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HEMATOLOGICAL AND IMMUNLOGICAL VARIATIONS IN RESPONSE TO SUBSTITUTION OF SOYBEAN MEAL BY COTTON SEED MEAL OR SUNFLOWER MEAL IN BROILER CHICKS DIET

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SUMMARY

The objective of this research was to study hematological and immunological variation in response to plant meal sources. Lohman broiler chicks were used in the study. All chicks received corn soybean meal diet (SBM-diet) during the first 7 days post hatch, thereafter, they were divided into three groups. The first group was fed SBM-diet, the second group was fed corn-cotton seed meal diet (CSM-diet) whereas the third group was fed corn-sunflower bean meal diet (SFM-diet). The hematological parameters that were measured at five weeks of age showed that SBM-diet and SFM-diet groups had significantly higher total red blood cells and hematocrit value than those of CSM-diet group. Also SBM-diet group had significantly higher Hemoglobin concentration than both SFM-diet and CSM-diet group.

Despite the total white blood cells was not different between groups, the humoral antibody response against sheep red blood cells for CSM-diet group was inferior to that in SBM-diet and SFM-diet groups. Also in vitro splenic lymphocyte activity in response to pokwed mitogen was significantly higher in the SFM-diet group as compared to the other groups. On the other hand, no differences were observed between groups in response to concanvalin-A.

Keywords: *broiler chicks, soybean meal, cottonseed meal, sunflower meal, hematology, immune response.*

INTRODUCTION

The cost of feed in today's commercial broiler flocks makes up approximately 70% of total broiler cost. Protein is one of the major cost components of the broiler diet, so poultry nutrition researchers have been looking for the cheapest and beneficial source of protein. Plant meal is one of the major feedstuffs widely used to encounter the protein requirement; but each meal dose not have a completely advantage. For example, soybean meal is commonly used for poultry feeds but it is very expensive, has a lower metabolizable energy, lysine and methionine and more

than half of phosphorus exist as phytate phosphorus (Hirabayashi *et al,* 1998;Leeson and Summers, 1991).

Though cottonseed meal is rich in protein and relatively high in energy, but it has some undesirable characteristics. It contains gossypol, gossypol-likebigments and cyclopropene fatty acid and is deficient in lysine (El-Boushy and Raternik, 1989). Gossypol is a polyphonic compound that is located inside the pigment glands of cotton seeds (Ryan *et al,* 1986; Fernandez *et al,* 1995). The cyclopropene fatty acid is only a concern when CSM still contains a substantial amount of the oil. When the oil is almost totally extracted, the remaining cyclopropene fatty acid in the CSM is negligible (Phelps, 1966). High amount of fibrin CSM yields low available energy for the animal, also lysine can been unavailable because of binding to gossypol (Fernandez *et al,* 1995). Sunflower meal is very cheap, free from toxic components and antinutritional factors which may affect the broiler performance (Gheyasuddin *et al,* 1970), but it has high fiber content (Soliman *et al,* 1996) and quite low in energy, lysine and threonine. Sunflower meal is also quite dark in color and so large additions to diets can result in marked changes in diet color (Lesson and Summers, 1991).

The aim of this study was to investigate the hematological and immunological changes in response to replacing soybean meal by cottonseed meal or sunflower meal.

MATERIALS AND METHODS

This experiment was carried out at the poultry Research Center, Faculty of Agriculture, Cairo, University, Giza, Egypt.

Birds:

One hundred and fifty unsexed one-day-old Lohman broiler chicks were used in this study. Chicks were wing banded, distributed randomly into 3 treatments (50 chicks each), and raised in floor pens. Room temperature was maintained at 33°C during the first week and decreased by 0.5°C each day until the temperature was maintained at 24°C. The birds received 24 hrs of light and fed control corn-Soy diet (SMB-diet) containing 22% CP and 2956 ME Kcal/Kg during the first 7-day post hatching.

Treatments:

Three experimental diets were used in each of the two stages (1-4 and 4-7weeks of age). The SBM-diet was formulated to meet nutrient requirements of chicks and served as a control group (Table 1). In the second group, soybean, was replaced by cotton seed meal (CSM-diet) which used at 15% level in starter, while used at 25% level in finisher ration . The 15-25% CSM contain 330-550 mg free gossypol / kg diet (Hassan *et al.,* 1996) . In the third group, soybean was replaced by sunflower meal (SFM-diet), which used at 15% level in starter, while used at 25% level in finisher ration.

Table 1. Composition of the experimental diets

	Starter diets			Finisher diets		
Ingredient	SBM	CSM	SFM	SBM	CSM	SFM
Yellow corn	55.24	53.93	45.26	69.41	68.61	53.16
Soybean meal 44%	38.82	24.89	31.31	26.95	2.92	14.01
CSM[1]	---	15.0	---	---	25.0	---
SFM[2]	---	---	15.00	---	---	25.00
Limestone	0.17	---	---	0.56	0.69	0.43
Bone meal	2.39	2.64	2.58	1.66	1.61	1.79
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Salt (NaCI)	0.30	0.30	0.30	0.30	0.30	0.30
Vegetable oil	2.64	2.66	5.00	0.82	0.31	4.91
Methionine	0.19	0.21	0.18	0.05	0.08	0.03
Lysine	---	0.12	0.03	---	0.23	0.12
Total	100.00	100.00	100.00	100.00	100.00	100.00
Chemical analysis:						
CP%	22.05	22.10	22.10	18.00	18.00	18.00
ME Kcal/ Kg	2956	2967	2958	3000	3002	3000
EE%	5.05	5.48	7.14	3.67	3.07	7.79
CF%	4.05	4.62	7.33	3.50	5.12	8.94
Ca%	0.90	0.90	0.90	0.80	0.80	0.80
Available P%	0.45	0.44	0.45	0.35	0.35	0.35
Total P%	0.71	0.80	0.79	0.58	0.66	0.70
Methionine	0.54	0.55	0.54	0.36	0.36	0.35
Methionine+Cystin%	0.90	0.90	0.90	0.65	0.65	0.65
Lysine%	1.27	1.20	1.20	0.96	0.90	0.90
C\P ratio	134.1	133.8	133.8	166.7	166.7	166.7

[1]Cotton seed meal (CSM), contained 39.1% CP, 13.3% CF, 5.8% EE, 6.6% ash and 10.2% moisture as determined; and 0.22% free gossypol as reported by supplier.
[2] Sunflower meal (SFM), contained 27.9% CP, 24.5% CF, 1.7 EE, 6.3 ash, and 11.3 moisture as determined.

Hematological study:

At five weeks of age uncoted blood samples were collected from 15 chicks of each group to detect the following hematological parameters:

Total red blood cells (RBC s): Total RBC s was determined by a heamocytometer (Hartman and Lessler, 1963)

*Packed cell volume (PCV):*PCV value was determined using microhematocrit tubes (Hunsaker, 1969).

Hemoglobin concentration (Hb): Hb (g/100ml blood) was determined by the hemoglobinometer (Pilaski, 1972).

Mean corpuscular volume *(MCV),* mean corpuscular hemoglobin *(MCH)* and mean corpascular hemoglobin concentration *(MCHC)* were calculated according to the equation of Thompson and Proctor (1984) as follow:

MCV (micron^3)= PCV/RBC s count

MCH (pictogram (pg))= RBC s count/Hb concentration

MCHC (g/dl) = Hb/PCV

Immune Parameters

Antibody titration: At six week of age, twelve chicks from each treatment were immunized with 0.2 ml of 9% sheep red blood cells (SRBC) suspension in saline to measure primary antibody response. Blood samples were collected at 6 day postimmunization to detect antibody titer using microhemagglutination technique (Trout *et al.*, 1996). Antibody titer values were expressed as log 2 of the highest serum dilution giving total agglutination.

Total white blood cells (WBC's): Total circulating leukocytes counts were measured using cresyl blue stains and haemocytometer.

Lymphocyte proliferation (Mitogenic Responses): At seven weeks of age, chicks from each group were sacrificed by cervical dislocation. Spleens were dissected, trimmed off, fat and connective tissue and expressed through a course mesh screen into RPMI 1640 media. The suspension was layered onto 3 ml of histopaque 1077 and centrifuged at 1000 rpm for 3 min. The white blood cells (upper cell layer) were isolated by centrifugation over Histopaque- 1077 density gradient, and cell viability was assessed by the means of trypan blue dye exclusion. Cells were then enumerated and adjusted to 1 X 10^7 cell/ml in RPMI 1640 media containing penicillin[4] (100 units/ml), streptomycin (100 µg/ml), and 10% fetal bovine serum. Cells were cultured at a concentration of $1X10^6$ cells in 50 µl/well in 96-well culture plates under stimulation triplicate *in vitro* with concanavalin A (Con A) (2.5 pokweed mitogen (PWM) (a dilution of reconstituted stoke), or with medium alone. Cells were incubated for 48 h at 39C in a humidified incubator with 5% CO_2 then pulsed with 50 ul ^{3}H-thymidine (1 µCi,6.7 Ci/mmol) for 18h. Cells were harvested on glass fiber filters by using a Skatron cell harvester the ^{3} H-thymidine incorporation was measured using a liquid scintillation counter.

Statistical analysis:

The data were analyzed using the SAS (1988). General linear models Procedure with a one-way ANOVA model using plant meal as main effect. Means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

As shown in Table 2 both chicks of SBM-diet and SFM diet groups had similar total RBC count and PCV with significantly superior over those of chicks of CSM-diet group. The significantly highest Hb concentration was observed in SBM-diet group, whereas SFM-diet group had intermediate Hb concentration with significantly higher than that of CSM-diet group. On the other hand no significant differences were observed between group in MCHC, MCV, or MCH (Table 2).

Regarding the immunological response. Despite the total WBC count was not differed significantly between groups (Fig. 1), but there activity influenced by diet. e.g., the result of humoral antibody titer which detected against SRBC antigen at 6 days post immunization showed that CSM-diet group harvested the lowest titer as compared to that of SBM-diet or SFM-diet. However, no significant differences were

observed between anti-SRBC antibody titer of SBM-diet or SFM diet group (Figure 2).

Table 2. Hematological parameter in different diet groups

Item	Soybean meal	Cotton seed meal	Sunflower meal
RBC count x10^4	356±34[a]	226.8±34[b]	280±0.34[a]
PCV%	36.0±2-[a]	29±2-[b]	36.3±2[a]
Hb (g/dl)	11.2±0.5[a]	8.5±0.5[c]	10±0.5[b]
MCHC (g/dl)	26.9±2.1[a]	26.8±2.1[a]	25.8±2.1[a]
MCV (micron^3)	181.1±15.3[a]	127±15.3[a]	143±15.3[a]
MCH (picogran)	36.7±6.2[a]	44.5±6.2[a]	43.5±6.2[a]

Values within row with the same letter are not significantly different (P<0.05)

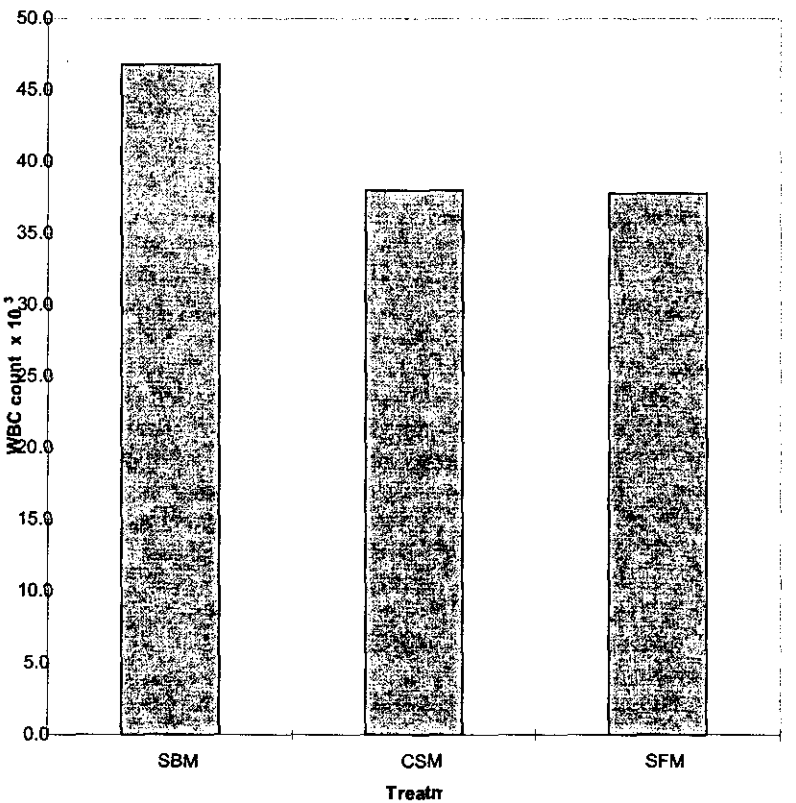

Fig. 1. The influence of protein source on White Blood Cells (WBC) count.

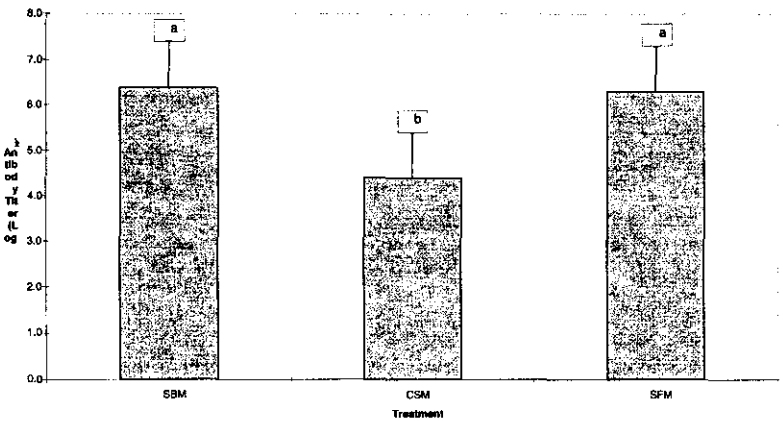

Fig. (2): The influence of protein source on anti-SRBC antibody titer.

The cell-mediated immune response that evaluated by mitogenic activity (lymphocyte proliferation) is shown in (Figure 3&4). ^{3}H-thymidine uptake by splenic lymphocytes which stimulated by PWM were significantly higher in SFM-diet group as compared to CSM-diet or SBM-diet group and no differences between former groups .On the other hand the mitogenic response to Con-A in SFM-diet group was superior followed by that of SBM-diet group and then CSM-diet group but no significant differences were observed between each other.

The results of the present study showed that using CSM-diet at 15-20% level reduced the total RBC count, PCV and Hb concentration. The explanation for this observation is attributed to the presence of gossypol that ranged between 330-550 mg/kg diet which binds iron in the diets and in the blood stream. Thus, it causes numerous problems related to the development of iron deficiency e.g. decrease heamopiosis in bone marrow (Watkins and Waldroup, 1995). Abdel-Latif *et al.*, (1997) confirm this observation and mitigate the toxicfication effect of gossypol by adding soluble iron compounds (ferrous sulfate) and found that ferrous sulfate reduced the deleterious effect of gossypol in PCV.

Host resistance to pathogens is complicated and involves both specific and nonspecific resistant factors. The humoral immune response is the principle specific immunity against extraclleular bacteria, whereas cell-mediated immunity (CMI) plays a major role in the responses against intracellular bacteria and viruses (Abbas *et al.*, 1994). The CMI usually functions earlier than humoral immunity after antigen stimulation. SRBC antigen which used in this experiment is multiantigenic determent and nonpathogenic T-cell dependent antigen. There is a positive association between Anti-SRBC antibody and resistance to some disease like Newcastle and Mark's disease (Gross *et al.*, 1980; Dunnington *et al.*, 1986).

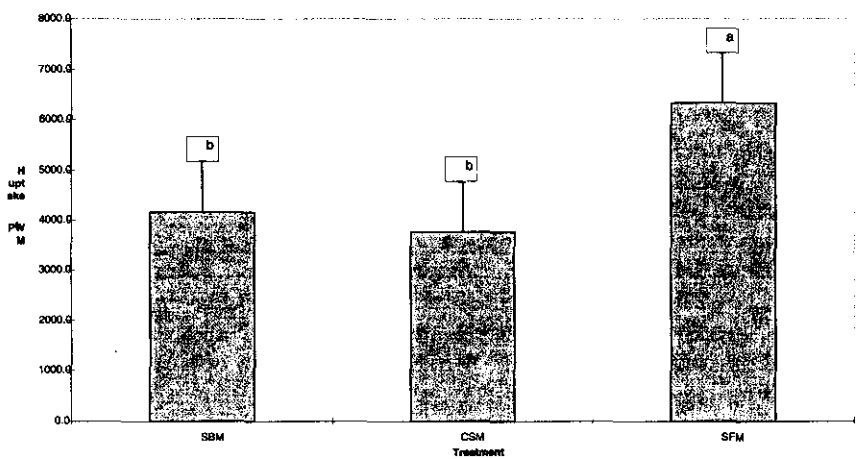

Fig. (3): The influence of protein source on ^{3}H uptake by proliferated lymphocyte in respo to PWM.

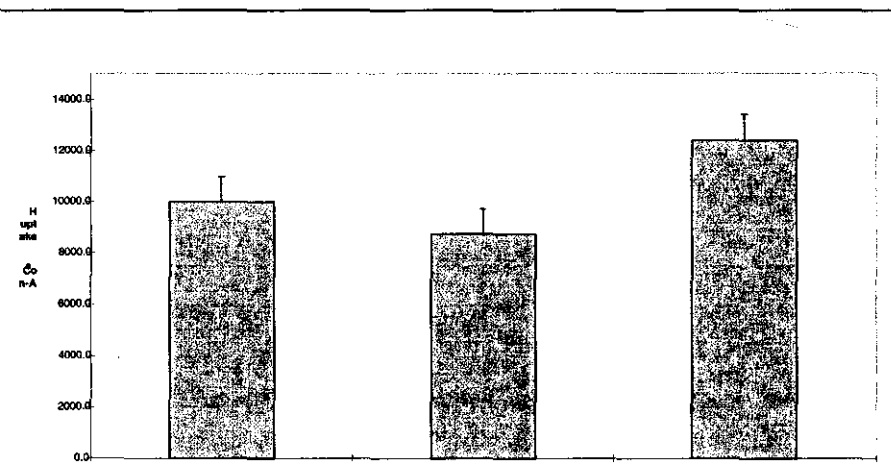

Fig. (4): The influence of protein source on ^{3}H uptake by proliferated lymphocyte in response to Con-A.

The mitogenic response to plant lactins is conventionally used to measure the cell-mediated immunity in mammals and aves. The Con-A and PWM mitogens stimulate T lymphocytes (Toivanen and Toivanen, 1973; Hovi *et al.,* 1978) by indirectly cross-linking T cell receptor complex (Abbas *et al.,* 1994). Con A may possibly involves the stimulation of the lymphocyte population that is mainly involved in CMI, whereas PWM may likely stimulate the lymphocyte population (s) that may be involved in both CMI and humoral immunity (Li *et al.,* 1999). Proliferation splenic lymphocyte is a true measure of cellular immune competence; therefore, they are suitable for monitoring the functional capabilities of immune cells in avian flocks (Lee, 1978; Sharma and Belzer, 1992; Talebi *et al.,*1995).

The current results show that humoral as well as cell mediated (lymphocyte proliferation) after activity by PWM in chicks of SFM-diet group was superior to those of chicks feed CSM-diet. The deleterious immunological effect of CSM may be atributed to gossypol which reduced RBC count and Hb concentration, retardation in oxidative reactions and deficiency in energy level that immunocompetance need. Abdel-Latif *et al.,* (1997) showed that binding between gossypol and iron are affected liver functions which explain as inhibition on plasma GOT and GPT (Transaminases enzymes) biosynthesis. Also the same researchers showed that gossypol reduced blood calcium level. Calcium ion play an important role in the transport of ions across the plasma membrane of lymphocytes during activation (Grinstein and Dixon 1989). Jones (1981) found that gossypol inhibit autocatalytic conversion of pepsinogen to pepsin. While Watkins and Waldroup (1995) showed that gossypol may bind with lysine to form maillard linkage, thus reducing the nutritional value of protein.

The previous researches have shown that mitoginc responses are affected by many other factor rather than nutrition , such as storage time (Raj *et al.,* 1997), the enhancement by erythrocytes in the cultures (Powell, 1980), the suppression by monocytes (Vainio and Ratcliffe, 1984;Schaefer *et al.,* 1985), serum sources and concentrations, incubation temperature, and length assay (Lee, 1974, 1978; Maheswaran *et al.,* 1975; Sharma and Belzer, 1992; Talebi *et al.,* 1995).

In conclusion, the present results explain the deleterious effect of CSM-on hematological and immunlogical parameter. Also, the results showed the benefits of substitution of SBM by SFM in poultry diet, which stimulate lymphocyte proliferation.

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تأثير إحلال كسب القطن وعباد الشمس بدلا من فول الصويا في علائق بدارى التسمين على خواص الدم والمناعة

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الغرض من هذا البحث هو دراسة التغيرات التي تحدث في خواص الدم والاستجابة المناعية تبعا لتغير مصدر البروتين النباتي. ولقد استخدم في هذه الدراسة كتاكيت تسمين لوهمان تم تغذيتها على عليقة نباتية من الذرة وكسب الصويا خلال ٧ أيام الأولى بعد الفقس. بعد ذلك تم تقسيمهم إلى ٣ مجاميع: المجموعة الأولى تم تغذيتها على عليقة فول الصويا (مجموعة المقارنة) والمجموعة الثانية تم تغذيتها على عليقة كسب قطن بينما المجموعة الثالثة : تم تغذيتها على عليقة كسب عباد الشمس .

ولقد أوضحت صفات الدم التي تم تقديرها عمر ٥ أسابيع إن استخدام عليقة فول الصويا وعليقة عباد الشمس أدى إلى زيادة معنوية في عدد كرات الدم الحمراء ونسبة المكونات الخلوية مقارنة بعليقة كسب القطن. أيضا في مجموعة فول الصويا كان مستوى نسبة الهيموجلوبين أعلى بصورة معنوية عن تلك الموجودة في مجموعة كسب عباد الشمس أو كسب القطن .

وعلى الرغم من عدم وجود اختلافات في عدد كرات الدم البيضاء بين المجاميع فإن الاستجابة المناعية المصلية ضد كرات الدم الحمراء في الغنم في مجموعة كسب القطن تدهورت واحتلت المرتبة الأخيرة عن تلك الاستجابة الموجودة في مجموعة كسب الصويا أو كسب عباد الشمس ومن ناحية أخرى فإن دراسة نشاط كرات الدم البيضاء الليمفاوية(خارج الجسم) المأخوذة من الطحال أبدت استجابة عالية لمادة البوكويد في مجموعة كسب عباد الشمس مقارنة بالمجاميع الأخرى بينما لم يلاحظ أي اختلافات في نشاط كرات الدم البيضاء الليمفاوية عند تنشيطها بمادة الكون-أ.