

## Metabolic Role of Non-Specific Immunestimulant (Inmunair) in Advanced Healthy of Broilers

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### Abstract

The objective of the present study was to evaluate the biochemical effect of Inmunair 17.5<sup>®</sup> administration on broilers. One hundred one day old Cobb broiler chicks were divided into 2 groups : control group (No treatment) and immunair group (Inmunair 17.5<sup>®</sup> treated :1.5ml/L to drinking water for 24 hours at first 5 days of life 1 day before vaccination and 2 days after vaccination). Blood samples were collected at 7,21 and 31 days of life. Body weight was registered at 10 and 34 days of life. Results revealed that immunair caused no significance on serum (TP, Alb, AST, ALP, Ca, Cholesterol, HDL, LDL, CRP, IL6) but caused significant decrease in Na all over experimental period, significant decrease in cortisol at 7 and 21 days of life, significant increase in IL2 at 31 days of life and high significant increase in immunoglobulins IgG and IgM all over the experimental period. The increase in body weight wasn't significant. In conclusion the obtained result show that immunair 17.5 can be used as a safe immunostimulant that can reduce stress after vaccination and cause antibody response in broilers.

**Keywords:** Immunestimulant; Inmunair; Lipid Profile; Liver Function

### Introduction

Immunostimulants are products that induce non antigen enhancement of innate or adaptative immunity [1] and tend to stimulate immunity with minimal or no tissue damage [2]. In this way, the administration of non-specific immunostimulants has been used in horses [1,3,4] for treatment of respiratory diseases and at length as adjuvant to veterinary vaccines [2]. The mechanism of action of non-specific immunostimulant is the activation of macrophages which subsequently activate terminal immune response such as antigen uptake, cytotoxicity, phagocytosis, cytokine release and antibody production [3-5].

Inmunair 17.5<sup>®</sup> (Propionibacterium acnes, and *E. coli* lipopolysaccharides) is one of the commercial products available in the Egyptian market as nonspecific immunostimulant for the chicken farms. Propionibacterium acnes, formerly known as Corynebacterium parvum, is a non-spore Gram positive bacteria and is consid-

ered an opportunistic pathogens [6]. *P. acnes* is a potent stimulator of the reticuloendothelial system (RES) [5,7,8] that enhanced both macrophage and lymphocyte function, natural killer cytotoxicity and cytokine production in laboratory animals. It has been recognised that *P. acnes* contribute the induction of pro-inflammatory cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-8 and TNF- $\alpha$  [6] and increase IL- 10 in mice infected with street rabies virus [8]. LPS is a large molecule consisting of a lipid component and a polysaccharide that contains repetitive glycan polymers called O-antigens. The polysaccharide component elicits a strong immune response in animals, including the production of cytokines by phagocytes and complement activation [9]. LPS can also cause changes in the physical condition of an animal, including piloerection, inactivity, loss of thermoregulation and hypotension in a condition known as endotoxic shock [9,10].

LPS has been investigated as an immunostimulant used at low doses. In fish, it has been shown to increase the phagocytic activity

of innate immune cells, and in addition, to increase the levels of lymphocytes, which are adaptive immune cells [11].

## Materials and Methods

### Birds

One hundred one day old Cobb broiler chicks were commercially purchased from El-Nile Company for poultry and ration. All chicks were housed in 2 separate ventilated rooms and maintained on a 24-h constant-light program and adjusted temperature.

### Inmunair 17.5®

Oral solution Inmunair 17.5® (LABORATORIOS CALIER, Spain for ATCO Pharma Co, Egypt) which consists of (inactivated cells of *Propionibacterium acnes* 0.17 mg, lipopolysaccharide from *E. coli* 0.05 mg, Thiomersal, 0.10 mg and Excipient q.s 1 ml).

### Experimental design

The chicks were allocated into 2 groups (50 birds for each); group (1) kept as control group (no treatment), group (2) supplemented with immunair 17.5® (1.5 ml/L to drinking water for 24 hours at first 5 days of life, 1 day before vaccination and 2 days after vaccination).

### Vaccination programme

The birds of the two groups were vaccinated against Newcastle disease at 7 days of age (HB1, Pfizer), lasota at 19 and 29 days of age and Gumboro disease (IBD) at 15 days of age.

### Serum collection

Blood samples were collected from two groups at 7, 21 and 31 days of age in a tube without anticoagulant for serum collection. Serum was collected after centrifugation at 3000 r.p.m for 15 minute and stored at -20°C until use.

### Body weight

The live body weight was determined by weighting chicks of the two groups after 10 days and 34 days of the experiment.

### Estimation of serum biochemical parameters

Liver function tests were determined by a colorimetric methods as described using commercial kits as Aspartate amino-transferase [12], Albumin [13], Alkaline phosphates [14] and Total protein [15]. Lipid profile test was determined calorimetrically that total

cholesterol and triacylglycerols were determined according to [16], HDL-cholesterol [17], LDL-cholesterol [18] and VLDL-cholesterol [19]. Serum CRP concentration was determined according to the method described by [15]. Interleukin 2, interleukin 6, immunoglobulin IgG and immunoglobulin IgM were determined by ELISA kits.

### Statistical analysis

The results were expressed as mean  $\pm$  SE (S.E = Standard error) using student's T- test according to [20]. Values were considered statistically significant when  $p < 0.05$ , 0.01 and 0.001.

## Results

### Effect of immunair 17.5® on some blood parameters

The obtained data in table 1 showed the effect of immunair 17.5 on some blood parameters:

- There were no significance in liver functions (AST, ALP), albumin and total protein between the two groups.
- There was significant decrease in Na concentration in immunair group all over the experimental period but there was no significance in Ca concentration between the two groups.
- There was no significance in cholesterol, HDL and LDL concentrations but there was significant increase in triglycerides and VLDL concentrations in immunair group at 31 days of age only.
- There was significant decrease in cortisol concentration in immunair group in 7 and 21 days of age but no significance at 31 days of age.
- There were no significance in IL6 and CRP concentrations but there was significant increase in IL2 concentration in immunair group at 31 days of age only.
- There was high significant increase in IgG concentration at 7 and 31 days of age, very high significant increase at 21 days of age, high significant increase in IgM at 7 and 21 days of age and high significant increase at 31 days of age (Table 1,2).

### Body weight

There was non significant increase in immunair group at 10 days of age (5%) and 34 days of age (8%).

Parameters	7 Days		21 Days		31 Days	
	Control group	Inmunair group	Control group	Inmunair group	Control group	Inmunair group
Total protein	6.95 ± 0.28	7.40 ± 0.21	7.54 ± 0.30	8.33 ± 0.59	7.68 ± 0.31	8.49 ± 0.60
Albumin	3.38 ± 0.15	3.51 ± 0.15	3.67 ± 0.16	3.69 ± 0.29	3.74 ± 0.16	3.76 ± 0.29
AST	82.13 ± 5.27	88.67 ± 3.89	89.05 ± 5.71	101.05 ± 6.83	90.78 ± 5.82	103.01 ± 6.96
ALP	25.02 ± 2.59	31.03 ± 2.76	27.12 ± 2.81	25.75 ± 0.59	27.65 ± 2.86	26.25 ± 0.61
Na	136.87 ± 3.91	116.53 ± 2.47 *	148.43 ± 4.27	127.15 ± 3.97 *	151.28 ± 4.32	129.62 ± 4.05 *
Ca	8.35 ± 1.05	8.43 ± 1.10	9.05 ± 1.14	8.19 ± 1.43	9.23 ± 1.16	8.35 ± 1.46
Cholesterol	58.65 ± 3.71	50.04 ± 4.16	63.59 ± 4.02	54.25 ± 4.51	64.82 ± 4.10	72.06 ± 5.86
Triglycerides	55.91 ± 5.03	58.94 ± 3.51	60.62 ± 5.46	63.90 ± 3.81	61.80 ± 5.56	83.65 ± 3.65 *
VLDL	11.18 ± 1.01	11.97 ± 0.57	12.12 ± 1.09	12.98 ± 0.62	12.35 ± 1.11	16.73 ± 0.73 *
HDL	22.10 ± 1.25	15.02 ± 2.87	23.96 ± 1.35	19.52 ± 1.62	24.42 ± 1.38	26.28 ± 2.63
LDL	25.37 ± 3.80	20.04 ± 3.58	27.51 ± 4.12	21.73 ± 3.89	28.04 ± 4.20	29.05 ± 8.95
CRP	3.97 ± 0.34	3.41 ± 0.47	4.31 ± 0.37	3.70 ± 0.51	4.39 ± 0.38	4.14 ± 0.26
Cortisol	6.90 ± 0.46	4.65 ± 0.48 *	7.48 ± 0.49	5.04 ± 0.52 *	7.62 ± 0.50	7.85 ± 1.08
IL-2	0.37 ± 0.03	0.45 ± 0.04	0.40 ± 0.03	0.49 ± 0.05	0.41 ± 0.03	0.62 ± 0.05 *
IL-6	5.06 ± 0.78	5.48 ± 0.54	5.49 ± 0.85	5.94 ± 0.58	5.60 ± 0.86	6.93 ± 0.34
IgG	181.69 ± 4.65	232.33 ± 2.05**	167.97 ± 2.47	261.18 ± 3.88***	177.87 ± 5.74	275.26 ± 17.17**
IgM	28.53 ± 2.12	44.92 ± 1.79**	26.80 ± 3.42	75.31 ± 8.57**	36.17 ± 2.17	85.46 ± 3.29***

**Table 1:** Effect of immunair 17.5<sup>®</sup> on some blood parameters after 7,21 and 31 days of age.

Data are presented as (Mean ± S.E). S.E = Standard error:\* Represents statistical Significant at P < 0.05, \*\* Represents statistical Significant at P < 0.01 and \*\*\* Represents statistical Significant at P < 0.001.

Groups	Experimental Period	
	10 Days	34 Days
Control group	466.67 ± 16.67	2000.00 ± 115.47
Inmunair group	493.33 ± 6.67	2166.67 ± 202.76

**Table 2:** Effect of immunair 17.5<sup>®</sup> on body weights after 10 and 34 days of age.

Data are presented as (Mean ± S.E). S.E = Standard error.

## Discussion

It is a significant to get a good immune response to prevent diseases in poultry with minimal or no damage. So, the use of immunostimulants is a resolution to enhance bird's resistance to infectious diseases. Our results revealed that there was no significance in liver functions between the two groups. These results are in agreement with [21] who investigated that oral administration of killed *P. acnes* (0.17mg/ml) has no significance on albumin and total protein in goat kids and [22] who demonstrated that there was

no significance in values of TP, albumin and AST after two intravenous administrations of (1 µg/kg bw) of *E. coli* LPS at 6 h interval in adult rabbits. He also demonstrated that there was significant increase in CRP and IL6 for 24 h after injection. It is known that increasing IL6 induces fever [23] and this doesn't agree with this study may be because we used LPS by oral route and with small concentration.

There was significant increase in IL2 at 31 days of experiment only. This is in agreement with [24] who showed that there was non significance in IL2 in pigs injected with LPS (1.5 µg kg<sup>-1</sup> IV).

This study revealed that there was significant decrease in cortisol at 7 and 21 days of age in immunair group that is in agreement with [25] who studied the effect of IV administration of immunostimulant containing *P. acnes* on weaned foals and found that cortisol as marker of stress decreased significantly with *P. acnes* administration.

There was significant increase in TG and VLDL at 31 days of age only but non significance in cholesterol, HDL and LDL (harmful cholesterol) all over experimental period so the risk factor for heart disease is very low.

There was significant decrease in Na concentration in immunair group allover experimental period but this decrease is in the normal value in the chicken which is that is, 122 - 160 mmol/l in chickens [26]. Which means that no disturbance of acid-base balance occurred.

There was high significant increase in immunoglobulins IgG and IgM allover the experimental period this is in agreement with [27] who studied the effect administration of immunair 17.5 on chickens infected with *Mycoplasma gallisepticum* and treated with tetracycline which give higher specific IgG and IgM values in immunair group. This also is with agreement with [28] who showed that injections of *E. coli* LPS (500 ng/kg of body weight) at 10 weeks of age in calves give a significant increase in total plasma IgG. However, such change was not observed in total serum IgM.

The increase in body weight was not high 5% at 10 days of age and 8% at 34 days of age. This is in agreement with [29] who found that the increase in body weight was low in broilers supplemented with immunair 17.5 1.5ml/L to drinking water for 24 hours at the first three days of every week. This is not in agreement with [30] who found that there was significant increase (14.3%) in body weight in New Zealand white rabbits at 13 weeks of age which received 1m/1liter in drinking water for three days at five weeks of age.

## Conclusion

The obtained result show that immunair 17.5 can be used as a safe immunostimulant that can reduce stress after vaccination and cause antibody response in broilers.

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