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ROLE OF PROBIOTICS ON HEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN GROWEING RABBITS

Abd-El-Mgied, Noha Y.[1]; Faten A.A. Ibrahim[2]; A. M. El-Kaiaty[2] and A. A. Sedki[1]

1- Animal Production Research Institute, Agriculture Research Center, Dokki, Egypt

2- Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt

ABSTRACT

The purpose of the present study was to determine if probiotic (Protexin) supplementation to drinking water at different levels (1.0, 2.5 and 5.0g /Litter of drinking water) would have a beneficial effect on immunological and hematological parameters of weaning rabbits. Fifty six, 5-week old New Zealand White rabbits (NZW) weighing an average of 580g were divided into four groups (14 rabbits each). Rabbits of group 1 received plain water. Rabbits of group 2, 3 and 4 received water supplemented with 1.0, 2.5 and 5.0g Protexin/Litter of drinking water, respectively The rabbit houses were made of available local materials (mud, wood *et*....). The study was conducted during fattening period, from 5-13 weeks, from February to April 2004

Obtained results showed that Protexin supplementation at 2.5g /Litter level improved significantly ($P \leq 0.05$) hematocrit values (Ht %), hemoglobin concentrations (Hb g/dl), total protein value (TP g/dl), albumin value (Alb g/dl), albumin/ globulin ratio (Alb/Glob %). Aspartate and alanine aminotransferase (AST&ALT m/l) and alkaline phosphatase levels (Alk-P IU/l) were also improved. However supplementation of 5.0g Protexin/Litter improved globulin value (Glob g/dl). No significant differences between treatments were observed in creatinine levels (Cr mg/dl). However, a significant reduction in urea- nitrogen values (UN mg/dl) was observed compared to the control group. All the immunological parameters were improved due to protexin treatments. Significant increase in lymphocyte percentages, and white blood cells counts (WBC_S 10^3 /mm^3) were observed at the 2.5g Protexin/Litter level. Improvement in humoral immune response (I.R.) at 2.5 and 5.0g Protexin/Litter levels and cell mediated immunity (CMI) at the 5.0g Protexin/Litter level were observed.

It can be concluded that supplementation of (Probiotics) Protexin at the 2.5g Protexin/Litter level improved hematological parameters. The 5.0g Protexin/Litter level showed a great role in enhancing the immune system

Keywords: Rabbits, Probiotics, Blood chemicals, Immune system,

INTRODUCTION

The problem of mortality, after weaning, is one of the main problems of rabbit production. Weaning of rabbits is often associated with reduced feed intake, little or no weight gain, insufficient pancreatic and intestinal enzyme production and /or activity and in some instances diarrhea, morbidity, and death, (Jensen *et al.,* 1997).

Rabbits have a very rapid growth rate during the first three months of life. Rapid growth makes these animals very sensitive to nonspecific syndromes, due to the higher rates of metabolism and cell division compared to that of other mammals. During this period of rapid growth, any nutrient deficiency or any kind of physiological or environmental stress or diseases is

likely to have a major effect on the weaned rabbits (Ismail, 1993). Additives of special interest in the formulation of animal diets include amino acids, antioxidants, vitamins, minerals, enzymes antibiotics, and drugs to prevent or to control disease. (Ali, 1999). Antibiotics have been used in the animal industry to prevent enteritis, diseases, infection by pathogens, maintain health and to improve meat quality. Antibiotics can eliminate undesirable microorganisms, that produce toxins or metabolic products that irritate and increase thickness of intestinal wall and subsequently decrease the absorption of nutrients (Lev *et al.*, 1957 and Stutz *et al.*, 1983). However, Chmitelin (1992) reported some of the disadvantages of using antibiotics including poor efficiency, risk of antibiotic resistance and residues in the meat. In addition, using antibiotics plays a havoc role in the digestive system, and hampers immune mechanisms of the body.

Administration of Probiotics to newly weaned rabbits improves the blood parameters and enhances the immune system (Pollman, 1986). These beneficial effects of Probiotics have been explained by: - 1-A change in enteric flora and reduction of *Escherichia Coli*, 2- A decrease in intestinal pH, 3-A production of antibiotic substances and 4-A reduction of ammonia levels in the gastrointestinal tract and blood. Lee *et al.*, (1999) reported that the mode of action of Probiotics may be through the production of antibiotic substances, that inhibits harmful bacteria metabolism and decrease intestinal PH.

The objective of the present study was to determine if a probiotic (Protexin) supplementation to drinking water at different levels (1.0, 2.5 and 5.0g /Litter) would have a beneficial effect on immunological and hematological parameters of weaned rabbits.

MATERIALS AND METHODS

Fifty six, 5-week old New Zealand White rabbits (NZW), with an average initial weight of about 580g, were used. Fourteen rabbits, for each treatment, were randomly placed in individual cages. Rabbits were fed on a commercial pelleted ration containing 17% CP and 2530 kcal DE. Protexin was administrated to animals through its suspension in drinking water at different levels according to the following groups: Rabbits of group 1 received plain water. Rabbits of group 2, 3 and 4 received water supplemented with 1.0, 2.5 and 5.0g Protexin/Litter respectively. The composition of Protexin is presented in Table (1). Feed and water were available *ad libitum*.

Blood samples, about 3 ml, were collected from the experimental animals (5 rabbits/ group) two months after starting the treatment from the marginal ear vein. The blood samples were collected in heparinized tubes and Hematocrit (Ht %) was measured immediately, then centrifuged and the blood plasma was decanted and stored at -20 °C until analysis. Hemoglobin (Hb) concentration, total protein (TP) and albumin (Alb) were determined by the colorimetric methods according to Merck (1974). Aspartate aminotransferase (AST) or (GOT) and Alanine aminotransferase (ALT) or (GPT) were determined using kits from Bio Merieux according to Reitman and Frankel (1957).

Table (1). The composition of Protexin.

Microorganisms	Microorganisms cfu[1] /Kg probiotic
Lactobacillus plantarum.	1.89×10^{10}
Lactobacillus delbruecki subsp. bulgaricus.	3.09×10^{10}
Lactobacillus acidophilus.	3.09×10^{10}
Lactobacillus rhamnosus	3.09×10^{10}
Bifidobacterium bifidum.	3.00×10^{10}
Streptococcus salivarus subsp. thermophilus.	6.15×10^{10}
Enterococcus faecium	8.85×10^{10}
Aspergillus oryzae	7.98×10^{9}
Candida pintolopesii	7.98×10^{9}

[1] Colony forming unit..

Alkaline phosphatase (Alk-P) activity was determined using kits from Bio Merieux according to Kind and King (1954). Creatinine (Cr) was determined by the colorimetric methods according to Folin (1934).Plasma urea (UN) levels were determined by the calorimetric methods according to Fawcest and Scott (1960). Immunological parameters and Leukocytes differential counts were determined according to Hawkey and Dennett (1989). White blood cells (WBC) were counted according to Feldman *et al.*,(2000). Humoral immune response (antibody titers against Newcastle Disease (ND), lasota strain), was determined at two months after starting the treatment (5 rabbits/ treatment) were vaccinated against Newcastle disease by Lasota strain via subcutaneously injection. Blood samples were collected from vaccinated rabbits after seven days and serum was separated and kept frozen until tested as described by (Atta, 1990). Cell mediated immunity (CMI) was determined, using 5 rabbits/ treatment, by injecting 50 µg of Phytohemagglutinin (dissolved in saline) subcutaneously into a defined area at the right ear by a volume of 0.05 ml. While the other ear (left ear) was used as a control and was injected with 0.05 ml. saline (El-Kaiaty, 1993). The thicknesses of both ears were measured before, 12 and 24 hours after Phytohemagglutinin (PHA) injection by a caliper (Cotter and Weinner, 1997). The response was calculated as a ratio as described by Bachman and Mashaly (1987) as follows:

$$\% \text{ Ear thickness increasing} = \frac{(\text{After injection} - \text{Before injection})}{\text{Before injection}} \times 100$$

Statistical analysis was conducted by one way analysis of variance using the general linear models (GLM) of (SAS, 1999). The differences among treatments means were separated according to Duncan's Multiple Range Test (Duncan, 1955). The significance level was set at 5%.

RESULTS AND DISCUSSION

I. Hematological parameters:

The effect of Protexin supplementation on hematological parameters for growing (NZW) rabbits are summarized in Table (2). The hematocrit values (Ht %) and hemoglobin concentrations (Hb) were significantly higher in the groups supplemented with Protexin than those of the control group. The highest (Ht) value and (Hb) concentration were obtained by adding 2.5g Protexin/Litter. These results are in agreement with the findings of El-Kholy (2003) when he supplemented Synertox to rabbits.

Result, in Table (2), show that the total protein (TP) values increased significantly ($p<0.05$) by the supplementation of 2.5 or 5.0g Protexin/Litter when compared to the control group. The highest TP value was observed by adding 2.5g Protexin/Litter. These results are in agreement with Hanna (2000), Metwally *et al.*, (2002) and El-Kholy (2003). The increase in plasma total proteins might reflect the increase in the hepatic function. The probiotic may act through affecting the metabolic rate beside its effect on the gastro-intestinal microbial activity. Similar results were observed for the albumin (Alb). Albumin levels increased significantly ($p<0.05$) by adding 2.5g Protexin/ Litter. On the contrary, the globulin (Glob) levels increased with the supplementation of Protexin but this increase was not significant. Globulin level was highest (although not significant) when adding 5.0g Protexin/Litter. The increase in globulin value reflects a good immunity status of the animal. However, the Alb/Glob ratio decreased with the supplementation of 5.0g Protexin/Litter. The lowest Alb/Glob ratio, as a good indicator for increasing the immunity, was observed by adding 5.0g Protexin/Litter which may be due to increased in globulin level. The addition of a probiotic (Protexin) decrease Alb/Glob ratio. This is agreement with the findings of El-Kholy (2002) when he supplemented the rabbits with Synertox which contain probiotic supplement. This improvement in blood parameters may indicate that an enhancement of immunity occurred corresponding to feeding tested biological additives as a result of improving feed consumption, absorption and utilization of nutrients.

The effect of Protexin supplementation on kidney function is summarized in Table (3). No significant differences between treatments in creatinine (Cr) levels were observed. This result is in agreement with the findings of El-Gaafary *et al.*, (1992) and Metwally *et al.*, (2002). However, plasma urea-nitrogen levels (UN) for growing NZW rabbits decreased significantly ($p\leq0.05$), than the control group, by the supplementation of Protexin. This result is in agreement with the findings of Gomaa *et al.*, (2003) who administrated Yeast to rabbit diets.

Alkaline phosphatase levels (Alk-p), aspartate aminotransferase levels (AST) or (GOT) increased significantly in all supplemented group (Table 3). However, this increase was still within the normal range, as indicated by no apparent signs of toxicity. The addition of probiotic (Protexin) improved liver functions. This is in agreement with the findings of Hanna (2000), Soliman *et. al*, (2000) and El-Kholy (2003).

Table (2): Least squares means (± S.E.) of some hematological parameters of NZW rabbits as affected by Protexin supplementation and Alb/Glob ratio (%) after two months of treatment.

Treatment	Ht %	Hb (g/dl)	TP (g/dl)	Alb (g/dl)	Glob (g/dl)	Alb/Glob ratio
Control	30.6^{b*}	10.8^{b}	5.6^{c}	3.1^{b}	2.6^{a}	1.18
1.0 g/Litter	31.0^{b}	11.7^{ab}	6.3^{bc}	3.4^{b}	2.9^{a}	1.18
2.5 g/Litter	35.2^{a}	12.8^{a}	7.4^{a}	4.6^{a}	2.8^{a}	1.66
5.0 g/Litter	33.8^{a}	12.6^{a}	6.5^{b}	3.4^{b}	3.1^{a}	1.12
S.E.	0.9	0.4	0.2	0.2	0.2	----
Overall means	32.7±0.6	11.9±0.3	6.5±0.2	3.6±0.2	2.8±0.1	1.19

*Means, within trait, between treatments, with different superscripts differ significantly (P≤0.05).

Table (3): Least squares means (± S.E.) of some blood constituents of NZW rabbits as affected by Protexin supplementation and AST/ALT ratios (%) after two months of treatment.

Treatments	Cr (mg/dl)	UN (mg/dl)	Alk-P (IU/l)	AST (m/l)	ALT (m/l)	AST/ ALT
Control	1.1^{a*}	16.1^{a}	97.2^{b}	21.6^{c}	13.6^{a}	1.59
1.0 g/Litter	1.1^{a}	13.9^{b}	103.3^{b}	25.7^{b}	13.8^{a}	1.87
2.5 g/Litter	1.1^{a}	13.7^{b}	112.9^{a}	28.6^{a}	15.5^{a}	1.85
5.0 g/Litter	1.1^{a}	13.9^{b}	112.0^{a}	26.1^{b}	14.2^{a}	1.84
S.E.	0.1	0.4	2.6	0.7	0.6	
Overall means	1.1±0.03	14.4±0.3	106.3±1.9	25.5±0.6	14.3±0.3	1.79

*Means, within trait, between treatments, with different superscripts differ significantly (P≤0.05).

No significant differences were observed in the alanine aminotransferase levels (ALT) or (GPT) due to Protexin supplementation (Table 3). Similar AST/ALT ratios were observed by adding all the 3 levels of Protexin.

II. Immunological parameters:

The effects of Protexin supplementation on Leukocytes differential counts for growing NZW rabbits are summarized in Table (4). A significant decrease in percentages of neutrophil, and a non significant decrease in monocyte, basophils, eosinophils and N/L ratio due to the supplementation of probiotic (Protexin) when compared to the control group were observed. However, lymphocyte percentage increased significantly by adding Protexin when compared with control group. This result is in agreement with the findings of Pestka (1993) and Pestka *et al.*, (2001). Lymphocytes are considered to be the main type of white blood cells. Lymphocyte count increase is considered as a good indicator of the increase in immunity. Similar results were reported by Abou El-Ella *et al.*, (2001) and Ismail *et al.*, (2003).

The effect of Protexin supplementation on white blood cell counts (WBC_S 10^3 /mm^3), humeral immune response (I.R.) and cell mediated immunity (CMI) for growing NZW rabbits are summarized in Table (5). WBC_S count increased significantly ($p<0.05$) by the supplementation of Protexin than the control group. This result is in agreement with the findings of **Fortun-**Lamothe and Drouet-Viard, 2002 and El-Kholy (2003).

The effect of Protexin supplementation on antibody titers against *Newcastle Disease vaccination* (Lasota strain) for growing NZW rabbits are summarized in Table (5). The results showed significant ($p\leq0.05$) increase in antibody production titers, due to the supplementation of Protexin, than the control group. The highest humoral Immune response (I.R.) was obtained by adding 2.5 or 5.0g Protexin/ Litter. These results indicate that the (I.R.) increased gradually with the increase in the supplementation levels of Protexin. These results are in agreement with the findings of Guerrero and Hoyos (1990), Perdigon *et al.*, (1995) and Fortun-Lamothe and Drouet-Viard, (2002).

The effect of Protexin supplementation on cell mediated immunity (CMI) for growing NZW rabbits is presented in Table (5). At 12 and 24 hours after Phytohemagglutinin (PHA) injection, there was an increase in the ear thickness reaction to Phytohemagglutinin (PHA) injection due to the supplementation of Protexin. The group that received 5.0 g Protexin/Litter had higher response than the other groups at 24 hour after injection PHA. These results indicated that the ear thickness increased gradually with the increase in the supplementation levels of Protexin, as an indicator to T-cell lymphocytes activity. This activity apparently is associated with increasing the dose of Protexin. Similar results were reported by Perdigon *et al.*, (1995), Pestka *et al.*, (2001) and Herich *et al.*, (2002).

Table (4): Least squares means (± S.E.) of leukocytes differential counts (%) and N/L ratios of NZW rabbits as affected by Protexin supplementation after two months of treatment.

Treatments	Neutrophil	Lymphocyte	N/L ratio	Monocyte	Basophil	Eosinophil
Control	37.8[b]*	56.0[c]	0.68	3.0[a]	1.6[a]	1.6[a]
1.0 g/Litter	33.4[b]	61.6[b]	0.54	2.8[a]	1.2[a]	1.0[a]
2.5 g/Litter	30.2[c]	64.8[a]	0.47	2.2[a]	1.4[a]	1.4[a]
5.0 g/Litter	34.2[b]	59.8[b]	0.57	2.4[a]	1.2[a]	1.8[a]
S.E.	0.8	0.8	----	0.3	0.4	0.3
Overall means	33.9±0.7	60.6±0.8	0.56	2.6±0.2	1.4±0.2	1.5±0.1

*Means, within trait, between treatments, with different superscripts differ significantly (P≤0.05).

Table (5): Least squares means (± S.E.) of white blood cells (WBCs x10^3 /mm^3) and antibody titers against Newcastle Disease (ND) (Lasota strain) of NZW rabbits as affected by Protexin supplementation after two months of treatment and Phytohemagglutinin (PHA) response (% increase in ear thickness) after injection after 12 and 24 hours post injection.

Treatments	WBCs	Antibody titers against ND	% increase in ear thickness			
			After 12 hours of injection		After 24 hours of injection	
			Injection PHA	Injection saline	Injection PHA	Injection saline
Control	4.5[b]*	1.0[c]	1.82	0.91	4.55	0.91
1.0 g/Litter	5.4[a]	2.2[b]	8.33	2.31	20.4	4.63
2.5 g/Litter	5.7[a]	4.0[a]	12.26	3.77	23.6	4.72
5.0 g/Litter	5.5[a]	4.0[a]	11.38	3.25	27.4	4.72
S.E.	0.1	0.4	--	--	--	--
Overall mean	5.3±0.1	2.8±0.3	--	--	--	--

* Means, within trait, between treatments, with different superscripts differ significantly (P≤0.05).

Conclusion

From the present results, it can be concluded that, using probiotic (Protexin) in drinking water at 2.5 or 5.0 g/Litter level improved hematological and immunological parameters of NZW rabbits.

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دور منشطات النمو علي المقاييس الهيماتولوجية والمناعية في الإرانب النامية

نهي يحيي عبد المجيد[1]، فاتن عبد الفتاح أحمد إبراهيم[2]، أحمد محمد القياتي[2] و عبد المنعم عبد الرحمن صدقي[1]

١- معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية – الدقي

٢- قسم الإنتاج الحيواني – كلية الزراعة – جامعة القاهرة – الجيزه

الهدف من هذة الدراسة تحديد إذا كان إضافة البروبيوتك (البروتكسن) لماء الشرب في مستويات مختلفة ١و٢,٥و٥ جم /لتر لها تأثير نافع علي الصفات الهيماتولوجية والمناعية في الإرانب المفطومة . تم أستخدام ٥٦ أرنبا من نوع نيوزيلندي أبيض عمر ٥ أسابيع ومتوسط وزن ٥٨٠ جم حيث قسمت علي ٤ مجاميع تجريبية . تم أضافة ٣ مستويات من البروتكسن إلي المياه كالتالي (١و٢,٥و٥ جم/لتر للمجاميع ٢و٣و٤ علي الترتيب) و مجموعة مقارنة بدون إضافة. التجربة أستمرت من عمر ٥ حتي ١٣ أسبوع، من فبراير إلي ابريل ٢٠٠٤.

أظهرت النتائج أن أضافة٢,٥ جم من البروتكسن/ لتر ماء تحسن قيم كل من الهيماتوكريت، الهيموجلوبين، البروتين الكلي، الألبيومين والنسبة بين الألبيومين والجلوبيولين وكذلك أنزيمات الكبد والالكالين فوسفاتيز بينما ٥ جم بروتكسن أظهر تحسن في مستوي الجلوبيولين . لم يلاحظ وجود فروق معنوية بين المعاملات في مستوي الكرياتينين وأيضا أظهرت انخفاض في مستوس اليوريا. وكذلك تحسنت جميع الصفات المناعية زادت نسبة الخلايا الليمفاوية وخلايا كرات الدم البيضاء(ليمفوثايتس)بإضافة ٢,٥ جم بروتكسن /لتر وتحسنت المناعة الخلوية والمناعة المصلية ع عند مستوي ٢,٥، ٥جم بروتكسن/لتر ماء.

من هنا يمكن القول أن أضافة البروتكسن في مستوي ٢,٥ جم /لتر ماء أظهر تحسن في المقاييس الهيماتولوجية ومستوي ٥ جم / لتر أظهر تحسن في كفاءة الجهاز المناعي.