# HEMATOLOGICAL RESPONSE OF SAUDI ARABIAN FOWL TO PROTEIN REARING REGIMENS

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## Summary

The purpose of this investigation was to study the hematological response of Saudi Arabian Baladi fowl to protein rearing regimens. Males and females were subjected to the following 4 protein rearing regimens: conventional, C; reverse protein, RP; 2 single-stage low protein, SS<sub>1</sub> and SS<sub>2</sub> using 15% and 12% CP diets, respectively. Regimen effect was highly significant ( $p \le .01$ ) on BW, PCV, TP and U-Ac and significant ( $p \le .05$ ) on TL. Serum chol levels were not affected by regimen. In general SS<sub>2</sub> birds showed the lowest values for all parameters studied, except for PCV. However, the differences were not significant in each case. Age and sex effects were highly significant ( $p \le .01$ ) for all parameters, however, the regimen X sex interaction was not significant except for PCV. Regimen X age interaction, on the other hand, was highly significant ( $p \le .01$ ) only for BW, TP and U-Ac concentrations. The data may suggest that low levels of protein in the rearing regimen is an important factor influencing levels of the blood parameters studied. The data also indicate a lack of clear relationship between hen-day egg production and the blood parameters studied.

(Key Words: Saudi Arabian Fowl, Protein Regimen, Blood Parameters, Egg Production).

#### Introduction

Variations in the performance of egg type chickens as affected by protein rearing regimens, have been reported previously (Christmas et al., 1982; Leeson and Summers, 1982; Doran et al., 1983; Robinson et al., 1986). However, the associated changes in blood chemistry have not been adequately investigated. It has been shown that serum proteins are not only influenced by the level of protein nutrition (Leveille and Sauberlich, 1961) but also by the sex and stage of development of birds (Grant and Anastassiadis, 1962). High dietary protein levels are shown to încrease serum total proteins (Keyser et al., 1968; Panigraphy et al., 1969) whereas alterations in level of dietary protein induced wide fluctuations in blood uric acid and ammonia (Okumura and Taski, 1969). Featherston (1969) showed that plasma uric acid increased in response to increased dietary protein, but failed to show a concurrent Packed cell volume, on the other hand, was reported to rise with age and decrease with increasing protein level in the diet from 15 to 20 or 28% CP (Ganguar and Guru, 1973). It was also reported (Bell, 1957) that laying hens have a lower PCV than nonlaying hens, and that the lowest red blood cell count coinciding with the period of highest egg production. Sturkie (1986) reported that PCV are influenced by age and sex.

Very high levels of blood lipids were also reported in mature hens during periods of egg formation (Christie and Moore, 1972). This might be explicable on the basis that liver lipid content in a hen normally increases at the onset of lay in response to an increase in estrogen (Sturkie and Muller, 1976).

Increases in plasma cholesterol are part of the classical stress of all higher vertebrates (Seyle, 1950). Yeh and Leveille (1973) showed that hypocholesterolemic effect of high protein diet was at least partly mediated by increased cholesterol turnover which resulted in a speedier removal of cholesterol from blood stream and excretion in the form of fecal cholesterol and bile acids. Some workers have examined the serum cholesterol content of chickens but there was

change in blood ammonia levels.

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no information relating it to protein rearing regimens. Particularly limited information is available on blood constituents of Saudi Arabia Baladi chickens; however, preliminary data were made on some mineral elements, total protein, albumin, globulin, bilirubin and GOT in males and females up to 22 wks of age (Shawer et al., 1985, 1987) together with studies on serum cholesterol levels at 16 and 32 wks of age (Attia et al., 1988).

The lack of reference levels of blood chemical indices, specific to adult male and female Saudi Arabian Baladi chickens necessiates research to establish these reference levels at different ages and for different protein rearing regimens.

#### Materials and Methods

Four group of 18 to 21 cockere's and 4 groups of 16 to 26 pullets each, were used in this study. All birds were wing banded at hatch. These groups were randomly selected from a random bred closed flock of Saudi Arabian Baladi chickens that had been subjected to 4 protein rearing regimens. The conventional regimen (C), followed the NRC (1984) recommendations. The other 3 regimens received an 18% CP diet for the first week of age. Thereafter, the reverse protein regimen (RP) was fed 12, 15 and 18% CP diet at 1 to 6, 6 to 14 and 14 to 20 wks of age, respectively. The single-stage low protein regimens, SS, and SS<sub>2</sub> received 15 and 12% CP diets from 1 to 20 wks of age, respectively. The composition of the experimental diets is shown in table 1. The selected groups for blood studies therefore represented male and female birds for each of these feeding regimens. At 20 weeks of age all birds received a practical layer diet containing Feed and water were provided ad 17% CP libitum. Birds were kept in floor pens of an environmentally controlled house, where the temperature ranged from 21 to 28°C. A photoperiod of 10 hr/day was provided until 20 wks of age. Light was then increased by 0.5 hr each day to reach 15/hr/day at 30 wks and then maintained at this level until the end of lay. Records were kept for daily egg production on pen basis. Individual body weights (BW) were determined at 14, 20, 30 and 49 wks of age. Blood samples from the hirds were drawn by heart puncture at 14, 20, 30 and 49 wks of age. Feed was not

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS USED IN THE REARING PERIOD (%)

	Crude Protein(%)					
Ingredient	18	1.5	12			
Corn, yellow ground	43.7	45.6	43.1			
Barley, ground	30.5	35.5	47.0			
Soybean meal	14.2	12.0	3.0			
Fish meal	5.0	_	_			
Animal fat	1.0	1.0	1.0			
Alfalfa	2.5	2.5	2.5			
Dicalcium phosphate	1.3	1.6	1.6			
Limestane	1.4	1.3	1.3			
Salt	.25	.25	.25			
Sodium bicarbonate	.10	.10	.10			
<b>M</b> icro-mix <sup>1</sup>	.15	.15	.15			
DL-Methionine	.03	.0.5	.04			
Lysine	-	-	.03			
Calculated analysis:						
Metabolizable energy						
(Kcal/kg)	2900	2900	2900			
Protein (%)	18	1.5	12			

<sup>&</sup>lt;sup>1</sup>Supplied the following per kilogram of diet: vitamin A, 10,000 IU; vitamin D<sub>3</sub>, 2000 ICU; vitamin E, 10 mg; vitamin B<sub>1</sub>, 0.5 mg; vitamin B<sub>2</sub>, 3 mg; Pantothenic acid, 61 mg; Niacin, 10 mg; vitamin K<sub>3</sub>,0.2 mg; vitamin B<sub>12</sub>, 0.01 mg; choline, 200 mg; Manganese, 30 mg; Zinc, 30 mg; Iron, 10 mg; Copper, 1 mg; Iodine, 0.3 mg; Cobalt, 0.1 mg; Selenium, 0.03 mg.

withdrawn before obtaining blood. Packed cell volume (PCV) was determined by a microhematocrit method. Samples for serum collection were allowed to clot overnight at 4°C, then the serum was seperated by centrifugation, harvested and stored at --20°C until analyzed. The following determinations were performed: total proteins (TP) by the Biaret method (Weichsellaun, 1946); total lipids (TL) as outlined by Merck (1974), and cholesterol (Chol) by the method of Pearson et al. (1953). Uric acid (U-Ac) was determined spectrophotometrically using commercial reagent Kits (biomerieux, Marcy L' Etoile 69260 Charbonemier, Les Bains, France).

Data were subjected to statistical analysis, King Saud University Computer Center, using general linear model procedure, SAS User's Guide (1986) according to the following model:  $Y_{ijk} = U + T_i + A_j + S_k + (SxT)_{ki} + (SxA)_{kj} + (TxA)_{jj} + eijk$ 

where

U = overall mean

Y<sub>ijk</sub> body weight and blood parameters of i<sup>th</sup> treatment of j<sup>th</sup> Age and K<sup>th</sup>

T<sub>i</sub> = the effect of treatment i, (1, ..., 4);

 $A_1$  = the effect of age j, (j = 1, ..., 4)

 $S_k$  = the effect of sex k, (k = 1,2)

(SxT = the interaction of sex and treatment effects.

(SxA)= the interaction of sex and age effects.

(TxA)= the interaction of treatment and age effects.

ei'k = the random error.

Duncan multiple range test (1956) was used to

seperate means when statistical significance was indicated. Person correlation among parameters were also estimated.

#### Results and Discussion

Statistical analysis showed highly significant (p = .01) regimen effect on BW, PCV, TP and U-Ac and significant  $(p \leq .05)$  effect on TL. Serum cholesterol levels were not affected by the regimen (table 2). Age and sex effects were highly significant  $(p \leq .01)$  for all parameters except age X sex effect on TP; however, the regimen X sex interactions were not significant except for PCV values. Regimen X age interactions, on the other hand, were highly significant  $(p \leq .01)$  only for BW, TP and U-Ac concentrations. Figure 1 indicates the production status of the C, RP, SS<sub>1</sub> and SS<sub>2</sub> layers bled during the study.

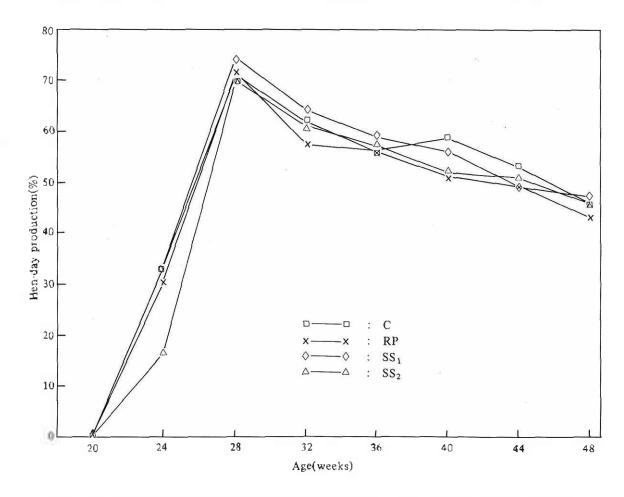


Figure 1. Effect of protein rearing regimen on egg production of Saudi Arabian Baladi chickens,

## ALSOBAYEL ET AL.

TABLE 2. LEAST SQUARES MEANS AND STANDARD ERRORS OF SAUDI ARABIAN BALADI BODY WEIGHT'S AND SELECTED BLOOD CONSTITUENTS AS AFFECTED BY REARING REGIMEN AGE AND SEX

Factor	BW(g)	PCV(%)	TP(g/100)	TL(g/100)	Chol(mg/100)	U-Ac(mg/100)
Regimen	**	**	**	*	NS	**
С	1231 <sup>a</sup>	32.7 <sup>ac</sup>	4.712	1.24A	135.3	7.74 <sup>A</sup>
	(135) <sup>d</sup>	(125)	(121)	(113)	(117)	(121)
RP	1196 <sup>a</sup>	32.8 a	4.88 <sup>a</sup>	1.07 <sup>AB</sup>	131.5	7.78 <sup>A</sup>
	(118)	(105)	(101)	(96)	(86)	(102)
SS	1196 <sup>a</sup>	31.3 b	4.84 <sup>a</sup>	$1.19^{AB}$	129.8	7.02B
	(152)	(135)	(134)	(134)	(129)	(138)
$SS_2$	1059b	31.5bc	4.33 <sup>b</sup>	.92 <b>B</b>	121.4	6.99B
	(136)	(128)	(122)	(118)	(113)	(129)
Age (weeks)	* *	**	**	* *	**	**
]4	<b>7</b> 67 <sup>a</sup>	27.7 a	4.16 <sup>8</sup>	.53 <sup>a</sup>	122.7 <sup>a</sup>	6.86 <sup>a</sup>
	(162)	(144)	(132)	(136)	(116)	(136)
20	1055 <sup>b</sup>	32.4bc	4.42 <sup>b</sup>	.65 <sup>a</sup>	136.6 b	6.21°
	(158)	(142)	(126)	(119)	(122)	(135)
30	1337 <sup>c</sup>	34.8 <sup>d</sup>	5.12 <sup>c</sup>	1.8 b	143.2 <sup>b</sup>	8.39 <sup>b</sup>
	(119)	(110)	(110)	(100)	(102)	(111)
49	1524 <sup>d</sup>	33.5°	5.05°	1.44°	115.6ª	8.06 <sup>b</sup>
	(102)	(115)	(111)	(106)	(105)	(108)
Sex	* *	**	**	**	* *	**
Female	1054 <sup>a</sup>	28.0 <sup>a</sup>	4,87 <sup>a</sup>	1.68 <sup>a</sup>	139.0 a	6.80 <sup>a</sup>
	(320)	(291)	(288)	(279)	(266)	(285)
Male	1286 <sup>b</sup>	36.3 <sup>h</sup>	4.52b	.55 <sup>b</sup>	119.7 <sup>b</sup>	8.06 <sup>b</sup>
	(221)	(220)	(191)	(182)	(179)	(205)
SEM	±4.36	±.18	±.29	+.05	±1.83	±.12
Interaction						
$R \times A$	* *	NS	* *	NS	NS	**
$S \times A$	**	**	NS	**	* *	* *
R x S	NS	* *	NS	NS	NS	NS

a,b,c Means within each trait and within each factor level without common letter are significantly different  $(p \le .01)$ .

A,B Means within each trait and within each factor level without common letter are significantly different  $(p \le .05)$ .

d<sub>Number</sub> of observations. \* $(p \le 05)$  \*\*  $(p \le .01)$ 

## Body weight

Body weights were significantly (p 1.01) smaller in  $SS_2$  birds compared to other regimens (table 2). This might suggest that the longer duration of the 12% CP feeding was directly related to body weight reduction of  $SS_2$  birds. Similar findings, with low protein diets, were reported by Keshavarz (1984) and Douglas et al. (1985).

#### Packed cell volume

Birds in both single-stage low protein regimens had lower PCV values compared to the C and RP regimens. The decrease in PCV in single-stage low-protein regimens is at variance with the results of Gangwar and Guru (1973); these workers noticed decreased PCV upon increasing dietary protein level from 15 to 20 or 28% CP in White Leghorn chicks. Such discrepancies could be due to variation in protein levels and/or breed differences. Our PCV estimates were lower than reported by Sturkie and Textor (1960) for mature White Leghorn males (48%) and females (31%), respectively. PCV measurements increased significantly (p = .01) with age up to 30 wks, then showed a slight decrease at 49 wks of age. The significant increase in PCV of males over females might be partly due to the stimulatory effect of erythropoiesis in males and/or the depressing effect of estrogen in females (Taber et al., 1943; Domm and Taber, 1946).

## Total proteins

TP concentrations of the different age groups of hirds ranged from 4.16 to 5.12 g/100 which is slightly higher than reported by Shawer et al. (1985). These differences, however, might be due to age effect since the latter studies were confined to 22 wks of age only. TP values of the C, RP and SS<sub>1</sub> birds significantly ( $p \le 01$ ) exceeded the SS2 birds. The higher TP levels of the C, RP and SS, birds might reflect the capability of these regimens for maintenance of adequate protein synthesis. Age also had a significant  $(p \le .01)$  effect on TP with marked increases at 30 and 49 wks of age. A similar finding regarding increased protein concentration with age was shown by Swensen (1970). TP concentrations in females was significantly (p < .01) higher than in males. Strukie (1986) reported that the increase in plasma proteins in response to estrogen administration is caused by an appreciable increase in the globulin fractions.

## Total lipids

TL estimates of the SS2 birds were significantly  $(p \le .05)$  lower than that of the C birds and numerically less than the RP and SS, birds. It is therefore suggested that the 12% CP feeding for the entire rearing period might have slightly reduced the ovarian activity of the SS2 birds. TL estimates increased rapidly at 30 wks of age, then showed a slight decrease at 49 wks of age, relating this observation to egg production curves (figure 1) might lead us to suggest that these changes which occurred subsequent to onset of egg production were probably related to estrognic effect on TL. This is in agreement with Sturkie (1986) who reported that total lipids of birds reached highest levels in females during periods of egg production. The lower TL values in males might have been due to the lack of estrogen and/or the higher levels of testosterone (Griminger, 1976). Also in this respect Balnave (1971) recorded TL value of 2548 mg/100 ml for active layers and 1112 mg/100 ml for females with quiscent ovaries.

## Serum cholesterol

There were no major differences in chol concentrations among the C, RP and SS<sub>1</sub> birds while the SS<sub>2</sub> birds showed lower values. This is at variance with Yeh and Leville (1973) who showed that high protein diets reduced chol level in blood. It is worth noting that the pattern of regimen effect on serum cholesterol is similar to that of TL, suggesting that the rate of lipid metabolism and turnover is somewhat associated with cholesterol metabolism.

## Uric acid

The SS2 birds showed the lowest U-Ac values. This is in agreement with Okumura and Tasaki (1969). A rather rapid increase in U-Ac concentrations occurred at 30 wks of age and remained more or less at this level until the termination of the experiment. This might be indicative of increased protein metabolism to fulfill physiological requirements for production. Uric acid levels were significantly (p  $\leq$  .01) higher in males than females. This is in agreement with the data of McFaraland et al. (1979) for White leghorns. Similarly higher male U-Ac values were reported by McFaraland and Coon (1980) in comparative studies of high and low uric acid line chickens. Clearly protein requirement of hens increases after 20th week, due to egg production, whereas

#### ALSOBAYEL ET AL.

TABLE 3. SIMPLE CORRELATIONS AMONG STUDIED PARAMETERS OF MALE AND FEMALE SAUDI ARABIAN BALADI CHICKENS<sup>8</sup>

Variable	BW	PCV	ΥP	TL	Cho	U-Ac	HD
			**	* *	* *	* *	**
$\mathbf{BW}$		,138	+.589	+.593	+.183	+.184	.170
		$(289)^{b}$	(286)	(278)	(265)	(282)	(320)
	* *		**	**			
PCV	+.707		172	336	118	069	+.041
	(173)		(276)	(268)	(254)	(273)	(291)
	* *	**		**	**	**	**
TP	+.436	+.456		+.555	+.444	+.271	210
	(155)	(171)		(271)	(259)	(275)	(288)
					**	**	
TL	032	148	036		+.642	+.169	.067
	(146)	(160)	(151)		(252)	(270)	(279)
				**			*
Cho	135	045	+.006	+.355		+.061	123
	(148)	(155)	(151)	(154)		(257)	(266)
	<b>*</b>	**	**				* *
U-Ac	+.294	+.347	+.289	060	141		281
	(169)	(179)	(165)	(164)	(166)		(285)

<sup>&</sup>lt;sup>a</sup>Female values above diagonal; male values below diagonal HD. bNumber of samples.

cocks are almost full grown at this age and do not have the production requirements of females; therefore elevated serum U-Ac levels in males reflect to some extent the degradation of excess dietary protein. Okumura and Tasaki (1969) stated that uric acid in blood is indeed a reflection of the direction of protein metabolism in birds.

Simple phenotypic correlations between all factors measured were calculated for both males and females (table 3). In males, positive and significant ( $p \le .01$ ) correlations were found between BW and PCV, TP and TL. PCV was positively correlated with TP and U-Ac. The low level of TL in males (table 2) would explain the lowered magnitude of correlation between BW and TL in males (table 3). In females BW was positively and significantly correlated ( $p \le .01$ ) with all blood parameters apart from a significant ( $p \le .01$ ) negative correlation between BW and PCV.

These correlations were low in magnitude except for TP and TL. To the contrary, PCV was negatively correlated with all blood parameters and only significant ( $p \le .01$ ) with TP and TL. It is worth noting that phenotypic correlations between BW and TP, for both males (+.436) and females (+.589) were moderate in magnitude positive and highly significant indicating the TP in serum is closely related to BW. Differences in estimates of male and female blood parameters might be a reflection of hormonal status and/or production rate in females.

The data may suggest that the low level of protein in the rearing regimen is an important factor influencing levels of blood parameters, especially if it is fed during the entire rearing period. The data also indicate a lack of clear relationship between hen-day egg production and the blood parameters; as very low correlations were obtained.

**<sup>\*</sup>**(p ≤ .05)

 $<sup>(10, \</sup>ge q)**$ 

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