

**Original Paper****Dose dependent effect of phytase supplementation on hematological parameters, serum biochemical analysis, carcass characteristics, and chemical meat analysis of Hubbard broiler chickens**Aya M. Ahmed<sup>1</sup>, Nasser Khedr<sup>1</sup>, Ahmed M. Hegazy<sup>2</sup><sup>1</sup> Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh 13736, Qalyubia, Egypt.<sup>2</sup> Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh 13736, Qalyubia, Egypt.**ARTICLE INFO****Keywords**

Broilers,  
Carcass characteristics,  
Chemical meat analysis,  
Exogenous phytase.

Received 04/07/2024

Accepted 22/07/2024

Available On-Line  
01/10/2024**ABSTRACT**

Since the start of the current century, incorporating enzymes that degrade phytate in chicken diets has greatly enhanced the sustainability of chicken meat production. The current study aims to ascertain the optimal effects of phytase supplementation on the complete blood count (CBC), liver function tests, Ca and P, carcass characteristics, and chemical meat analysis of Hubbard broiler chickens. A total of 270 one-day-old Hubbard broilers were distributed randomly among six groups. The experimental groups were Group 1 (G1), which served as the control group and were fed standard basal diets. G2, G3, G4, G5, and G6 were supplemented with standard basal diets containing 50, 75, 100, 150, and 200g/ton of phytase, respectively. On the 35th day, five birds from every group were selected and slaughtered. The results revealed that elevating the inclusion of phytase up to 150 and 200g/ton significantly increased carcass yield, protein% of breast meat, and decreased abdominal fat compared to 50, 75, and 100g/ton. Phytase supplementation did not affect CBC or serum biochemical parameters. In conclusion, incorporating phytase at higher doses in broiler diets can improve carcass yield, improve the protein content of breast meat, and have no adverse effect on CBC or liver function tests.

**1. INTRODUCTION**

The poultry industry stands out as one of the most rapidly expanding and significant agro-based sectors globally. Broiler production continues to rise annually, driven by heightened demand from both local consumers and export markets (El Enshasy et al., 2018). Consumer choice is the current concern of researchers as they establish new quality and nutritional targets that enable optimal production performance with minimal financial expenditure. Finding substitutions for high-cost feedstuffs and enhancing nutrient bioavailability with enzymatic supplementation might be a solution to maintain growth performance and quality within healthy, natural, and low-cost resources (Grigore et al., 2019).

Phosphorus (P) poses a significant limitation in poultry nutrition because the majority of P present in plant-derived feed ingredients exists in the form of phytic acid. Regrettably, most of this phytic acid remains indigestible for monogastric animals, serving as an anti-nutritional factor that hampers the absorption of diverse minerals (Dersjant-Li et al., 2015). Hence, inorganic phosphorus is commonly supplemented to fulfil the bird's physiological phosphorus needs. However, besides escalating feed expenses, excessive phosphorus supplementation in feed is not utilized by animals and is consequently excreted, posing environmental risks. Concerns regarding both environmental impact and economic factors have led to a reduction in the use of inorganic phosphorus, thus advocating for phytases as a more sustainable alternative (Jing et al., 2018).

Currently, exogenous phytases are regularly incorporated into broiler diets, often at higher inclusion rates. This significant advancement stems from phytases' ability to improve phosphorus utilization, consequently decreasing phosphorus excretion. This trend is further fueled by a growing recognition of the potent anti-nutritional characteristics of phytate. This trend has been reinforced by a growing recognition of phytate's potent anti-nutritive properties. Exogenous phytases effectively neutralize the wide array of anti-nutritive properties associated with dietary phytate by breaking down phytate through hydrolysis, resulting in positive effects stemming from phytate degradation. Phytases improve the utilization of minerals such as P, sodium, and calcium, as well as enhance protein digestion, and facilitate the intestinal absorption of amino acids and glucose to different degrees (Selle et al., 2023).

Additional phosphorus release, thorough and swift degradation of phytic acid, and the production of myo-inositol are suggested mechanisms for the favorable outcomes observed in broiler growth and development (Cowieson et al., 2011). Supplementing broiler chicken diets with phytase levels exceeding industry recommendations ( $\geq 1,500$  phytase units (FTU)/kg) enhances even more the digestibility and absorption of nutrients beyond phosphorus, such as protein, amino acids, and energy, resulting in additional benefits known as extra-phosphoric effects (Walk and Rama Rao, 2020). The present study aimed to explore the impact of dose-dependent phytase supplementation on

\* Correspondence to: [aya.abdo@fvtm.bu.edu.eg](mailto:aya.abdo@fvtm.bu.edu.eg)

CBC, liver function tests, Ca and P, carcass characteristics, and chemical meat analysis of Hubbard broiler chickens.

## 2. MATERIALS AND METHODS

### Ethical Approval

The experimental protocol was approved by the Institutional Animals Ethical Committees of the Faculty of Veterinary Medicine, Benha University, No. BUFVTM 06-12-2022.

### Phytase enzyme

Axtra PHY GOLD (phytase enzyme) was sourced from (Danisco Animal Nutrition, International Flavors and Fragrance (IFF) Inc., NY, US), which is a 6-phytase (EC 3.1.3.26) originating from *Trichoderma reesei*, with a minimum activity of 30,000 FTU/g of the product. Each 1 g of Axtra PHY GOLD contains 10 FTU.

### Birds and housing

A total of two hundred seventy-one-day-old Hubbard broiler chickens, averaging  $43.76 \pm 0.86$  g body weight, were obtained from El Ahram Company, Giza, Egypt. They were wing-banded, placed on fresh wood shaving litter, and provided with clean food and water. The birds were accommodated in a broiler house where environmental conditions aligned closely with the specifications outlined in the Hubbard Broiler Management Guide (2022). The chickens were raised under uniform growing conditions, which included a constant lighting schedule (23 hours of lighting and 1 hour of darkness), mechanical ventilation,

maintaining at least 50% air humidity, and ensuring a comfortable temperature condition (initially starting at 34°C and gradually reduced to 22°C throughout the experimental period). All birds were properly immunized against infectious bursal disease (IBD) and Newcastle disease (ND). Dry mash feed and water were supplied ad libitum every day.

### Experimental design

Following a completely randomized design, 270 unsexed Hubbard broiler chicks aged one day were divided into six experimental groups. Each treatment comprised three replications, with each replication consisting of 15 birds. The first group (G1) served as the control and was fed a standard basal diet. The second, third, fourth, fifth, and sixth groups were given the standard basal diet, supplemented with 50, 75, 100, 150, and 200g/ton of phytase. Three stages comprised the rearing period: days 1 to 10 (starting), days 11 to 24 (growing), and days 25 to 35 (finishing) of age. All diets were formulated with a corn and soybean meal base and offered in mash form. Diets were designed to satisfy the birds' suggested nutrient requirements, based on Hubbard's requirements (2022). The components of the feed and the chemical makeup of the experimental diets are detailed in Table 1. At the end of the trials, five birds per treatment were chosen randomly for blood sampling. The birds were humanely slaughtered by cutting the jugular vein. Blood samples were obtained from each bird for analysis, including CBC, liver function tests, and measurements of Ca and P levels.

Table1. Ingredient and nutrient composition in the starter, grower, and finisher phases of different experimental groups.

	G1	G2	G3	G4	G5	G6
<b>Starter, 0-10 d</b>						
<i>Ingredients per Ton</i>						
yellow corn	525.40	541.80	545.87	546.40	550.25	555.45
SBM46	341.00	350.00	350.00	350.00	349.00	347.00
Wheat bran	33.00	33.00	35.00	35.00	35.00	33.00
vegetable oil	25.00	25.00	25.00	25.00	25.00	25.00
Corn gluten meal	24.00	8.00	3.00	2.50	-	-
Mono calcium phosphate	17.25	9.60	8.65	8.00	7.30	6.85
Limestone	15.75	15.30	15.20	15.40	15.50	15.50
Sodium bicarbonate	3.25	1.95	1.75	2.25	2.40	1.50
DL-Methionine	3.10	3.30	3.40	3.40	3.45	3.45
L- Lysine	3.10	2.60	2.55	2.45	2.35	2.40
premix	3.00	3.00	3.00	3.00	3.00	3.00
Sodium chloride	2.25	2.35	2.35	2.35	2.40	2.40
L -Threonine	1.20	1.35	1.45	1.45	1.50	1.55
Choline chloride	1.10	1.10	1.10	1.10	1.10	1.10
Anti-mycotoxin	1.00	1.00	1.00	1.00	1.00	1.00
Anticoccidial	0.25	0.25	0.25	0.25	0.25	0.25
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10
Energy enzymes	0.10	0.10	0.10	0.10	0.10	0.10
Anti clostridial	0.10	0.10	0.10	0.10	0.10	0.10
Protease enzyme	0.05	0.05	0.05	0.05	0.05	0.05
Axtraphy Gold	-	0.05	0.08	0.10	0.15	0.20
<i>Chemical composition (%)</i>						
ME (Kcal \ Kg diet)	3,002	3,059	3,067	3,048	3,073	3,084
CP	23.14	23.10	23.05	23.10	23.09	23.12
Calcium	1.03	1.05	1.06	1.05	1.05	1.05
Available phosphorus	0.50	0.50	0.51	0.50	0.50	0.50
phytate	0.26	0.26	0.26	0.26	0.26	0.26
<b>Grower, 11-24 d</b>						
<i>Ingredients per Ton</i>						
yellow corn	567.15	582.07	586.18	591.87	597.20	599.74
SBM46	294.00	311.00	307.00	304.00	299.00	296.00
Wheat bran	35.00	34.00	35.00	33.00	34.00	35.00
vegetable oil	35.00	35.00	35.00	35.00	34.00	34.00
Corn gluten meal	21.00	-	-	-	-	-
Mono calcium phosphate	16.30	8.60	7.65	7.00	6.30	5.90
Limestone	14.50	13.40	13.35	13.50	13.65	13.65
Sodium bicarbonate	2.70	2.05	1.90	1.70	1.90	1.70
DL -Methionine	2.70	2.95	3.00	3.00	3.00	3.04
L- Lysine	2.95	2.25	2.28	2.30	2.30	2.30
premix	3.00	3.00	3.00	3.00	3.00	3.00
Sodium chloride	2.10	2.30	2.26	2.25	2.25	2.25
L -Threonine	0.90	0.73	0.70	0.68	0.65	0.62
Choline chloride	1.10	1.00	1.00	1.00	1.00	1.00
Anti-mycotoxin	1.00	1.00	1.00	1.00	1.00	1.00
Anticoccidial	0.25	0.25	0.25	0.25	0.25	0.25
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10
Energy enzymes	0.10	0.10	0.10	0.10	0.10	0.10
Anti clostridial	0.10	0.10	0.10	0.10	0.10	0.10
Protease enzyme	0.05	0.05	0.05	0.05	0.05	0.05
Axtraphy Gold	-	0.05	0.08	0.10	0.15	0.20

<i>Chemical composition</i>							
ME (Kcal / Kg diet)	3,103	3,152	3,167	3,157	3,180	3,185	
CP (%)	21.12	21.10	21.16	21.12	21.12	21.12	
Calcium (%)	0.95	0.95	0.96	0.95	0.95	0.95	
Available phosphorus (%)	0.47	0.47	0.48	0.47	0.47	0.47	
phytate (%)	0.24	0.25	0.25	0.25	0.25	0.25	
<b>Finisher, 25-35 d</b>							
<i>Ingredients per Ton</i>							
yellow corn	594.20	601.56	607.77	612.33	616.64	620.11	
SBM46	253.00	286.00	282.00	278.00	274.00	271.00	
Wheat bran	34.00	35.00	35.00	35.00	35.00	35.00	
vegetable oil	44.00	45.00	44.00	44.00	44.00	44.00	
Corn gluten meal	32.00	-	-	-	-	-	
Mono calcium phosphate	14.30	6.50	5.50	5.00	4.25	3.85	
Limestone	13.00	12.00	11.90	12.00	12.15	12.20	
Sodium bicarbonate	2.55	1.70	1.55	1.35	1.55	1.36	
DL-Methionine	2.00	2.33	2.36	2.39	2.45	2.45	
L- Lysine	2.50	1.45	1.45	1.50	1.50	1.50	
premix1	3.00	3.00	3.00	3.00	3.00	3.00	
Sodium chloride	2.20	2.55	2.55	2.50	2.50	2.50	
L -Threonine	0.65	0.34	0.31	0.29	0.26	0.23	
Choline chloride	1.00	0.92	0.93	0.94	0.95	1.00	
Anti-mycotoxin	1.00	1.00	1.00	1.00	1.00	1.00	
Anticoccidial	0.25	0.25	0.25	0.25	0.25	0.25	
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10	
Energy enzymes	0.10	0.10	0.10	0.10	0.10	0.10	
Anti clostridial	0.10	0.10	0.10	0.10	0.10	0.10	
Protease enzyme	0.05	0.05	0.05	0.05	0.05	0.05	
Axtraphy Gold	-	0.05	0.08	0.10	0.15	0.20	
<i>Chemical composition</i>							
ME (Kcal /Kg diet)	3,204.	3,236.	3,247.	3,235	3,264	3,270	
CP (%)	20.01	20.01	20.07	20.01	20.03	20.02	
Calcium (%)	0.85	0.85	0.86	0.85	0.85	0.85	
Available phosphorus (%)	0.42	0.42	0.43	0.42	0.42	0.42	
Phytate (%)	0.24	0.24	0.24	0.24	0.24	0.24	

#### *Complete Blood Count (CBC)*

Heparinized blood samples were collected for haematological analysis. The total leukocyte count (TLC) and red blood cells (RBCs) count were determined using a Neubauer hemocytometer with a 1:200 dilutions of Natt and Herrick solution. Hemoglobin (Hb) concentration was measured following the method described by Campbell (1995). The packed cell volume (PCV) was assessed using a microhematocrit capillary tube and a Hematocrit reader. The differential leukocyte count (DLC) was performed according to the procedure outlined by Schalm and Jain, (1975) and was expressed as a percentage of the total leukocytes.

#### *Liver function tests*

The alanine aminotransaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were measured according to Siekmann et al., (2002).

#### *Serum calcium and phosphorus concentration*

Total calcium and phosphorus were determined according to Young (1997).

#### *Carcass Characteristics*

At the end of the trial, five birds per group, each with weights closest to the average body weight for their respective treatment, were randomly chosen. They were weighed, subjected to a 12-hour fasting period, and then euthanized using the halal neck-cut method following the procedure outlined by Ali et al. (2011), and then eviscerated. As a percentage of live body weight, the dressing percentage was calculated. Carcass parts, including breast muscles, thighs, and abdominal fat, as well as visceral organs such as the liver, gizzard, small intestine, spleen, thymus, and bursa of fabricius, were removed and weighed separately. These weights were subsequently expressed as a percentage of the pre-slaughter weight, following the methodology described by Biesek et al.,(2020).

#### *Chemical analysis of breast meat*

On day 35, another five birds per group were collected randomly, weighed, slaughtered, and deskinning, and samples were taken from the right pectoralis major muscle

for chemical analysis. The right pectoralis muscles (100g) were homogenized immediately and subsequently frozen at -80 °C for chemical analyses. Pooled samples of homogenized breast muscles underwent analysis to determine their moisture, fat, protein, and ash contents using the standard procedure established by the Association of Official Analytical Chemists (AOAC, 2000). The calcium content of meats was ascertained using spectrometry of atomic absorption following the European standard (ISO 6869:2000), while the phosphorus content was analyzed using a spectrometric approach by the International Standards (ISO 6491:1998).

#### *Statistical analysis*

The data were analyzed using analysis of variance (ANOVA) with the statistical software SPSS for Windows (Version 16.0, SPSS Inc., 2007). Tukey's multiple-comparison test was utilized to evaluate differences among means, with significance set at  $P \leq 0.05$ . This statistical methodology facilitated a thorough investigation of the data, enabling the identification of significant differences between groups.

### **3. RESULTS**

#### *Complete blood count*

The effects of phytase supplementation on the CBC of Hubbard broiler chickens are illustrated in Table 2. There were no statistical differences between the experimental groups in RBCs, TLC, Hb, PCV, lymphocyte, heterophile, monocyte, and basophile.

#### *Liver function tests*

The effects of phytase supplementation on liver function tests of Hubbard broiler chickens are illustrated in Table 3. There were no statistical differences between the experimental groups in ALT, AST, and LDH levels.

#### *Serum calcium and phosphorus concentration*

The effects of phytase supplementation on serum Ca and P of Hubbard broiler chickens are illustrated in Table 4. There

were no statistical differences between the experimental groups in serum Ca and P levels.

Table 2. Effect of phytase supplementation on complete blood count (CBC) of Hubbard broiler chickens at the end of the experiment (Mean ± SD), (N=5).

Items	G1	G2	G3	G4	G5	G6
Hb (g/dl)	8.85±0.34 <sup>a</sup>	9.6±0.29 <sup>a</sup>	10.76±0.60 <sup>a</sup>	9.22±0.38 <sup>a</sup>	9.54±0.43 <sup>a</sup>	9.91±0.54 <sup>a</sup>
PCV (%)	28.50±1.29 <sup>a</sup>	31.00±0.82 <sup>a</sup>	34.25±1.71 <sup>a</sup>	29.75±1.26 <sup>a</sup>	30.50±1.29 <sup>a</sup>	31.75±1.71 <sup>a</sup>
RBCs×10 <sup>6</sup> (mm <sup>3</sup> )	2.94±0.74 <sup>a</sup>	2.74±1.4 <sup>a</sup>	3.99±0.65 <sup>a</sup>	2.11±0.48 <sup>a</sup>	2.89±0.29 <sup>a</sup>	3.37±0.63 <sup>a</sup>
TLC (mm <sup>3</sup> )	27.9±1.52 <sup>a</sup>	22.02±1.55 <sup>a</sup>	19.99±3.33 <sup>a</sup>	38.2±6.99 <sup>a</sup>	22.91±5.89 <sup>a</sup>	19.72±8.30 <sup>a</sup>
Heterophile (%)	25.86±2.06 <sup>a</sup>	26.13±8.19 <sup>a</sup>	24.26±7.16 <sup>a</sup>	18.90±3.48 <sup>a</sup>	22.10±1.64 <sup>a</sup>	28.18±3.70 <sup>a</sup>
Lymphocyte (%)	66.14±1.77 <sup>a</sup>	64.25±4.46 <sup>a</sup>	66.03±3.10 <sup>a</sup>	74.02±2.41 <sup>a</sup>	70.61±2.84 <sup>a</sup>	65.13±3.38 <sup>a</sup>
Monocyte (%)	3.63±1.38 <sup>a</sup>	5.22±2.18 <sup>a</sup>	5.44±2.99 <sup>a</sup>	3.98±0.85 <sup>a</sup>	4.49±2.53 <sup>a</sup>	3.80±1.49 <sup>a</sup>
Eosinophile (%)	2.75±0.65 <sup>a</sup>	3.00±1.08 <sup>a</sup>	3.08±1.06 <sup>a</sup>	2.17±0.99 <sup>a</sup>	2.38±0.90 <sup>a</sup>	1.94±0.72 <sup>a</sup>
Basophile (%)	1.63±0.48 <sup>a</sup>	1.41±1.18 <sup>a</sup>	1.2±0.96 <sup>a</sup>	0.93±0.16 <sup>a</sup>	0.42±0.72 <sup>a</sup>	0.96±0.32 <sup>a</sup>

Means with different superscripts in the same row are significantly different ( $P \leq 0.05$ ).

G1: Control group (basal diet); G2: basal Diet+50g/ton phytase; G3: basal Diet+75g/ton phytase; G4: basal Diet+100g/ton phytase; G5: basal Diet+150g/ton phytase; G6: basal Diet+200g/ton phytase.

Table 3. Effect of phytase supplementation on liver function tests of Hubbard broiler chicks at the end of the experiment (Mean±SD), (N=5).

Items	G1	G2	G3	G4	G5	G6
LDH (U/L)	1327.78±402.12 <sup>a</sup>	1383.33±531.29 <sup>a</sup>	1333.8±391.82 <sup>a</sup>	1672.25±595.05 <sup>a</sup>	1713.75±533.97 <sup>a</sup>	1232.5±247.01 <sup>a</sup>
AST (U/L)	33.25±11.62 <sup>a</sup>	25.50±4.97 <sup>a</sup>	25.00±5.16 <sup>a</sup>	22.5±8.06 <sup>a</sup>	27.75±15.48 <sup>a</sup>	29.75±10.44 <sup>a</sup>
ALT (U/L)	56.67±12.96 <sup>a</sup>	59.10±22.32 <sup>a</sup>	46.76±6.63 <sup>a</sup>	51.56±10.90 <sup>a</sup>	64.37±11.65 <sup>a</sup>	69.40±19.98 <sup>a</sup>

Means with different superscripts in the same row are significantly different ( $P \leq 0.05$ ).

G1: Control group (basal diet); G2: basal Diet+50g/ton phytase; G3: basal Diet+75g/ton phytase; G4: basal Diet+100g/ton phytase; G5: basal Diet+150g/ton phytase; G6: basal Diet+200g/ton phytase.

Table 4. Effect of phytase supplementation on serum Ca and P of Hubbard broiler chicks at the end of the experiment (Mean ± SD), (N=5).

Items	G1	G2	G3	G4	G5	G6
Ca (mg/dl)	8.28±1.30 <sup>a</sup>	7.83±0.84 <sup>a</sup>	9.24±1.07 <sup>a</sup>	7.98±0.54 <sup>a</sup>	9.02±1.01 <sup>a</sup>	9.35±1.09 <sup>a</sup>
P (mg/dl)	9.26±2.88 <sup>a</sup>	4.74±1.91 <sup>a</sup>	6.99±3.73 <sup>a</sup>	6.19±1.40 <sup>a</sup>	5.81±1.68 <sup>a</sup>	7.99±4.22 <sup>a</sup>

#### Carcass characteristics

The effects of phytase supplementation on the carcass characteristics of Hubbard broiler chickens are illustrated in Table 5. The results revealed that G5 and G6 that received higher doses of phytase supplementation exhibited increased live weight and dress weight percentages compared to the

other experimental groups. Also, abdominal fat percentage was significantly decreased in G4, G5, and G6 compared to G1, G2, and G3. However, phytase supplementation did not affect breast and thigh weight, internal organ weight (liver, gizzard, heart, spleen, thymus, and bursa), or the intestinal length and diameter of different parts of the intestine.

Table 5. Effect of phytase supplementation on Carcass characteristics of Hubbard broiler chickens at the end of the experiment (Mean ± SD), (N=5).

Items	G1	G2	G3	G4	G5	G6
Live weight(g)	1830.20±38.73 <sup>a</sup>	1885.6±31.74 <sup>b</sup>	1955.2±30.43 <sup>a</sup>	1882.60±37.13 <sup>b</sup>	1966.20±6.02 <sup>a</sup>	1970.00±11.36 <sup>a</sup>
Dressed weight (%)	76.21±0.67 <sup>b</sup>	74.38±2.30 <sup>b</sup>	75.46±1.1 <sup>b</sup>	73.91±0.82 <sup>b</sup>	79.93±1.31 <sup>a</sup>	80.09±0.61 <sup>a</sup>
Breast weight (%)	25.4±1.36 <sup>a</sup>	25.81±2.24 <sup>a</sup>	25.98±1.56 <sup>a</sup>	23.56±2.96 <sup>a</sup>	26.18±1.03 <sup>a</sup>	28.21±1.48 <sup>a</sup>
Thigh weight (%)	27.05±2.26 <sup>a</sup>	28.58±1.66 <sup>a</sup>	27.64±1.75 <sup>a</sup>	28.00±4.11 <sup>a</sup>	27.5±2.79 <sup>a</sup>	27.09±0.85 <sup>a</sup>
Gizzard weight (%)	2.46±0.07 <sup>a</sup>	2.70±0.32 <sup>a</sup>	2.54±0.18 <sup>a</sup>	2.65±0.28 <sup>a</sup>	2.44±0.20 <sup>a</sup>	2.23±0.12 <sup>a</sup>
Liver weight (%)	2.15±0.10 <sup>a</sup>	2.20±0.30 <sup>a</sup>	2.10±0.26 <sup>a</sup>	2.16±0.19 <sup>a</sup>	2.27±0.27 <sup>a</sup>	2.28±0.22 <sup>a</sup>
Heart weight (%)	0.51±0.03 <sup>a</sup>	0.50±0.02 <sup>a</sup>	0.51±0.02 <sup>a</sup>	0.55±0.03 <sup>a</sup>	0.50±0.07 <sup>a</sup>	0.49±0.07 <sup>a</sup>
Thymus weight (%)	0.41±0.19 <sup>a</sup>	0.38±0.12 <sup>a</sup>	0.43±0.19 <sup>a</sup>	0.32±0.06 <sup>a</sup>	0.28±0.13 <sup>a</sup>	0.41±0.09 <sup>a</sup>
Bursa weight (%)	0.15±0.02 <sup>a</sup>	0.15±0.06 <sup>a</sup>	0.12±0.06 <sup>a</sup>	0.15±0.04 <sup>a</sup>	0.13±0.04 <sup>a</sup>	0.18±0.05 <sup>a</sup>
Spleen weight (%)	0.11±0.03 <sup>a</sup>	0.14±0.02 <sup>a</sup>	0.12±0.02 <sup>a</sup>	0.11±0.03 <sup>a</sup>	0.1±0.01 <sup>a</sup>	0.09±0.03 <sup>a</sup>
Fat weight (%)	1.26±0.06 <sup>a</sup>	1.12±0.11 <sup>a</sup>	1.06±0.09 <sup>a</sup>	0.62±0.08 <sup>b</sup>	0.61±0.06 <sup>b</sup>	0.7±0.11 <sup>b</sup>
Intestinal length (cm)	169.20±11.10 <sup>a</sup>	163.80±16.77 <sup>a</sup>	187.20±9.34 <sup>a</sup>	164±14.76 <sup>a</sup>	180.4±8.88 <sup>a</sup>	184±6.48 <sup>a</sup>
Duodenum diameter (cm)	1.82±0.33 <sup>a</sup>	1.86±0.21 <sup>a</sup>	2.06±0.22 <sup>a</sup>	1.94±0.23 <sup>a</sup>	2.08±0.15 <sup>a</sup>	2.06±0.11 <sup>a</sup>
Jejunum diameter (cm)	1.82±0.11 <sup>a</sup>	1.72±0.08 <sup>a</sup>	1.66±0.18 <sup>a</sup>	1.76±0.18 <sup>a</sup>	1.80±0.07 <sup>a</sup>	1.92±0.24 <sup>a</sup>
Ilium diameter (cm)	1.32±0.28 <sup>a</sup>	1.34±0.31 <sup>a</sup>	1.44±0.09 <sup>a</sup>	1.72±0.18 <sup>a</sup>	1.64±0.39 <sup>a</sup>	1.72±0.25 <sup>a</sup>

Means with different superscripts in the same row are significantly different ( $P \leq 0.05$ ).

G1: Control group (basal diet); G2: basal Diet+50g/ton phytase; G3: basal Diet+75g/ton phytase; G4: basal Diet+100g/ton phytase; G5: basal Diet+150g/ton phytase; G6: basal Diet+200g/ton phytase

#### Chemical meat analysis

The effects of phytase supplementation on the chemical meat analysis of Hubbard broiler chickens are illustrated in Table 6. Different levels of phytase supplementation did not significantly affect the chemical composition of broiler meat

(moisture%, fat%, ash%, calcium%, and phosphorus%). Compared to other experimental groups, G5 and G6 received higher phytase doses (150 and 200 g/ton, respectively). There was a marked increase in protein percentage.

Table 6. Effect of phytase supplementation on chemical meat analysis of Hubbard broiler chicks at the end of the experiment (Mean ± SD), (N=5).

Items	G1	G2	G3	G4	G5	G6
Moisture (%)	73.37±0.34 <sup>a</sup>	73.83±0.33 <sup>a</sup>	73.97±0.33 <sup>a</sup>	73.53±0.37 <sup>a</sup>	73.45±0.20 <sup>a</sup>	73.63±0.21 <sup>a</sup>
Protein (%)	18.9±0.29 <sup>b</sup>	19.3±0.37 <sup>b</sup>	19.63±0.34 <sup>b</sup>	19.87±0.46 <sup>b</sup>	20.85±0.29 <sup>a</sup>	21.07±0.45 <sup>a</sup>
Fat (%)	2.87±0.21 <sup>a</sup>	2.67±0.12 <sup>a</sup>	2.50±0.14 <sup>a</sup>	2.33±0.17 <sup>a</sup>	2.00±0.08 <sup>a</sup>	2.00±0.14 <sup>a</sup>
Ash (%)	1.93±0.17 <sup>a</sup>	2.07±0.21 <sup>a</sup>	2.17±0.12 <sup>a</sup>	2.27±0.17 <sup>a</sup>	2.50±0.08 <sup>a</sup>	2.47±0.17 <sup>a</sup>
Ca (mg/100g)	75.00±8.04 <sup>a</sup>	70.33±3.68 <sup>a</sup>	63.33±6.34 <sup>a</sup>	60.00±5.35 <sup>a</sup>	57.00±4.90 <sup>a</sup>	55.00±4.55 <sup>a</sup>
P (mg/100g)	203.00±4.55 <sup>a</sup>	208.33±4.64 <sup>a</sup>	210.67±3.68 <sup>a</sup>	212.00±2.94 <sup>a</sup>	217.00±2.45 <sup>a</sup>	220.00±5.10 <sup>a</sup>

Means with different superscripts in the same row are significantly different ( $P \leq 0.05$ ).

G1: Control group (basal diet); G2: basal Diet+50g/ton phytase; G3: basal Diet+75g/ton phytase; G4: basal Diet+100g/ton phytase; G5: basal Diet+150g/ton phytase; G6: basal Diet+200g/ton phytase.

## 4. DISCUSSION

Approximately sixty percent of the P in diets based on maize and soybean meal is bound to phytate, rendering it inaccessible to broiler chickens. Phytase supplements

represent the most efficient method for enhancing P utilization and availability in the diet (Selim et al., 2022). Therefore, the main purposes of the current investigation were to ascertain the dose-dependent impact of phytase supplementation on CBC, liver function tests, Ca, and P,

carcass characteristics, and chemical meat analysis of Hubbard broiler chickens.

The complete blood count is recognized as an essential tool for assessing the physiological condition of birds (Chowdhury et al., 2005). Hematological indices play a crucial role in indicating and reflecting the impacts of dietary treatments on animals. These indices provide insights into the type, quality, and quantity of feed consumed by the animals, ensuring they meet their physiological, biochemical, and metabolic requirements (Ewuola and Egbunike, 2008). The current hematological results showed that Hb, PCV, RBCs count, TLC, lymphocyte, heterophile, monocyte, and basophile were not significantly affected by dietary phytase supplementation compared to the control group. Those results coincide with those of Al-Harhi et al., (2020), who indicated that Hb, PCV, MCH, and MCHV, were not significantly affected by phytase supplementation. Similarly, Fijabi et al. (2018) found no significant variations in hematological parameters when broiler chicks were fed varying amounts of phytase enzyme at 0, 250, 500, 750, and 1000 FTU/kg. Chuka (2014) revealed no significant difference in hematological parameters between broilers fed probiotics and commercial phytase. The result of this study indicated that all the haematological parameters measured fell within the normal range (reference range) for control chickens, suggesting that the experimental animals tolerated the diets well. In contrast, Baloch et al. (2021) discovered that adding dietary phytase to broiler diets significantly elevated the levels of Hb, PCV, RBCs, and WBCs when compared to the control group.

The result of the current dietary phytase supplementation indicated a non-significant impact on serum ALT, AST, and LDH levels in broiler chickens. These results align with Hossain et al. (2022), who stated that serum ALT and AST levels in broiler chickens were not significantly affected by phytase administration. Serum levels of ALT, AST, and LDH are considered indicators of the health condition of the liver. The lack of a significant effect of the treatment on these serum enzymes implies that liver functions were not impacted by phytase administration (Hossain et al., 2022). Attia et al. (2011) demonstrated that different levels of fungal phytase did not significantly affect plasma ALT and AST. Also, Ciurescu et al. (2020) concluded that dietary phytase incorporation had no impact on LDH, ALT, or AST levels in comparison to the non-supplemented group. In contrast, Ghahri et al. (2012) found that dietary phytase supplementation significantly increased serum AST activity and reduced ALT, ALP, and LDH activities.

The results of the current investigation show that the levels of Ca and P in the serum were not affected by phytase administration. These results align with those of Ghazalah and Alsaady, (2008), who similarly observed that the plasma levels of calcium and phosphorus were not significantly altered by dietary phytase supplementation. A similar finding was also reported by Kliment and Angelovicova (2011), who found that microbial phytase supplementation (0.1%) in broiler feed mixtures did not affect blood calcium or phosphorous. Rezaei et al. (2007) demonstrated that broilers supplemented with 500 FTU/kg phytase showed no significant difference in blood phosphorus compared to the control group.

The outcomes of the current investigation demonstrated that higher doses of phytase supplementation (150 and 200 g/ton) significantly increased live weight and dressed carcass weight, while abdominal fat was significantly decreased compared to other experimental groups. These findings align with those of Marchal et al. (2021), who stated that birds

receiving a diet supplemented with 2,000 FTU/kg phytase showed higher live weight (8.0%), dressed carcass weight (17.8%), and considerably lower fat yield by the 42<sup>nd</sup> days of the experiment. Similarly, Campasino et al. (2014) noted that the carcasses of broilers receiving diets enhanced with phytase were heavier. These results coincide with those of Shirzadi et al. (2009), who discovered that incorporating phytase into the diets of broiler chickens enhanced the quality of the meat and produced the highest percentages of dressed carcasses. Also, Ennis et al. (2020) showed that giving broiler chicks 1500 FTU of phytase/kg of the diet increased processing yields, raised tender yields about carcass weights, and greatly decreased the amount of abdominal fat compared to the control group.

The present study revealed that dietary phytase administration had no impact on the percentage of breast and thigh weight, internal organ weight (including liver, gizzard, heart, spleen, thymus, and bursa), or intestinal length. These results are supported by Srikanthithasan et al. (2020), who found that after 35 days of the experiment, the weight percentages of the breast and thigh muscles were not significantly impacted by any phytase-supplemented meals. Furthermore, there was no notable variance in the weights of the gizzard and small intestine among broilers that were provided with phytase-containing diets. Similarly, Nourmohammadi et al. (2010) demonstrated that broiler chickens given a phosphorus-deficient diet supplemented with phytase up to 1000 FTU/kg showed no significant impact on carcass parameters by the 35<sup>th</sup> day of the experiment. Attia et al. (2011) noted that different doses of fungal phytase did not significantly alter the liver, spleen, pancreas, gizzard, or abdominal visceral fat. Conversely, Baloch et al. (2021) concluded that the relative weights of the heart, gizzard, spleen, and intestine were significantly increased by adding phytase to the broiler diet.

The study found that birds in G5 and G6 had a higher protein percentage in their breast meat compared to the other experimental groups. There were no significant differences between the experimental groups in the percentages of moisture, fat, ash, calcium, and phosphorus in the meat. Kriseldi et al. (2021) demonstrated that supplementing broiler diets with phytase and increasing nutrient density together improve growth performance and carcass quality. This enhancement is likely due to the combined effects of higher nutrient density, which supplies essential amino acids and energy as building blocks of muscle accretion, and phytase, which boosts protein synthesis through the release of inositol and an increase in hypothalamic dopamine levels. These results agree with Metwally et al. (2020), who discovered that the inclusion of phytase at a level of 1500 FTU/kg resulted in an elevation of the crude protein percentage in meat, while simultaneously reducing the fat and dry matter content of the meat. Also, Attia et al. (2020) observed that dietary phytase supplementation had no effects on the chemical composition of the meat of 64-day-old Sasso chickens. However, these results were different from those of Mohammed et al. (2021), who found that the chemical properties of broiler meat (DM, EE, ash, Ca, and P) were significantly different in groups that were given phytase at 1000 and 1500 FTU/kg feed.

## 5. CONCLUSIONS

The current study demonstrated that incorporating high doses (150 and 200 g/ton) of phytase in broiler diets improved live body weight and dressed carcass percentage,

and enhanced meat quality (protein%) without any changes in CBC, liver enzymes, Ca, or P.

## 6. REFERENCES

- Al-Harthi, M. A., Attia, Y. A., El-Shafey, A. S., Elgandy, M. F., 2020. Impact of phytase on improving the utilisation of pelleted broiler diets containing olive by-products. *Italian Journal of Animal Science*, 19,1, 310–318. <https://doi.org/10.1080/1828051X.2020.1740896>
- Ali, S. A. M., Abdalla, H. O., Mahgoub, I. M., Medani, W., 2011. Effect of slaughtering method on the keeping quality of broiler chickens' meat. *Egypt Poultry Science*, 31,5, 727–736.
- Attia, Y., .A., Zeweil, H. S., Alsaffar, A. A., El-Shafy, A. S., 2011. Effect of non-antibiotic feed additives as an alternative to flavomycin on productivity, meat quality and blood parameters in broilers. *Archiv Fur Geflugelkunde*, 75,1, 40–48.
- Attia, Y. A., Bovera, F., Iannaccone, F., Al-harthi, M. A., Alaqil, A. A., Zeweil, H. S., Mansour, A. E., 2020. Performance , Nutrient Digestibility and Blood Profile of Broilers Fed Different Levels of. *Animals*, 10,580, 1–13. <https://doi.org/10.3390/ani10040580>
- AOAC. 2000. Official methods of analysis. 17th ed. Gaithersburg: AOAC. Google Scholar
- Baloch, F. N., Baloch, H. N., Khan, A. U., Baloch, N. M., Baloch, I. A., Baloch, N. K., Qambrani, A. Q., Samejo, A. K., 2021 Effect of Phytase Enzyme on Organs Growth Performance and Blood Profile of Broiler. *Advances in Enzyme Research*, 09,03, 37–49 .<https://doi.org/10.4236/aer.2021.93004>
- Biesek, J., Kuzniacka, J., Banaszak, M., Adamski, M., 2020. The Quality of Carcass and Meat from Geese Fed Diets with or without Soybean Meal. *Animals*, 10,2, 200. <https://doi.org/10.3390/ani10020200>
- Campasino, A., York, T., Wyatt, C., Bedford, M. R., Dozier, W. A., 2014. Effect of increasing supplemental phytase concentration in diets fed to Hubbard × Cobb 500 male broilers from 1 to 42 days of age. *Journal of Applied Poultry Research*, 23,4, 705–714. <https://doi.org/10.3382/japr.2014-00999>
- Campbell, T. W., 1995. Avian hematology and cytology, 2nd Ed, Iowa state Uni. Press, Ames.
- Chowdhury, Smith, T. K., Boermans, H. J., Woodward, B., 2005. Effects of feed-borne Fusarium mycotoxins on hematology and immunology of laying hens. *Poultry Science*, 84,12, 1841–1850. <https://doi.org/10.1093/ps/84.12.1841>
- Chuka, E., 2014. Comparative Study of the Effects of Probiotic and Commercial Enzyme on Growth Rate, Haematology and Serum Biochemistry of Broiler Chicken. *Journal of Food Processing & Technology*, 05,09. <https://doi.org/10.4172/2157-7110.1000367>
- Ciurescu, G., Vasilachi, A., Grosu, H., 2020. Efficacy of microbial phytase on growth performance, carcass traits, bone mineralization, and blood biochemistry parameters in broiler turkeys fed raw chickpea ,Cicer arietinum L., cv. Burnas diets. *Journal of Applied Poultry Research*, 29,1, 171–184. <https://doi.org/10.1016/j.japr.2019.10.004>
- Cowieson, A. J., Wilcock, P., Bedford, M. R., 2011. Super-dosing effects of phytase in poultry and other monogastrics. *World's Poultry Science Journal*, 67,2, 225–236. <https://doi.org/10.1017/S0043933911000250>
- Dersjant-Li, Y., Awati, A., Schulze, H., Partridge, G., 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *Journal of the Science of Food and Agriculture*, 95,5, 878–896. <https://doi.org/10.1002/jsfa.6998>
- EL Enshasy, H., Dailin, D. J., Abd Manas, N. H., Wan Azlee, N. I., Eyahmalay, , Jennifer, Yahaya, , Sarah Afiqah, Abd Malek, R., Siwapiragam, V., Sukmawati, D., 2018. Current and Future Applications of Phytases in Poultry Industry: A Critical Review. *Journal of Advances in VetBio Science and Techniques*, 3,3, 65–74. <https://doi.org/10.31797/vetbio.455687>
- EN ISO 6869:2000. International standards animal feeding stuffs: determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc: method using atomic absorption spectrometry. Geneva: International Organization for Standardization. [cited 2016 Dec 15]. <https://www.iso.org/obp/ui/#iso:std:iso:6869:ed-1:v1:en>
- Ennis, C. E., Jackson, M., Gutierrez, O., Cantley, S., Wamsley, K. G. S., 2020. Phytase and carbohydrase inclusion strategies to explore synergy within low-energy diets to optimize 56-day male broiler performance and processing. *Journal of Applied Poultry Research*, 29,4, 1045–1067. <https://doi.org/10.1016/j.japr.2020.09.013>
- Ewuola, E. O., Egbunike, G. N., 2008. Haematological and serum biochemical response of growing rabbit bucks fed dietary fumonisin B1. *African Journal of Biotechnology*, 7,,23
- Fijabi, O. E., Fakayode, T. O., Eniola, O. M., and Omojola, A. B. et al., 2018. Performance, blood profile and microbial characteristics of broiler chicken fed phytase phytase supplemented diets. *Nigerian Journal of Animal Production*, 45,2, 129–136. <https://doi.org/10.51791/njap.v45i2.504>
- Ghahri, H., Rostami, D., Zandiyeh, M. A., Abbasi, R. H., 2012. The effects of phytase on performance, serum mineral levels, enzyme activities and immune function of broilers fed nutritionally marginal diets. *Middle East Journal of Scientific Research*, 11,11, 1481–1490. <https://doi.org/10.5829/idosi.mejsr.2012.11.11.6468>
- Ghazalah, A. A., Alsaady, M. A., 2008. Effect of dietary metabolizable energy and microbial phytase levels on broiler performance, nutrients digestibility and minerals utilization. *Egyptian Poultry Science Journal*, 28, 815–832.
- Grigore, D.M, Ciurescu, G., Lavinia,I., Silvia, M., Iuga, M., Mironeasa, S., Narcisa, B., 2019. Effect of low-fiber sunflower meal and phytase addition on broiler carcass traits, and meat quality. *Archiva Zootechnica*, 22,1, 32–47.
- Hossain, S., Hossain, M. A., Akter, N., Akter, M., 2022. Effects of Phytase Super Dosing on Performance, Plasma Mineral Contents and Bone Mineralization in Broiler Chicken. *International Journal of Poultry Science*, 21,1, 1–9. <https://doi.org/10.3923/ijps.2022.1.9>
- Hubbard., 2022. Hubbard efficiency plus Management guide broiler.' Available at [broiler\\_guide\\_efficiency\\_plus\\_en\\_imp\\_20220120\\_ld.pdf\\_hubbardbreeders.com](http://broiler_guide_efficiency_plus_en_imp_20220120_ld.pdf_hubbardbreeders.com)
- Jing, M., Zhao, S., Rogiewicz, A., Slominski, B. A., House, J. D., 2018. Assessment of the minimal available phosphorus needs of pullets during the pre-laying period. *Poultry Science*, 97,2, 557–567. <https://doi.org/10.3382/ps/pex313>
- Kliment, M., Angelovicova, M., 2011. The effect of microbial phytase on blood performance of broiler chickens. *Scientific Papers Animal Science and Biotechnologies*, 44,1, 58. <https://doi.org/10.1016/j.psj.2020.12.059>
- Kriseldi, R., Bedford, M. R., Dilger, R. N., Foradori, C. D., MacKay, L., & Dozier, W. A. 3rd., 2021. Effects of phytase supplementation and increased nutrient density on growth performance, carcass characteristics, and hypothalamic appetitive hormone expression and catecholamine concentrations in broilers from 1 to 43 days of age. *Poultry Science*, 100,12, 101495.
- Marchal, L., Bello, A., Sobotik, E. B., Archer, G., Dersjant-Li, Y., 202. A novel consensus bacterial 6-phytase variant completely replaced inorganic phosphate in broiler diets, maintaining growth performance and bone quality: data from two independent trials. *Poultry Science*, 100,3, 100962.
- Metwally, M. A., Farghly, M. F. A., Ismail, Z. S. H., Ghonime, M. E., Mohamed, I. A. 2020. The effect of different levels of optizyme and phytase enzymes and their interactions on the performance of broiler chickens fed corn/soybean meal: 1-broiler performance, carcass traits, blood constituents and nitrogen retention efficiency. *Egyptian Journal of Nutrition and Feeds*, 23,1, 123–136. <https://doi.org/10.21608/EJNF.2020.95831>
- Mohammed, M., Alshamiri, A., Awad, S., Ali, M., Abdalla, H. O., Ahmed, H. B., Authority, E., 2021. The effect of supplementing different levels of phytase enzyme on performance , some carcass properties and economics of broiler chickens. *Agrobiological Records*, 7182,4, 14–22. <https://doi.org/https://doi.org/10.47278/journal.abr/2020.025>
- Nourmohammadi, R., Hosseini, S. M., & Farhangfar, H., 2010. Influence of citric acid and microbial phytase on growth

- performance and carcass characteristics of broiler chickens. *American Journal of Animal and Veterinary Sciences*, 5,4, 282–288. <https://doi.org/10.3844/AJAVSP.2010.282.288>
32. Rezaei, M., Borbor, S., Zaghari, M., Teimouri, A., 2007. Effect of Phytase Supplementation on Nutrients Availability and Performance of Broiler Chicks. *International Journal of Poultry Science*, 6,1, 55–58. <https://doi.org/10.3923/ijps.2007.55.58>.
  33. Schalm, O. W. and N. C. Jain., 1975. *Veterinary haematology*. 3rd Edn. Lea and Febiger, Philadelphia.
  34. Selim, S., Abdel-Megeid, N. S., Khalifa, H. K., Fakiha, K. G., Majrashi, K. A., Hussein, E., 2022. Efficacy of Various Feed Additives on Performance, Nutrient Digestibility, Bone Quality, Blood Constituents, and Phosphorus Absorption and Utilization of Broiler Chickens Fed Low Phosphorus Diet. *Animals*, 12,14. <https://doi.org/10.3390/ani12141742>
  35. Selle, P. H., Macelline, S. P., Chrystal, P. V., Liu, S. Y., 2023. The Contribution of Phytate-Degrading Enzymes to Chicken-Meat Production. *Animals*, 13,4, 1–20. <https://doi.org/10.3390/ani13040603>
  36. Shirzadi, H., Moravej, H., Shivazad, M., 2009. Comparison of the effects of different kinds of NSP enzymes on the performance, water intake, litter moisture and jejunal digesta viscosity of broilers fed barley-based diet. *Journal of Food, Agriculture and Environment*, 7,3, 615–619.
  37. Siekmann, L., Bonora, R., Burtis, C., Ceriotti, F., Clerc-Renaud, P., Féraud, G., Ferrero, C., Forest, J.-C., Franck, P., Gella, F., Hoelzel, W., Jørgensen, P., Kanno, T., Kessner, A., Klauke, R., Kristiansen, N., Lessinger, J.-M., Linsinger, T., Misaki, H., Schumann, G., 2002. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C. Part 1. The Concept of Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes. *Clinical Chemistry and Laboratory Medicine: CCLM / FESCC*, 40, 631–634. <https://doi.org/10.1515/CCLM.2002.109>
  38. Srikanthithasan, K., Macelline, S. P., Wickramasuriya, S. S., Tharangani, H., Li-Ang, Jayasena, D. D., Heo, J. M., 2020. Effects of adding phytase from aspergillus Niger to a low phosphorus diet on growth performance, Tibia characteristics, phosphorus excretion, and meat quality of broilers 35 days after hatching. *Journal of Poultry Science*, 57,1, 28–36 <https://doi.org/10.2141/jpsa.0180143>
  39. Spss, I., 2007. *SPSS version 16.0*. Chicago, IL: SPSS Incorporated
  40. Walk, C. L., Rama Rao, S. V., 2020. Increasing dietary phytate has a significant anti-nutrient effect on apparent ileal amino acid digestibility and digestible amino acid intake requiring increasing doses of phytase as evidenced by prediction equations in broilers. *Poultry Science*, 99,1, 290–300. <https://doi.org/10.3382/PS/PEZ489>
  41. Young, D. S., 1997. Effects of Drugs on Clinical Laboratory Tests. *Annals of Clinical Biochemistry*, 34,6, 579–581. <https://doi.org/10.1177/000456329703400601>