 11.19, 11.72 and 13.11 mg/dl for control, low, medium and high doses, respectively. Packed cell volume (PCV) also increased ($P<0.05$) by Echinacea treatment. The respective values for control, low, medium and high doses were 40.3, 41.3, 41.0 and 42.0%, and red blood cell (RBC) count were 4.85, 5.04, 5.26 and 5.78 $\times 10^6$. Calculated indices of erthropoietic status including mean corpuscular hemoglobin concentration, MCHC were 26.23, 27.12, 28.55 and 31.19 pg/dl for the four treatment groups, respectively. Contrariwise, there were significant decrease in mean corpuscular volume, MCV and the values for control, low, medium and high doses were 83.79, 82.35, 79.41 and 73.85 um^3 respectively. There was no significant change in mean corpuscular hemoglobin (MCH) due to Echinacea treatment.

It has been reported that Echinacea contents play their roles via increasing cell nuclear contents of transcription factors, including some pro-inflammatory agents such as NFKB, AP-1, AP-2 and ST, these factors are known as cytokinase and chemokines (Sharma et al., 2006). The increase that occured in the current hematological parameters could be attributed to the fact that the active ingredients in Echinacea purpurea play stimulatory roles on the level of nucleus of many cells in the body. In a study by Chow et al., (2006) they found increases in erythrocytes and lymphocyte numbers in pregnant mice given Echinacea purpurea. Whitehead et al., (2007) studied the effect of 4 weeks of oral Echinacea supplementation on serum erythropoietin and erythropoietic status and found that serum erythropoietin was greater ($P < 0.001$) at days 7, 14, and 21 and reflected a 44%, 63%, and 36% increase, respectively. On the other hand, Maass, et al., (2005) did not find any significant differences of blood picture recieved dried herb Echinacea purpurea as feed additive in diets of sows, piglets, and grower/finisher pigs.

Effect of Echinacea extract on total and differentiation leukocyte counts:

Data in Table (3) and Figure (2) represented the total leukocytes counts and its differential distribution. The treatment with Echinacea caused significant increase in white blood cell count (5.65, 7.19, 7.54 and 7.70 $\times 10^3$/ml).

Echinacea extract treatment in medium and high doses showed significant higher value in lymphocytes percentage compared with control and the respective values for control, low, medium and high doses were 64.5, 64.8, 69.6 and 71.2%. Significant increases were also found in eosinophils percentage of growing rabbits due to only high dose treatment (2.8, 3.6, 3.5 and 5.3% for control, low, medium and high doses, respectively). While, neutrophils, monocytes and basophils showed significant decrease for Echinacea treatment, the values were significantly lower in high dose group. The respective values were 27.6, 26.2, 22.8 and 20.1% for neutrophils, 3.2, 3.1, 2.5 and 2.1% for monocytes and 1.9, 2.3, 1.6 and 1.3% for basophils in control, low, medium and high doses groups, respectively. These results are compactable with the results of many investigators such as Böhmer, et al., (2008) who found that hens and pigs treated with Echinacea purpurea juice as feed additive had significant higher values in total numbers of leukocytes and lymphocytes percentage compared to control. Also, in pigs the phagozytosis rate showed significantly higher values in the Echinacea group compared with the control group. They add that the Echinacea extract probably has a different ratio of components causing this effect besides a higher concentration of alkamids.

Scientific studies indicates that Echinacea derived polysaccharides, alkylamides and cichoric acid (a caffeic acid derivative also known as chicoric acid, 2,3-o-di-caffeoyl-tartaric acid) each possess health-promoting properties. For example, alkylamides from Echinacea have been shown to stimulate phagocytosis in mice granulocytes at concentrations of about 0.1 ppm (Bauer et al. 1988). Similarly, cichoric acid has been shown to increase phagocytosis in granulocytes, and may stimulate the immune system at concentrations as low as 0.01 ppm . In addition to that, Cundell et al. (2003), found a significant increase of lymphocytes after one week in rats fed with dried Echinacea preparations. Increase of phagocytic activity is a characteristic reaction associated with efficacy of Echinacea (Barrett, 2003). Allen (2003) found a stimulation of the phagocytic response in chickens provided with ground root preparations. Thus, Bauer et al. (1988) reported that phagocytosis in mice was enhanced after treatment with ethanolic

extracts of Echinacea. Additionally, the dosage of Echinacea has an important influence, therefore, Gaisbauer et al. (1990) could prove in human whole blood that only a low dose of Echinacea could enhance phagocytosis. A higher dosage reduced the number of granulocytes with phagocytic activity.

Effect of Echinacea extract on plasma components:

Plasma biochemical changes in growing rabbit supplemented with Echinacea extract was shown in Table (4). The treatment with Echinacea caused significant increase in plasma total protein (TP) compared with control. The values of TP were 5.14, 5.93, 5.71 and 6.08 gm/dl for control, low, medium and high doses, respectively. Globulin (G) also significantly increased by Echinacea treatment compared with control. The respective values for control, low, medium and high doses were 2.08, 2.98, 2.71 and 3.2gm/dl. Contrariwise, there were significant decrease in urea (mg/dl) compared to control and the values for control, low, medium and high doses were 39.6, 38.2, 35.8 and 35.3 gm/dl, respectively. Similar results were found in plasma activity of AST and ALT especially in medium dose, the values for control, low, medium and high doses were 33.2, 34.2, 29.8 and 30.4 IU for AST and 52.6, 50.2, 43.9 and 46.9 IU for ALT. There were no significant change in plasma values of glucose, albumin, A-G ratio, cholesterol, total lipids and creatinine due to Echinacea treatment.

These results agree with the findings of Nasir and Grashorn (2008) who found that broiler serum total protein and globulin contents were significantly ($P < 0.05$) higher in *Echinacea purpurea* supplemented group as compared to control, while no significant treatment effect was observed on albumin contents. The levels of ALT was insignificantly different. They reported that improvement of serum total protein, and globulin contents showed positive effects of Echinacea fermented juice supplementation through drinking water on protein metabolism, with no harmful effects on liver enzymes. On the other hand, Maass, et al., (2005) investigated the inclusion of the dried herb Echinacea purpurea as feed additive in diets of sows, piglets, and grower/finisher pigs on plasma enzymes and they did not find significant differences compared with control.

Economic Efficiency:

The results presented in Table (5) showed that growing rabbits treated with low, medium and high doses of Echinacea extract showed the highest value of economical efficiency (31, 41.7 and 68.3%) compared with control group.

CONCLUSION

It may be concluded from present study that giving growing rabbits a natural herb, namely Echinacea purpurea improved growth performance and induced immune stimulating effects as indicated by increasing lymphocytes. Mortality rate of treated groups was markedly decreased which participate in increasing of economic efficiency of the treatment. Further multidiscipline studies must be undertaken to isolate the different Echinacea constituents and investigate the single as well as the integrate combinations of such constituents on animals performance.

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Table (1): Effect of different doses of Echinacea extract on growth performance of growing rabbits (M± SE).

Item	Control	Low dose	Medium dose	High dose
Number of rabbits at 35 days (Weaning age)	12	12	12	12
Number of rabbits at 90 days (Marketing age)	10	11	11	12
Initial body weight (g)	622 ±9.1	635±14.8	625±8.5	627±7.6
Final body weight (g)	1423 ±15.8^{C}	1583±55.7^{B}	1637±23.2^{AB}	1696±21.1^{A}
Average body weight gain (g/day)	22.9 ±0.48^{C}	27.1±0.70^{B}	28.9±0.63^{A}	30.6±0.55^{A}
Average feed intake (g/kg BW)	85.3 ±4.03	94.4±4.18	92.5±3.79	88.7±3.91
Average FC (g feed/g gain)	3.77±0.196^{A}	3.54±0.175^{AB}	3.26±0.168^{BC}	2.95±0.153^{C}
Mortality Rate (MR) %	17	8.5	8.5	0

Means within the same row with different superscript are significantly different

Table (2): Effect of different doses of Echinacea extract on hematological parameters of growing rabbits (M ± SE).

Item	Control	Low dose	Medium dose	High dose
Hb (mg/l)	10.6 ± 0.17^{c}	11.2 ± 0.25b^{c}	11.7 ± 0.34^{b}	13.1 ± 0.26^{a}
RBC (X 10^{6}/ml)	4.9 ± 0.08^{c}	5.1 ± 0.07^{bc}	5.3 ± 0.13^{b}	5.8 ± 0.13^{a}
PCV (%)	40.3 ± 0.18^{c}	41.3 ± 0.14^{b}	41.0 ± 0.13^{b}	42.0 ± 0.18^{a}
MCH (pg)	22.0 ± 0.64	22.4 ± 0.63	22.6 ± 0.73	22.8 ± 0.44
MCV (fl)	83.8 ± 1.46^{a}	82.4 ± 1.19^{a}	79.4 ± 1.98^{a}	73.9 ± 1.69^{b}
MCHC (%)	26.2 ± 0.48^{c}	27.1 ± 0.60^{bc}	28.6 ± 0.82^{b}	31.2 ± 0.62^{a}

Means within the same row with different superscript are significantly different

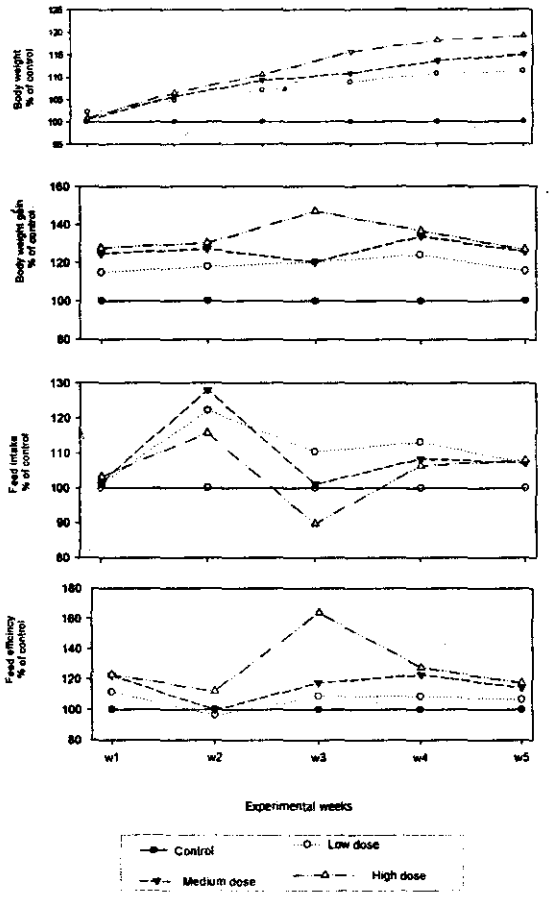
Table (3): Effect of different doses of Echinacea extract on blood total leukocytes counts and its differentiation of growing rabbits.

Item	Control	Low dose	Medium dose	High dose
WBC (× 10^3/ml)	5.7 ± 0.06^{b}	7.2 ± 0.21^{a}	7.5 ± 0.27^{a}	7.7 ± 0.27^{a}
Lymphocyte (%)	64.5 ± 0.71b	64.8 ± 0.66b	69.6 ± 1.33a	71.2 ± 1.53a
Neutrophils (%)	27.6 ± 0.48^{a}	26.2 ± 1.07^{ab}	22.8 ± 1.24^{bc}	20.1 ± 1.81^{c}
Monocytes (%)	3.2 ± 0.12^{a}	3.1 ± 0.33^{ab}	2.5 ± 0.39^{ab}	2.1 ± 0.51^{b}
Eosinophils (%)	2.8 ± 0.12^{b}	3.6 ± 0.24^{b}	3.5 ± 0.22^{b}	5.3 ± 0.58^{a}
Basophils (%)	1.9 ± 0.19^{ab}	2.3 ± 0.20^{a}	1.6 ± 0.24^{bc}	1.3 ± 0.12^{c}

Table (4): Effect of different doses of Echinacea extract on blood plasma composition of growing rabbits (M±SE).

	Control	Low dose	Medium dose	High dose
Glucose(gm/dl)	100.8 ± 2.02	101.2 ± 3.11	103.0 ± 1.71	102.3 ± 1.37
Total Protein (gm/dl)	5.14 ± 0.07^{c}	5.93 ± 0.09^{ab}	5.71 ± 0.18^{b}	6.08 ± 0.07^{a}
Albumin (gm/dl)	3.05 ± 0.16	2.95 ± 0.06	3.00 ± 0.04	2.88 ± 0.08
Globulin (gm/dl)	2.08 ± 0.19^{c}	2.98 ± 0.12^{ab}	2.71 ± 0.15^{b}	3.20 ± 0.10^{a}
A-G ratio	1.47 ± 0.102	0.99 ± 0.070	1.11 ± 0.361	0.90 ± 0.100
Cholesterol (mg/dl)	85.4 ± 3.46	76.7 ± 5.67	71.3 ± 9.14	71.6 ± 9.05
Total Lipids (mg/dl)	295 ± 8.4	293 ± 8.61	298 ± 1.32	299 ± 4.58
Urea (gm/dl)	39.6 ± 0.95^{a}	38.2 ± 0.67^{ab}	35.8 ± 1.13^{bc}	35.3 ± 0.24^{c}
Creatinine (mg/dl)	1.13 ± 0.04	1.05 ± 0.03	1.13 ± 0.05	1.07 ± 0.06
AST (IU)	33.2 ± 0.54^{a}	34.2 ± 0.65^{a}	29.8 ± 1.23^{b}	30.4 ± 0.49^{b}
ALT (IU)	52.6 ± 1.36^{a}	50.2 ± 4.01^{ab}	43.9 ± 1.27^{b}	46.9 ± 0.85^{ab}

Means within the same row with different superscript are significantly different



Figur (1): Changes in body weigh, body weigh gain, feed intake and feed efficinecy during the treatment of growing rabbits with Echinacea extract

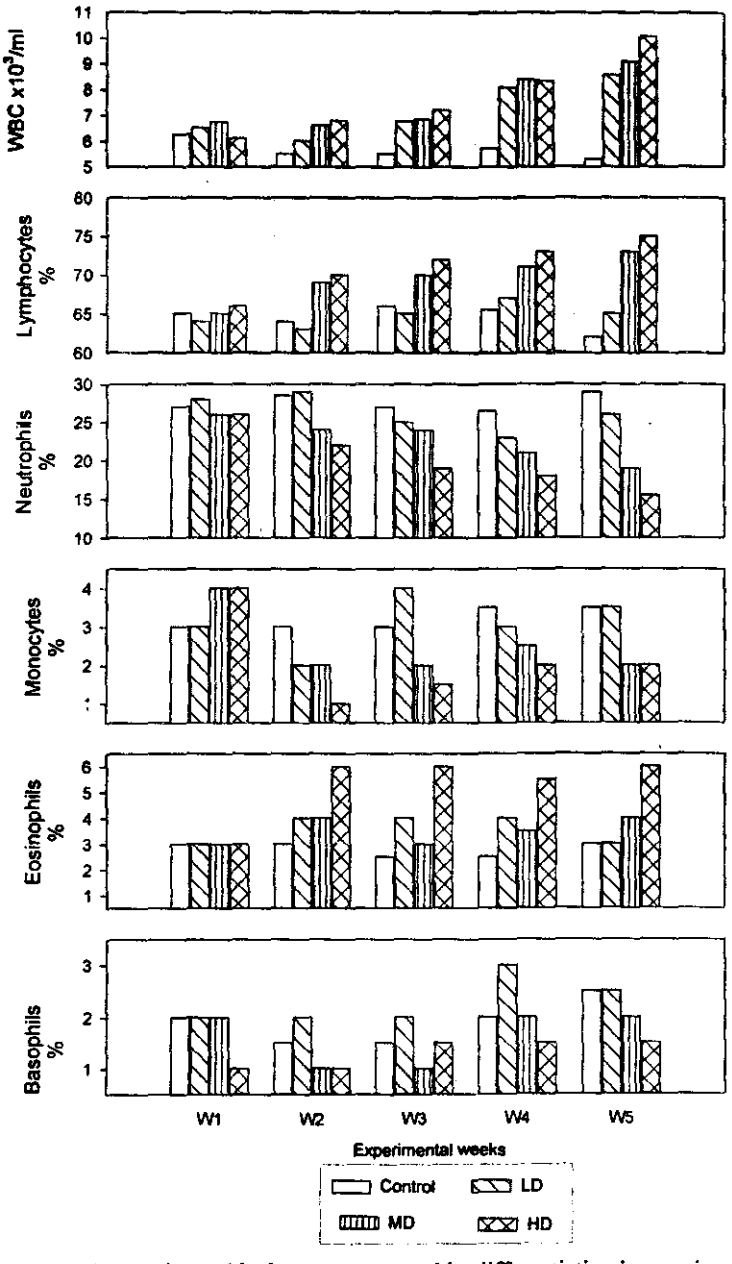


Figure 2: Changes in total leukocyte counts and its differentiation in growing rabbits treate with Echinacea extract

Table (5): Economic Efficiency for treatment of growing rabbit with low, medium and high doses of Echinacea purpurea.

Items	Control	Low	Medium	High
Growing No at marketing	10	11	11	12
Body weight gain (g/period)	801	948	1012	1069
Total body weight gain (g)	7690	10343	11041	12828
Price of weight gain (L.E)	**138.42**	**186.17**	**198.74**	**230.90**
Feed intake (g/ period)	43998	56621	55481	58562
Price of feed intake (L.E)	66.0	84.9	83.2	87.8
Echinacea intake (mg/period)	-	1500	3000	4950
Price of Echinacea (L.E)	-	6.43	12.86	21.21
Total costs (L.E)	**66.0**	**91.3**	**96.1**	**109.0**
Net return (L.E)	**72.42**	**94.87**	**102.64**	**121.9**
Economic Efficiency (y)	**109.7**	**103.9**	**106.8**	**111.8**
Economic Efficiency (%)	**100**	**131.0**	**141.7**	**168.3**

Price of 1 kg live weight = 18 L.E
Price of 1 kg feed = 1.5 L.E
Price of 20 tables Echinacea = 15 L.E
Price of feed intake = average daily feed intake x 55 day x price feed
Price of Echinacea intake = average daily intake x 55 day x price Echinacea.
Economic efficiency (y) = Net return / Total cost.
Economic efficiency (%) = As a percent of control.

الملخص العربي

تأثير إضافة مستخلص الاشينسيا علي صفات النمو وبعض مكونات الدم في الأرانب النامية

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تم دراسة تأثير اضافة مستخلص الاشينسيا علي الصفات النمو ومكونات الدم للأرانب النيوزيلاندي النامية. الحيوانات قسمت الي ٤ مجاميع، المجموعة الاولي هي المجموعة المقارنة المجموعة الثانية والثالثة والرابعة اعطيت ٢.٥، ٥.٠، ٧.٥ ملجرام اشينسيا /كيلوجرام من وزن الارانب عن طريق الفم يوميا. واتضح من النتائج ان وزن الجسم النهائي ومتوسط وزن الجسم المكتسب ومتوسط الغذاء المتحول تأثرت معنويا بالمعاملة بالاشنسيا مقارنا بالمجموعة المقارنة. كما ان معدل النفوق انخفض جوهريا نتيجة المعاملة وبدرجة اعتمدت علي الجرعة. المعاملة بالاشنسيا زادت معنويا من تركيز الهيموجلوبين، حجم الدم المعباء، عدد خلايا الدم الحمراء والبيضاء. العد التعريفي للخلايا البيضاء أظهر زيادة نسبة الخلايا من النوع الليمفوسيت والازينوفيلز. بينما اختلفت نسب الخلايا من نوع النتروفيلز والمونوسيت والبازوفيلز بين المجاميع المختلفة من المعاملة ومجموعة الكنترول. وتحليل بلازما الدم اوضح حدوث زيادة معنوية في تركيز البروتين الكلي والجلوبيولين في المجاميع المعاملة بينما انخفضت معنويا قيم تركيز اليوريا وانزيمات الكبد خاصا في المعاملة المتوسطة. الجدوي الاقتصادية اظهرت تفوق واضح في المجاميع المعاملة بالاشنشيا في كفاءتها الاقتصادية مقارنا بالمجموعة الغير معاملة.وعلية فان الدراسة الحالية توضح ان معاملة الارانب النامية بمستخلص الاشينسيا احدث تحسنا في الخصائص الانتاجية والصفات والفسيولوجية والكفاءة المناعية للارانب النيوزيلاندي النامية دون حدوث تأثير ضار علي الكبد والكلي