

## CLINICOPATHOLOGICAL STUDIES ON BIO-STIMULANT AGENT IN BROILER CHICKENS

Adel M. A. Eisa and Sahar S. Abd El-Hamied  
Animal Health Research Institute Tanta branch Zagazig branch

### ABSTRACT

*Sixty, one day old, commercial Hubbard broiler chickens were equally divided into 3 groups. Chicks of group 1 kept as a control; meanwhile chickens of group 2 and 3 administered a bio-stimulant agent (**Ultra Natural Plus**) with a dose 1ml/5 liters and 2ml/5 liters drinking water respectively from 20 - 25 day of age and a second dose from 35-40 day of age. Blood samples were collected from each group at 33 and 45 day old.*

*The bio-stimulant agent significantly increased body weight and improved feed conversion in both tested group at day 35 and 43. There was also an increase in total leukocytic and lymphocytic count associated with an increase in gamma globulin and total globulin with dose-related manner in both groups at day 35 and 45. No significant changes were recorded in serum gammaglutamyl transferase activity, calcium, phosphorus, creatinine or uric acid levels in all groups during the whole experimental period. These data indicated that, the studied bio-stimulant agent cause dose-related improvement in chicken performance and stimulate their immunity without altering the other tested biochemical parameters.*

### INTRODUCTION

Nowadays, plant extracts are assuming a position of prime importance in poultry industry. Most companies have been advocating the growth-promoting and immunostimulating effects of number of ingredients to improve physical performance. **Ultra Natural Plus** is the trade name of bio-stimulating agents for livestock and poultry. It contains natural ingredients of yucca schidigera extract, seaweed extract and many enzymes. The use of yucca schidigera extract in poultry feed is good alternative to maintain metabolic and environmental ammonia levels within acceptable limits to improve productivity parameters. (Al-Bar et al. 1993)

**Seaweed extract (*Ascophyllum nodosum*)** is a marine Kelp, an excellent source of over sixty minerals that are naturally chelated by the plant, plus twenty two amino acids, twelve vitamins and some polysaccharides. Kelp is an excellent source of protein bound iodine, which is especially important for stimulation of immunity and endocrine functions (Shan et al., 1999 and Turner et al., 2002). Addition of enzymes to poultry ration as beta-glucanase (Ouhida et al, 2000), hemi cellulase (Patel and McGinnis 1985) and phytase (Lan et al., 2002) enables the metabolic pathway to run efficiently and also act as regulator of individual process thus improves performance (Ravindran et al, 1999).

**Our study aimed to study the synergistic effects** of all these components on the performance, immunity and some biochemical parameters of the broiler chicken.

## **MATERIALS & METHODS**

**Drug:-**

**Bio-Stimulating agent (Ultra Natural Plus) \* CODE: PB-20**

**The drug is a product of Ultra Bio-Logics Inc. Rigaud, Quebec (Canada).**

**It was obtained from Biovet Co. Egypt.**

**Proprietary liquid solution of natural plant extracts and enzymes:**

**Ingredients:** Yucca schidigera extract, seaweed of ascophyllum nodosum, beta-glucanase, alpha-amylase, hemi-cellulase, phytase, pectinase, protease

Content and Activity% : Proprietary

Use Rates:

1 ml with 5 liters drinking water

1 ml with 2.5 liters drinking water

The drug was given orally in drinking water.

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## **Clinicological Studies on Bio-Stimulant Agent..**

### **Experimental chickens:-**

Sixty, one day old commercial Hubbard broiler chickens were used. The chicks were reared under standard hygienic conditions and fed on a balanced commercial ration. They were vaccinated against New Castle (Hitchiner at 7 day of age and Lasota at 16 *day*). Gumboro (at 13 day of age).

**These chicks were equally divided into 3 groups.** Chicks of group 1 were kept as a control meanwhile. chickens of group 2 and 3 administered **Ultra Natural Plus** 1 ml/5L and 2 ml/5L drinking water respectively from 20-25 day of age and a second dose from 35-40 day of age.

### **Growth performance:-**

**Chicks of all groups were weighted individually before the study** and at 35 and 45 day. Feed consumption per chick and feed conversion were also calculated at 35 and 45 day of age.

### **Blood sampling:-**

**Two blood samples were collected from each bird** via hem puncture. The first blood sample (0,5 ml) was taken on sodium salt of EDTA and used for leukogram studies. The second blood sample (5 ml) was taken without anticoagulant in clean and dry centrifuge tubes left to clot at room temperature and then centrifuged at 3000 r.p.m for 15 minutes. The separated sera were subjected to biochemical analysis.

### **Leukogram studies:-**

**Leukocytic counts were performed using an improved** Neubaur Haemocytometer and Natt & Herick solution, For differential leukocytic count, blood films were made on clean slides, dried on air, fixed with absolute methyl alcohol and stained with Giemsa stain. The percentage and absolute value for each type of white cells were calculated according to *Schalm (2000)*.

Table(4):some biochemical parameters ( mean values  $\pm$  S.E and % diff) in chickens administered Ultra Natural Plus 1mL/5L and 2mL/5L drinking water respectively from 20-25 day of age and a second dose from 35-40 day of age compared with control.

Time of sampling	Groups		GGT $\mu$ L	Calcium mg/dl	Phosphorus mg/dl	Uric Acid mg/dl	Creatinine mg/dl
35 day	Group1 (control)	Mean	51.30	9.35	4.89	5.39	1.15
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		S.E	0.67	0.340	0.08	0.16	0.05
	Group2	Mean	52.08	9.81	5.01	5.01	1.12
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		S.E	0.70	0.200	0.27	0.06	0.03
		%diff	1.50%	5.00%	2.30%	-7.10%	-2.90%
	Group3	Mean	51.34	9.74	4.98	4.89	1.08
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
S.E		0.95	0.22	0.07	0.16	0.04	
	%diff	0.07%	4.3%	1.8%	-9.3%	-6.8%	
45 day	Group1 (control)	Mean	52.74	10.12	4.71	5.82	1.18
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		S.E	0.92	0.080	0.06	0.1	0.04
	Group2	Mean	50.44	10.03	4.89	5.52	1.17
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		S.E	0.46	0.400	0.06	0.1	0.05
		%diff	-4.40%	-0.90%	3.90%	-5.20%	-0.50%
	Group3	Mean	50.30	10.4	4.87	5.4	1.12
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
S.E		0.65	0.300	0.17	0.05	0.03	
	%diff	-4.60%	2.60%	3.50%	-7.20%	-5.60%	

Table (3): proteinogram (mean values  $\pm$  S.E and % diff) in chickens administered Ultra Natural Plus 1mL/5L and 2mL/5L drinking water respectively from 20-25 day of age and a second dose from 35-40 day of age compared with control.

Time of sampling	Groups		Total protein gm/dl	Albumin gm/dl	Alpha globulin gm/dl	Beta globulin gm/dl	Gamma globulin gm/dl	Total globulin gm/dl
35 day	Group1 (control)	Mean	3.77	1.81	0.52	0.67	0.77	1.96
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	
		S.E	0.09	0.070	0.02	0.02	0.03	0.05
	Group2	Mean	3.72	1.54	0.5	0.74	0.94*	2.18*
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		S.E	0.16	0.150	0.03	0.03	0.07	0.06
		%diff	-1.70%	-15.00%	-4.90%	10.40%	23.00%	10.40%
	Group3	Mean	3.76	1.54	0.53	0.79*	0.96**	2.22*
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
S.E		0.07	0.110	0.03	0.04	0.05	0.07	
	%diff	-0.70%	-15.00%	1.15%	18.00%	26.00%	13.30%	
45 day	Group1 (control)	Mean	3.81	1.87	0.51	0.7	0.73	1.94
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	
		S.E	0.09	0.040	0.02	0.04	0.04	0.06
	Group2	Mean	3.84	1.79	0.49	0.69	0.87*	2.05
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		S.E	0.04	0.030	0.01	0.02	0.04	0.04
		%diff	0.70%	-4.20%	-4.70%	-1.90%	19.40%	5.30%
	Group3	Mean	3.94	1.78	0.53	0.7	0.93**	2.16*
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
S.E		0.05	0.070	0.02	0.02	0.03	0.03	
	%diff	3.30%	-4.80%	2.70%	0.00%	27.30%	11.00%	

\* Significant at P < 0.05

\*\* Highly significant at P < 0.01

Table(2):leukogram (mean values ± S.E and % diff) in chickens administered Ultra Natural Plus 1mL/5L and 2mL/5L drinking water respectively from 20-25 day of age and a second dose from 35-40 day of age compared with control.

Time of sampling	Groups		T.L.C Cell × 10 <sup>7</sup> / μl	Absolute differential count × 10 <sup>7</sup> cell / μl				
				Lymphocytes	Heterophils	Monocytes	Eosinophils	
35 day	Group1 (control)	Mean	23.81	14.76	6.09	2.08	0.88	
		±	±	±	±	±	±	
		S.E	0.16	0.190	0.05	0.03	0.02	
	Group2	Mean	25.17**	15.74**	6.33	2.18	0.92	
		±	±	±	±	±	±	
		S.E	0.33	0.160	0.16	0.05	0.02	
			%diff	5.50%	6.60%	3.90%	4.60%	4.90%
	Group3	Mean	25.57***	16.27***	6.23	2.16	0.91	
		±	±	±	±	±	±	
S.E		0.22	0.120	0.09	0.06	0.02		
		%diff	7.30%	10.20%	2.20%	3.60%	2.70%	
45 day	Group1 (control)	Mean	23.53	13.99	6.35	2.29	0.9	
		±	±	±	±	±	±	
		S.E	0.19	0.220	0.13	0.09	0.03	
	Group2	Mean	25.0**	15.8***	6.22	2.07	0.91	
		±	±	±	±	±	±	
		S.E	0.25	0.470	0.08	0.03	0.01	
			%diff	6.30%	12.90%	1.90%	-10.20%	1.33%
	Group3	Mean	25.95***	16.1***	6.58	2.36	0.91	
		±	±	±	±	±	±	
S.E		0.26	0.26	0.11	0.1	0.03		
		%diff	10.30%	15.10%	3.80%	2.90%	1.33%	

\*\* Highly significant at P < 0.01

\*\*\* Very highly significant at P < 0.001

Clinical Pathological Studies On Bio-Stimulant Agent...

Kafir Et-Sheikh Vet.Med.J. Vol. 1 No.1 (2003)

Table (1):Growth performance (mean values ±SE and % diff) in chickens administered Ultra Natural Plus 1mL/5L and 2mL/5L drinking water from 20-25 day and a second dose from 35-40 day of age compared with control.

Time of sampling	Groups		Body weight Kg	Feed consumption Gm/ bird	Feed conversion
35 day	Group1 (control)	Mean	1.304	3500	2.60
		±	±		
		SE	0.020		
	Group2	Mean	1.424***	3400	2.32
		±	±		
		SE	0.010		
		% diff	10.60%	-2.90%	-10.80%
Group3	Mean	1.488***	3350	2.28	
	±	±			
	SE	0.020			
		% diff	14.10%	-4.30%	-12.30%
45 day	Group1 (control)	Mean	1.586	4400	2.77
		±	±		
		SE	0.010		
	Group2	Mean	1.796***	3900	2.17
		±	±		
		SE	0.020		
		% diff	13.20%	-11.40%	-21.70%
Group3	Mean	1.796***	4000	2.22	
	±	±			
	SE	0.020			
		% diff	13.40%	-9.10%	19.90%

\*\*\* Very highly significant at P < 0.001

Abdel M.A. Elwa & Sohair S. Abdel El-Hamid

Kafir Et-Sheikh Vet.Med.J. Vol. 1 No.1 (2003)

### **Proteinogram:-**

Total protein was measured according to *Peters (1968)*. Electrophoretic analysis was carried out for determination of serum albumin, alpha, beta, and gamma globulins according to the technique described by *Davis (1964)*.

### **Clinico-biochemical studies:-**

Gammaglutamyl transferase (GGT) activity was measured according to *Szaz (1969)*. Serum uric acid was measured according to *Fossati (1980)*, Serum creatinine levels were measured according to *Henry (1979)*, Serum calcium was measured according to *Gindler and King (1972)*. Serum inorganic phosphorus was measured according to *Goldenberg (1966)*.

### **Statistical analysis:-**

The data obtained from this investigation were statistically analyzed by student "t" test according to *Snedecor and Cochran (1967)*.

## **RESULTS**

### **Growth performance finding:-**

Table (1) showed that both groups administered **Ultra Natural Plus** with doses of 1 ml and 2 ml / 5 liters drinking water, revealed a significant increase in body weight by 10.6% and 14.1% respectively at day 35 and by 13.2% and 13.4% respectively at day 45. Also there was a decrease in feed consumption in both groups along the entire period of the experiment which was more marked in group 3. The food conversion rate was improved at day 35 and 45 in both groups when compared with control.

### **Leukogram findings:**

**It has been observed from table (2) that Ultra Natural Plus** elicited a significant dose-related increase in total leukocyte and lymphocytic count at 35 and 45 day of age in both groups.

### **Proteinogram findings:**

Group 2 revealed a significant increase in gamma globulin levels at 35 and 45 day with a significant increase in total globulin at 35 day as shown in table (3). Group 3 recorded a significant increase in total gamma globulin and total globulin at 35 and 45 day of age, This was accompanied with a significant increase in total beta globulin at 35 day,

### **Biochemical findings:**

The serum gammaglutamyl transferase activity (GGT), calcium, phosphorus, creatinine and uric acid levels revealed non-significant changes in all groups during the experimental period as noticed in table (4).

## DISCUSSION

**Results of the experiment revealed an increase in the body weight** and an improvement in feed conversion in both groups administered **Ultra Natural Plus** with dose of 1ml/5L and 2ml/5L drinking wafer. Similar results were also recorded by *Pettersson and Amart (1989)*, *A.I-Bar et al., (1993)*.

**This increase was attributed to the natural components of Ultra Natural Plus**, which provides *and* synergistic effect between each other.

**Ultra Natural Plus contains extract from Yucca schidigera**, which have been used in animal feed industry to promote digestion and improve production. Yucca extract in poultry feeding can dramatically increase the performance of stock (*Al-Bar et al., 1993*).

**Enzymes in Ultra Natural Plus assist** digestive process thus improving feed conversion rate and promote growth, Our results accords with those recorded by *Patel and McGinnis (1985)* alter addition of hemi-cellulase enzyme to laying hen.

**Also, Petterssoit and Ama (1989) recorded an increase in body weight** and improved feed conversion in chicken fed on diet contains enzymes preparation (mainly beta-glucanase). They noticed that enzymes supplementation increased the digestibility of organic matter and crude protein.

**The growth-simulating effect of enzymes** was explained by *Sebastian efal., (1997)* who reported that phytase enzyme supplementation in broiler chickens diet aid in digestibility by making nutrient more available to chicks therefore improving feed conversion and promoting growth. In another investigation , *Ravindran et al, (1999)*.obtained the same results in broiler fed a diet supplemented with phytase and glucanase enzymes. They stated that glucanase might release extra nutrient from feed. About two third of the total phosphorus in cereal grains exist in phytate bound form.

**The availability of phytate phosphorus from plant** derived feedstuffs is low in chickens because of low or no phytase activity in digestive tract (*Common 1989*). Phytate can also bind with protein to form phytate protein complex that is less soluble resulting in decreased protein digestibility.

**Phytate has also been found to inhibit trypsin and pepsin** activities (*Singh and kirkorian, 1982*). So phytase supplementation improve phosphorus utilization and have positive effect on bioavailability of other minerals as calcium, zinc arid copper in chickens and subsequently resulted in good performance (*Lan et al 2002*) Regarding the leukogram findings, our result revealed significant increase in total leukocytic and lymphocytic count in both groups at 35 and 45 day of age. This result associated with significant increase in beta, gamma and total globulin, which were more pronounced in group 3.

**This results may be attributed to the immuno-stimulating** effects of seaweed extract (*Ascophyllum nodosum*) which markedly stimulate lymphocytes proliferation, enhance induction of T-lymphocytes and stimulate immunoglobulins production by B-cells (*Shan et al, 1999 and Turner et al, 2002*).

**The biochemical results in our study revealed** non-significant change in serum gammaglutamyl transferase activity, calcium, phosphorus, creatinine and uric acid levels in all groups. Such findings were also reported by *Coen et al, (1995)* who recorded no adverse effects when broiler chicks were fed dietary concentrations of beta-glucanase enzyme up to eight times the daily recommended dose. Also *Lan et al (2002)* detected non-significant change in blood calcium levels in broiler chicken supplemented with phytase - producing bacterial culture.

## CONCLUSION

It could be concluded that the Bio-stimulating agent used in this investigation (***Ultra Natural Plus Code PB-20***) elicited significant increase in body weight, improved feed conversion and stimulate the immunity with a dose related manner without any harmful alteration in the biochemical parameters of chickens.

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## CLINICOPATHOLOGICAL STUDIES ON BIO-STIMULANT AGENT IN BROILER CHICKENS

Kafr El-Sheikh Vet. Med. J. Vol.1 No.1 (2003)

Adel M. A. Eisa and Sahar S. Abd El-Hamied  
Animal Health Research Institute Tanta branch Zagazig branch

Manufacturer: Ultra Bio-Logics Inc.  
24 Seguin Street, Rigaud Quebec Canada J0P 1P0  
International Tel. 450-451-6277 Fax: 450-451-6167  
Internet: [www.ublcorp.com](http://www.ublcorp.com) Email: <http://www.ublcorp.com/message.html>