Functional-Food Supplementation and Health of Broilers

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Abstract: This study was conducted to evaluate the effect of a probiotic mixture and chromium as food supplementation on broiler chicks' performance. The experiment was conducted, to determine the effect of a probiotic mixture (BiovetYC) and chromium chloride supplementation on growth performance, carcass traits and immune response against Avian Influenza virus from 0- to 42-d-old broiler chicks as well as to determine the anti-stress effect of the dietary probiotic mixture and chromium chloride supplementation on broiler chicks (0-42days old) when subjected to high stocking density (15 birds/m^2 in open-system) as a stress factor. Growth performance, carcass traits and Avian Influenza immune response were recorded. At 42-d of age, 50 birds were randomly selected from each group for blood samples collection and slaughtered for carcass traits. Stress indicators in blood (cortisol and L/H ratio) were measured. The current results revealed: (1) The activation effect of the probiotic mixture on growth performance (2) Chromium chloride supplementation improves growth performance, carcass traits, and immune response and had a strong anti-stress effect. [Nature and Science 2010;8(5):181-189]. (ISSN: 1545-0740).

Keywords: Functional food; Performance; Immune response.

1. Introduction

The microbial populations in the gastrointestinal tracts of poultry play a key role in normal digestive processes and in maintaining animal health. In Greek probiotic means "for life" and can be defined as a live microbial feed supplement, which beneficially affect the host animal by improving its intestinal balance (Huang *et al.*, 2004). The inclusion of probiotics in foods is designed to encourage certain strains of bacteria in the gut at the expense of less desirable ones. The use of probiotics, yeast cultures and acidifiers in poultry feeds generated because of increased public awareness and objection to the use antibiotics as growth promotant feed additive.

The combine use of *lactobacillus* and yeast cultures in the feed and water has been shown to be effective in reducing morbidity and mortality and improving growth performance and production. (Choudhari, *et al*; 2008). Live yeast culture (*S. cerevisiae*) plus lactic acid producing bacteria (*L. acidophilus* and *S. faecium*) was supplemented in broiler (1 kg/ton) and the results showed improved weight gain and feed conversion. With laying hens *lactobacilli* resulted in an improvement in egg production and feed efficiency (Mohan *et al*., 1996). In commercial broilers the inclusion of *L.sporogens* @100 mg/kg feed resulted into increased body weight gain, improved FCR and humoral immune response in broiler chicks during 0-6 weeks of age (Panda *et al*., 2005). Over the last several years considerable attention has been given. The mechanism of action of probiotics had not been fully explained although there are several hypotheses. The health-promoting effect of probiotics in the gastrointestinal tract had been mainly associated with their capacity to stimulate the immune response and to inhibit the growth of pathogenic bacteria. (Barnes *et al.*, 1972 and Gérard *et al.*, 2008), as well as to modulate the immune markers (Dekker *et al.*, 2007). *Lactobacillus* administration had been shown to enhance nutrient absorption, improve growth rates and feed conversion in broiler chickens (Kalavathy *et al.*, 2003).

On the other hand, with the success of the Human Genome Project and the advances in molecular biology, a new discipline, namely nutrigenomics, in the field of nutrition research has emerged (Kaput *et al.*, 2007). The goal of nutrigenomics -- short for nutritional genomics -- is to develop foods and feeds that can be matched to genotypes of animals to benefit health and enhance normal physiological processes. Chromium has been demonstrated to enhance the expression of plasmalemmal calcium-ATPase in smooth muscle cells; it is possible that chromium may influence calcium homeostasis by increasing the calcium storage capacity through up-regulation of calsequestrin (Moore *et al*., 1998).

The intriguing possibility that supplementation chromium increased longevity and retarded aging by improving immune function and enhancing resistance to infectious diseases is being investigated (Burton *et al.*, 1996). Currently, the predominant hypothesis on the Cr (III) action is the chromodulin-mediated role on the insulin-activated glucose uptake by cells. (Hamilton and Wetterhahn, 1986 and EFSA, 2009). Besides the effects on blood glucose clearance, Cr(III) via insulin action is thought to participate also in the protein metabolism by stimulating the amino acids uptake by cells (Evans and Bowman, 1992).

In poultry, studies using supplementation with Cr-picolinate, Cr-yeast and Cr-nicotinate at doses up to 0.8 mg Cr kg-1feed did not show consistent effects on performance and carcass traits in chickens or turkeys for fattening (Lee *et al*., 2003 and Debski *et al*., 2004). Piva *et al*. (2003), could not show adverse effects in laying hens fed with a basal diet containing 3.4 mg Cr kg^{-1} feed after a five-week supplementation with 24.1 mg Cr kg^{-1} feed from Crchloride,36.3 mg Cr kg-1 feed from yeast and 47.5 mg Cr kg^{-1} feed from aminoniacinate,respectively.

Similarly to cattle, birds under stress conditions tend to show reduced mortality. The only relatively consistent effects of Cr (III) supplements occur in serum lipid profile and reduced egg cholesterol. (Lindemann, 2007).

The aim of current study was to examine the effects of a probiotic mixture as functional-food and a trivalent chromium chloride as nutrigenomics additives on growth performance (feed intake, body weight gain , food conversion rate), carcass traits (dressing %, liver somatic indices), immune response (against AIV) and stress conditions(high stocking density) in broiler chickens.

2 - Materials and Methods

These experiments were conducted from January to March of 2009. A total 2700 of commercial one day-old Hubbard chicks of both sexes were used. All pens contained litter top-dressed with 10 cm of clean wood shavings. The broiler chicks had ad libitum access to feed in mash form and water for the duration of the experiment .The chicks were brooded between 31 and 32°C on wk 1, and the temperature was lowered gradually each week until 24 to 27°C was achieved. The 2-phase feeding program consisted of starter, and finisher diets (Table 1) fed from 0 to 21, and 22 to 42.

The experiment was conducted to determine the effect of a probiotic mixture (Biovet) and chromium chloride supplementation on growth performance, carcass traits and AI immune response of broiler chicks (0-42-d-old) also, to determine the anti-stress effect of the dietary probiotic mixture and chromium chloride supplementation on broiler chicks when subjected to high stocking density (15 birds/m2 in open-system) as a stress factor. The experiment was consisted of 6 treatments x 3 replication, each 150 broiler chicks of both sexes per pen.

Table 1. *Composition of basal diets (%)*

Ingredients	Starter (0 – 21 d)	Finisher (22 – 42 d)
Yellow corn	50.86	58.66
Corn gluten	5.00	3.00
Soybean meal	35.0	30.00
Soy oil	5.80	5.00
Dicalcium phosphate	2.50	2.50
Lime stone	0.13	0.13
Common salt	0.33	0.33
DL-Methionine	0.05	0.05
L-lysine	0.03	0.03
Broiler premix[1]	0.30	0.30
<u>Nutrient Profile:</u>		
ME (kcal/kg)	3184.21	3187.81
Crude protein%	22.82	20.00
C / P ratio	139.5	159.4
Crude fat%	7.3	6.1
Crude fibre%	4.3	4.8
Total ash%	6.1	6.2
Calcium%	0.9	0.9
Non-phytate phosphorus%	0.46	0.48

[1] Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D_3 , 9790 IU; vitamin E, 121 IU; B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60.

On 0-d, chicks were randomly divided into 18 floor pen groups containing 150 birds each. Mortalities were recorded daily, and the weights of the dead birds were recorded weekly to adjust gain, feed consumption (feed intake, and gain: feed) and body weights .At 42-d of age, 50 birds were randomly selected from each group for blood sample collection and slaughtered for evaluation of carcass traits, stress indicators in blood (cortisol and L/H ratio). All chicks were s\c immunized with killed vaccine of Avian Influenza (H5N1) virus at age of 7 days (each chick was given 0.05 mL of vaccine). On day 21, 28, 35 and 42, blood samples were collected from the wing vein of 15 chicks per the two replicates and serum antibody titer against Influenza (H5N1) virus were determined by Haemagglutination Inhibition (HI) test and were expressed as logarithm base 2 (OIE,2005).

Experiment

This experiment was conducted to:

1- Evaluate the effect of a probiotic mixture and chromium chloride supplementation on growth performance, carcass traits and AI immune response of broiler chicks in 0-42-d-old commercial broilers. The supplemented and non-supplemented diets were assigned to be isocaloric and isonitrogenous. All groups were stocked at 10 birds/m², group A consumed non-supplemented diets and served as a negative control group. Group B fed on probiotic mixture supplemented diets and served as a positive control group. Group C fed on chromium chloride supplemented diets with 10 birds/m^2 and served as a positive control group. Probiotic mixture was used in the diet to provide 5 g/kg. Chromium chloride was included in the diet to provide 200 ppm (0.2 g/kg) supplemental.

2- To determine the anti-stress effect of the dietary probiotic mixture (Biovet) and chromium chloride supplementation on broiler chicks in 0- to 42-d-old broilers and stocked at 15 birds/m^2 as a stress factor. Group D consumed non-supplemented diets with 15 birds/m^2 and served as a negative control group. Group E fed on probiotic mixture supplemented diets. Group F fed on chromium chloride supplemented diets with 15 birds/m^2.

Probiotic

A commercially available probiotic mixture (Biovet) was used. Each kg of the probiotic mixture contains a combination of Lactobacillus sporogenes ($75x10^8$ c.f.u.), Lactobacillus acidophilus ($330x10^9$ c.f.u.), Saccharomyces cerevisiae $125x10^9$ c.f.u.), alpha-amylase 5 g and sea weed powder 100g. Biovet is a commercial feed additive used by an inclusion rate of 500 g/tone and marketed by WOCKHARDT Limited, Mumbai, India.

Chromium chloride

Chromium chloride (anhydrous Merck-Germany) was used in the diet in the rate of 200 mg/kg in basal diet (NRC, 1997 and Ahmadi et al., 2004).

Basal diets

Corn-soybean meal based diets (Table 1) as mash form were formulated to meet the nutrient requirements of broilers (NRC, 1994). Feed and water were provided *ad-libitum* during the entire experimental period (42 d).

Growth performance

Feed consumption (FC) throughout the experimental period (6 wks), feed efficiency (FE), and final Live body weight (LBW) as well as body weight gain were determined for each group separately (Feddes et al., 2002).

Immune response

Hemagglutination (HA) and Hemagglutination Inhibition (HI) tests. The recommended method use V-bottomed micro well plastic plates were applied. In which the final volume for both types of HA and HI test was 0.075 ml. The reagents required for these tests are isotonic PBS (0.1 M), pH 7.0-7.2 and RBCs. Positive and negative control antigens and antisera should be run with each test. The test was applied to quantify AIV antibodies in chicken sera according to OIE (2005).

Carcass traits

Carcass yield (dressing %) was obtained by expressing the dressed carcass weight as a percentage of live body weight (Brake *et al.*, 1993). The birds were then processed by removing the head, neck, shanks and feet, and eviscerated by cutting around the vent then carefully removing the viscera.

Haematological analysis

Blood samples were collected from randomly selected 50 birds from each group at the end of the experimental period and the number of heterophils and lymphocytes were determined using the haemocytometer method. Also the plasma cortisol level was determined (Gross and Siegel, 1983)

Statistics analysis

All data were statistically analyzed using SPSS® version 11.0 software for personal computer (2005). Means were compared by the Post-Hoc test.

3- Results

Growth performance

Results in group A,B and C, showed that addition of the probiotic mixture and chromium chloride supplementation improved significantly *(P<0.05)* performance (feed gain and feed conversion), increase immune response and carcass traits in group B and C compared with group A. Broiler chicks in group C recorded highest performance, immune response and carcass traits compared with group B.

The highest performance (growth rate and feed efficiency) noticed in group C post Cr supplementation. Results in group D, F and E (Anti-stress) showed that, the lowest performance parameters and highest feed conversion (*P<0.05*) were recorded in the high stocking density group D, which consumed non supplemented diets. Furthermore, BiovetYC affected positively the chicken performance under the stress of high stocking density in group E and the bird performance parameters recorded in that group were significantly different from those in the control group D.

Immune response

Antibody titers against Influenza virus (H5N1) are shown in Table 4, at age 21 days, antibody titer was significantly affected by supplemental chromium and probiotic mixture (groups C and B respectively) in comparison with group A *($P < 0.05$)*. At age 28 and 35 days, chicks in group C were showed a highly significant improvement of antibody titers against Influenza virus (H5N1) *($P < 0.05$)*.

Carcass traits

Table 5 demonstrated the results of dressing, liver weights and their percentages of live body weights (somatic index). Dressing weights were improved significantly ($P<0.05$) versus group A with chromium and probiotic supplementation in groups B and C. The highest carcass traits ($P<0.05$) were recorded with chromium supplementation in group C. These results confirmed that chromium supplementation had a benefit health effect and enhanced normal physiological processes.

Dressing weights were improved significantly in groups B and C ($P<0.05$) versus group A with chromium and probiotic supplementation. Group E recorded significant difference ($P<0.05$) compared with the control negative group D.The highest carcass traits ($P<0.05$) were recorded in the high stocking density group F, which consumed chromium supplemented diets. These results revealed that the chromium used had strong anti-stress effects and thereby the overcrowded conditions in group F which did not negatively affect carcass traits of broiler chickens.

Stress indicators

Blood Stress indicators in (Table 6) significantly increased ($P<0.05$) in group D, while there was no significant difference between the other groups Conclusively; the present research showed: (1) the activation effect of the probiotic mixture on growth performance and stress (2) Cr^{3+} supplementation improved aspects of growth performance, carcass traits, immune response and has a strong anti-stress effect.

Table 2. *Effect of the dietary supplementation of probiotic mixture and chromium chloride on weekly body weight, feed intake, body weight gain and feed conversion factor (FCR) in broiler chickens (mean±SD)*

Age (d)		Group A	Group B	Group C
	Body weight (g/bird)	138.50±2.7	142.33±3.9	148.8±3.3
7	Feed Intake (g/bird)	127.21±4.5	126.51±6.40	127.14±5.2
	Body weight gain (g/bird)	98.5±1.87	100.34±1.68	100.12±1.82
	FCR	1.29	1.25	1.25
	Body weight (g/bird)	385.87±3.5 ^{a}	404.1±4.8 ^{b}	407.1±4.8 ^{b}
14	Feed Intake (g/bird)	365.4±6.7 ^{a}	374.3±7.50^{a}	374.3±7.50 ^{a}
	Body weight gain (g/bird)	247.37±7.5 ^{a}	263.76±5.13 ^{b}	263.76±5.13 ^{b}
	FCR	1.48	1.41	1.40
	Body weight (g/bird)	720.45±4.65 ^{a}	823.43±6.12 ^{b}	840.42±4.5 ^{b}
21	Feed Intake (g/bird)	557.2±8.84	563.81±2.93	564.90±2.14
	Body weight gain (g/bird)	334.58±2.18^{a}	423.33±4.14 ^{b}	434.84±5.82 ^{b}
	FCR	1.67 ^{a}	1.34 ^{b}	1.31 ^{b}
	Body weight (g/bird)	1095.00±9.84^{a}	1124.50±5.72^{b}	1174.50±7.38 ^{b}
28	Feed Intake (g/bird)	884.60±8.93 ^{a}	718.80±124.24^{b}	867.30±10.65 ^{a}
	Body weight gain (g/bird)	375.0±4.44 ^{a}	305.07±8.50 ^{b}	355.08±13.68 ^{c}
	FCR	2.36	2.39	2.32
	Body weight (g/bird)	1654.20±6.80^{a}	1720.23±10.10 ^{b}	1835.78±6.45 ^{c}
35	Feed Intake (g/bird)	877.10±6.65	864.9±8.82	876.68±10.43
	Body weight gain (g/bird)	559.20±9.76 ^{a}	609.73±12.90^{b}	582.29±4.19 ^{a}
	FCR	1.57	1.45	1.44
	Body weight (g/bird)	2000.5±20.92 ^{a}	2160.45±14.00^{b}	2202.88±24.58^{b}
42	Feed Intake (g/bird)	675.35±13.74^{a}	818.57±14.77^{b}	825.86±23.52^{b}
	Body weight gain (g/bird)	346.30±16.87^{a}	446.27±15.50^{b}	467.07±22.86^{b}
	FCR	1.95 ^{a}	1.85 ^{b}	1.81 ^{b}
	Body weight (g/bird)	2000.5±20.92 ^{a}	2160.45±14.00^{b}	2242.88±25.66 ^{c}
Total	Feed Intake (g/bird)	3486.86±17.89	3449.87±13.37	3492.13±24.08
	Body weight gain (g/bird)	1960.5±8.17 ^{a}	2118.48±16.24^{b}	2287.85±16.34 ^{c}
	FCR	1.78 ^{a}	1.61 ^{b}	1.58 ^{b}

Figures in the same row with different letters are statistically significantly different ($P<0.05$)

Table 3. Effect of the dietary supplementation of probiotic mixture on weekly body weight, feed intake, body weight gain and feed conversion factor (FCR) in broiler chickens under stress conditions (mean±SD)

Age (d)		Group D	Group E	Group F
7	Body weight (g/bird)	139.2±4.1	140.32±3.8	140.32±3.8
	Feed Intake (g/bird)	131.62±6.37	125.50±6.40	125.50±6.40
	Body weight gain (g/bird)	98.2±2.19	99.42±178	100.32±1.68
	FCR	1.32	1.28	1.25
14	Body weight (g/bird)	383.3±5.8 ^{a}	388.2±4.7 ^{a}	402.1±4.7 ^{b}
	Feed Intake (g/bird)	384.5±9.23 ^{a}	366.3±8.60 ^{b}	370.3±7.50 ^{b}
	Body weight gain (g/bird)	248.1±14.32 ^{a}	241.88±5.12 ^{b}	261.78±5.12 ^{b}
	FCR	1.59	1.47	1.41
21	Body weight (g/bird)	710.5±5.23 ^{a}	760.53±8.14 ^{b}	820.43±6.12 ^{c}
	Feed Intake (g/bird)	540.6±8.68	558.80±2.83	558.80±2.83
	Body weight gain (g/bird)	326.2±2.25 ^{a}	350.43±5.14 ^{b}	418.33±4.14 ^{c}
	FCR	1.66 ^{a}	1.64 ^{a}	1.34 ^{b}
28	Body weight (g/bird)	1001.60±10.48 ^{a}	1110.52±5.82 ^{b}	1120.50±5.72 ^{b}
	Feed Intake (g/bird)	887.20±7.22 ^{a}	875.80±124.24 ^{a}	715.80±124.24 ^{b}
	Body weight gain (g/bird)	291.10±17.02 ^{b}	380.17±7.56 ^{b}	300.07±8.50 ^{b}
	FCR	3.05 ^{a}	2.34 ^{b}	2.39 ^{b}
35	Body weight (g/bird)	1561.89±9.99 ^{a}	1710.23±10.10 ^{b}	1710.23±10.10 ^{b}
	Feed Intake (g/bird)	826.55±9.46	854.9±8.82	854.9±8.82
	Body weight gain (g/bird)	561.29±8.51 ^{a}	589.73±12.90 ^{b}	589.73±12.90 ^{b}
	FCR	1.47	1.5	1.45
42	Body weight (g/bird)	1803.35±24.52 ^{a}	2050.25±16.11 ^{b}	2150.45±14.00 ^{b}
	Feed Intake (g/bird)	484.92±13.77 ^{a}	714.76±13.87 ^{b}	814.57±14.77 ^{c}
	Body weight gain (g/bird)	242.46±26.86 ^{a}	350.44±14.60 ^{b}	440.22±14.50 ^{c}
	FCR	2.00 ^{a}	1.9 ^{a}	1.85 ^{b}
Total	Body weight (g/bird)	1804.35±24.52 ^{a}	2000.45±14.00 ^{b}	2150.45±14.00 ^{c}
	Feed Intake (g/bird)	3258.39±18.79 ^{a}	3439.87±13.37 ^{b}	3439.87±13.37 ^{b}
	Body weight gain (g/bird)	1764.35±28.22 ^{a}	2000.46±1 ^{a} 6.26 ^{b}	2110.45±15.24 ^{c}
	FCR	1.86 ^{a}	1.8	1.63 ^{b}

Figures in the same row with different letters are statistically significantly different (*P<0.05*)

Table 4. The immune response of broiler chickens vaccinated by AIV inactivated oil-emulsion vaccines H5N1 Immune response§ (log-2) of post-vaccination

Group	Titers(Log2)				
Group	At age14	At age21 d	At age 28 d	At age35 d	At age42
A	1.8 ±0.22 [a]	2.2±0.42 [a]	3.2±0.52 [a]	3.31±0.72 [a]	2.56±0.44 [a]
B	2.2±0.34 [a]	4.4±0.44 [b]	4.2±0.72 [b]	3.81±0.72 [a]	3.76±0.42 [b]
C	2.3±0.24 [a]	4.4±0.42 [b]	4.8 ±0.52 [b]	6.31±0.92 [b]	5.56±0.48 [b]
D	1.8 ±0.24 [a]	2.4±0.42 [a]	3.2±0.62 [a]	2.8±0.72 [a]	2.26±0.62 [a]
E	2 ±0.24 [a]	3.4±0.42 [b]	3.2±0.52 [a]	3.31±0.94 [a]	2.56±0.42
F	2.2±0. 48 [a]	4.4±0.72 [b]	6.2±0.82 [b]	4.31±0.62 [b]	4.56±0.42 [b]

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All chicks were S\c immunized with 0. 5 mL of killed vaccine of Avian Influenza (H5N1) virus at age of 7 days. On day 21, 28, 35 and 42, blood samples were collected from the wing vein of 15 chicks per the two replicates and serum antibody titres against Influenza (H5N1) virus were determined by Haemagglutination Inhibition (HI) test and were expressed as logarithm base 2. Figures in the same column with different letters are statistically significantly different ($P<0.05$).

Table 5. *Effect of the dietary supplementation of probiotic mixture and chromium chloride on dressing weight percentage, liver weight and percentage (mean±SD)*

Group	Group A	Group B	Group C	Group D	Group E	Group F
Dressing weight (%)	73.31	74.50	76.45	73.80	74.37	75.25
Liver weight (g)	45.8±2.19 [a]	53.5±3.11 [b]	55.8±2.19 [b]	42.95±4.21 [a]	44.5±3.02 [a]	48.5±3.11 [b]
Liver weight (%)	2.31	2.53	2.72	2.39	2.42	2.53

Figures in the same row with different letters are statistically significantly different ($P<0.05$)

Table 6. *Effect of the dietary supplementation of probiotic and chromium chloride on physiological stress indicators (mean±SD)*

Parameter	Group A	Group B	Group C	Group D	Group E	Group F
H/L Ratio	0.39 ± 0.04[a]	0.38 ± 0.05 [a]	0.37 ± 0.06 [a]	0.82 ± 0.09[b]	0.45 ± 0.06[a]	0.41 ± 0.06[a]
Cortisol level (ng/ml)	3.79 ± 0.17 [a]	3.69 ± 0.09 [a]	3.79 ± 0.09 [a]	8 ± 0.12[b]	4.82 ± 0.08[a]	4.12 ± 0.08[a]

Figures in the same row with different letters are statistically significantly different ($P<0.05$).

4-Discussion

The highest growth rate and feed efficiency were noticed in group C post Cr supplementation was confirmed by many researchers (Lien *et al*., 1999; Uyanik *et al*., 2002; Sahin *et al.*, 2002; Amayta *et al.*, 2004; Jackson *et al.*, 2008; Samanta *et al*., 2008).

In addition, Lien *et al*., (1999) had demonstrated that a supplement of 1600 mg/kg of chromium picolinate in the ration influences the growth, carcass, serum traits and lipid metabolism of broilers, while Jackson *et al*., (2008) had used Cr as chromium propionate and recorded improved feed efficiency in the later phases of growth and decreased mortality as well as no effect on carcass traits.

Chromium supplementation in diet had been related to increased protein deposition (Seerley, 1993; Ward *et al*., 1995), with decrease in muscle fat (Ward *et al*., 1995). It also affects body mass and feed conversion ratio (Moore *et al*., 1998 and Hossain *et al.,* 1998). The enhancing effect of Chromium supplementation could attributed to its involvement in stimulating the biological activity of insulin by increasing the insulin-sensitive cell receptors or binding activity (Mertz *et al*.,1974;Lien *et al.*,1999) . Insulin can also stimulate anabolism and inhibit catabolism (Lien *et al.*, 1999). The improved BW, FI, FE, in group A is confirmed by Yeo and Kim (1997).

Moreover, chromium is involved in protein metabolism and may have a role in nucleic acid metabolism (Lukaski, 1999). Its role in stress condition in animals and birds is more appreciated where it alleviate the negative influence of environmental and nutritional impacts.

The observed improvement in immune responses with chromium supplemented to broilers in groups B and C especially at 28 and 35 days old is coincide by results of (Luo *et al*., 1999 and Toghyani *et al.*, 2007).As well previously elevated antibodies titer against Newcastle disease virus in broiler chicks with supplement of 2 or 10 mg\kg chromium either in the form of CrCl3 or yeast (Guo *et al*., 1999) and infectious bronchitis titer in broiler chicks fed on 400 ug\kg Cr picolinate (Lee *et al.*, 2003). Beside Cr supplementation enhance the IFN-c m-RNA expression in response to Newcastle disease vaccine according to (Bahgat *et al.,* (2008).

The highest carcass traits recorded in group F confirmed that the probiotic mixture used had anti-stress effects and thereby the overcrowded conditions in group E did not negatively affect carcass traits of broiler chickens. These data coincided with that findings reported by Yeo and Kim (1997), and may reflect the activation effect of the probiotic mixture on the liver cells which improved the overall growth performance.

The results also, showed that Cr supplementation improved aspects of growth performance, carcass traits, (Steel and Rosebrough, 1981; Hossain *et al.*, 1998; Lein *et al.*, 1999; Uyanik *et al.*,2002;Sahin *et al.*,2002; Amayta *et al.*,2004; Jackson *et al.*,2008;Samanta *et al.*,2008) and immune response (Lou *et al.*,1999; Toghyani *et al.*,2007; Bahgat *et al.*,2008;Kheiri and Toghyani,2009) .The review by the NRC (NRC. 1997) regarding the variability of Cr supplementation agreed with some researchers (Kim, *et al*,1996; Lien *et al.*,1999;Sahin *et al*.,2002 and Kim *et al*.,2002). (Lee *et al*., 2003) reported that CrPic had no effect on growth, but feed efficiency was improved. Also, researchers (Chen et al., 2001; Sahin *et al.*, 2002; Kroliczewska *et al*., 2004) had shown that Cr as CrPic improved growth performance of broilers during heat stress. Similarly, others (Kim *et al.*, 2002) had reported that 1,600 ppb Cr as CrPic improved gain in broilers. However, not all research showed positive effects of Cr as CrPic on growth performance in broilers (Sahin *et al.*, 2002). The use of probiotic in broiler chick's diets significantly improved the daily body weight gain, feed intake and feed efficiency as reported by Yeo and Kim (1997).The obtained results confirmed that the chromium supplement had good anti-stress effects and thereby the overcrowded condition in group F did not negatively affect performance, immune response and carcass traits of broiler chickens. Although, probiotics as feed additive can act as an anti-stress factor and has positive effects on broiler chickens performance as well as carcass traits, chromium chloride supplementation significantly acted as a powerful anti-stress factor and improved performance (decreased mortality rate, feed intake, feed gain, feed conversion, and body weight), immune response and carcass traits as well as its economic nature. Similarly, CrPic and Cr yeast had been reported to decrease mortality in broilers (Kim *et al*., 1996a; Kim *et al*., 1996b; Jackson *et al*., 2008). These results confirmed that the probiotic mixture used had anti-stress effects and thereby the overcrowded conditions in group E which did not negatively affect carcass traits of broiler chickens. These results might reflect the activation effect of the probiotic mixture on the liver cells which improved the overall growth performance .This may be due to the anti-stress effect of: (1) the chromium supplement where, its role in stress condition in animals and birds is more appreciated as it helps to reduce the negative influence of environmental and nutritional stress (Seerley, 1993; Ward *et al.*, 1995), (2) the anti-stress effect (Gross and Siegel, 1983).

5-Conclusion

The present research confirmed: (1) the activation effect of the probiotic mixture on growth performance (feed intake, body weight gain , food conversion rate), carcass traits (dressing %, liver somatic indices), and stress conditions (stocking density) in broiler chickens and stress (2) Cr3+ supplementation improved aspects of growth performance, carcass traits, immune response (against AIV) and has a strong anti-stress effect.

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