تأثير إضافة إنزيم الفيتيز الميكروبي لعلائق الدواجن على بعض القياسات الفسيولوجية و البيئية

رسالة مقدمة من

إيناس إبراهيم محمد إسماعيل

بكالوريوس في العلوم الزراعية (إنتاج دواجن), جامعة القاهرة ٢٠٠٠

لاستكمال متطلبات ا لحصول على درجة الماجستير
في العلوم البيئية

قسم العلوم الزراعية
معهد الدراسات و البحوث البيئية
جامعة عين شمس

٢٠٠٦

صفحة الموافقة على الرسالة

تأثير إضافة إنزيم الفيتيز الميكروبي لعلائق الدواجن على بعض القياسات الفسيولوجية و البيئية

رسالة مقدمة من

إيناس إبراهيم محمد إسماعيل
بكالوريوس في العلوم الزراعية (إنتاج دواجن), جامعة القاهرة ٢٠٠٠

لاستكمال متطلبات الحصول على درجة الماجستير
في العلوم البيئية
قسم العلوم الزراعية

وقد تمت مناقشة الرسالة و الموافقة عليها:

اللجنة	**التوقيع**
أ.د./ جعفر محمود الجندي أستاذ إنتاج الدواجن,قسم الإنتاج الحيواني, كلية الزراعة, بمشتهر, جامعة بنها.	....
د./ نبيل محمد حسن المدني أستاذ مساعد تغذية الدواجن, قسم إنتاج الدواجن ,كلية الزراعة, جامعة عين شمس.	....
أ.د./ إبراهيم الوردانى السيد حسن أستاذ فسيولوجي الدواجن, قسم إنتاج الدواجن, كلية الزراعة, جامعة عين شمس (المشرف الرئيسي).	....

٢٠٠٦

تأثير إضافة إنزيم الفيتيز الميكروبي لعلائق الدواجن على بعض القياسات الفسيولوجية و البيئية

رسالة مقدمة من

إيناس إبراهيم محمد إسماعيل

بكالوريوس في العلوم الزراعية (إنتاج دواجن), جامعة القاهرة ٢٠٠٠

لاستكمال متطلبات الحصول على درجة الماجستير
في العلوم البيئية
قسم العلوم الزراعية

تحت إشراف:

١ـ أ.د/ إبراهيم الوردانى السيد حسن
أستاذ فسيولوجي الدواجن, كلية الزراعة, جامعة عين شمس.

٢ـد./ عبد المجيد مخيمر عبد المقصود
أستاذ مساعد فسيولوجي الدواجن, كلية الزراعة, جامعة عين شمس.

٣ـ د./ مجدي سيد حسن حسن
باحث بمعهد بحوث الإنتاج الحيواني, مركز البحوث الزراعية .

ختم الإجازة

أجيزت الرسالة بتاريخ / / ٢٠٠٦

موافقة مجلس المعهد	**موافقة مجلس الجامعة**
/ / ٢٠٠٦	/ / ٢٠٠٦

٢٠٠٦

الملخص العربي

أجريت هذه الدراسة بمعمل الفسيولوجى التابع لقسم إنتاج الدواجن – كلية الزراعة – جامعة عين شمس . و كان الهدف منها هو دراسةَ تأثيراتَ إنزيمِ الفيتيز الميكروبي المضاف لعلا يق الدواجن على بعض القياسات الفسيولوجية و البيئية .

استخدم فى هذه الدراسة ٢٧٠ كتكوت لحم عمر يوم (سلالة الروس) , حيث قسمت عشوائا إلى ٩ مجموعات تشمل كل مجموعة ٣٠ كتكوت فى ٣ مكررات بكل منها ١٠ كتاكيت استخدم ثلاث مستويات من الفسفور المتاح (٠,٤٥-٠,٣٠-٠,١٥ %)،و ثلاث مستويات من إنزيم الفيتيز الميكروبي (صفر-٥٠٠-٧٥٠) وحدة دولية فيتيز/كيلو جرام علف. تم تغذية الكتاكيت ورعايتها بطريقة موحدة لجميع المعاملات.

و يمكن تلخيص أهم النتائج المتحصل عليها كالاتى :

١- وزن الجسم :

أظهرت النتائج زيادة معنوية و ملحوظ فى أوزان المجموعات المغذاة على علائق بها ٧٥٠ وحدة دولية من إنزيم الفيتيز الميكروبي مع ٠,١٥% فسفور متاح و نفس النتائج بالنسبة للوزن المكتسب كما لوحظ ان وزن مجموعة المقارنة (٠,٤٥% فسفور بدون فيتيز) كانت الأكثر انخفاضا مقارنة بباقى المجموعات.

٢- الاستهلاك الغذائي و معامل التحويل الغذائي:

كان استهلاك الغذاء للمجموعات المغذاة على (٧٥٠,٥٠٠ وحدة دولية فيتيز /كجم عليقة) مرتفعا بصورة معنوية خلال فترة التجربة من عمر يوم و حتى ٦ اسابيع من العمر, كما تحسنت الكفاءة الغذائية بإضافة الفيتيز الميكروبى.

٣- خصائص الذبيحة :

- الأعضاء التمثيلية (كنسبة من وزن الجسم)

لم تلاحظ اى زيادة معنوية فى الأوزان النسبية لبعض اعضاء الجسم التى لها صلة مباشرة بعملية التمثيل الغذائى (الغدة الدرقية , الكبد , الكلية) عند عمر ٢, ٤, و ٦ أسابيع .

- الأعضاء المناعية (كنسبة من وزن الجسم)

كان الوزن النسبى للغدة الثيموثية مرتفعا فى عمر ٤ أسابيع و ذلك فى المجاميع المغذاة على ٧٥٠ وحدة دولية فيتيز . أما عند عمر ٦ أسابيع , فلم يكون هناك اى تاتير معنوى لكل المعاملات.

لم يؤثر إضافة إنزيم الفيتيز الميكروبى (٥٠٠ , ٧٥٠ وحدة دولية فيتيز /كجم علف) فى الوزن النسبي لغدة البرسا على عمر أسبوعان . و كانت الزيادة بصفة عامة غير معنوية .

كذلك لم يكن هناك تاثير معنوى للتداخل بين إنزيم الفيتيز الميكروبى ومستوى الفسفور المتاح على الوزن النسبى لغدتى البرسا و غدة الطحال على أعمار ٢, ٤, ٦ أسابيع.

٤- مقاييس الدم :

- أظهرت النتائج انه بزيادة نسب إنزيم الفيتيز الميكروبى يزداد معنويا محتوى بلازما الدم من الكالسيوم فى كل الأعمار المختبرة.
- لم يتاثر تركيز بلازما الدم معنويا بإضافة الفسفور المتاح عند أعمار ٢ , ٤ أسابيع. بينما كان تأثير إضافة العلائق المخفضة (٠,١٥, ٠,٣٠ %) من الفسفور المتاح معنويا على خفض مستوى الفسفور فى الدم .
- انخفض محتوى بلازما الدم من الكوليسترول باستخدام الفوسفور المتاح – بينما ارتفع محتوى بلازما الدم من الكوليسترول بإضافة إنزيم الفيتيز الميكروبى.
- لم تلاحظ اية زيادة معنوية فى محتوى البلازما من البروتين الكلى عند كل الأعمار فى المجاميع المختبرة.
- انخفض محتوى بلازما الدم من الدهون الكلية بانخفاض محتوى العليقة من الفسفور المتاح و زيادة نسب إنزيم الفيتيز الميكروبى.

٥- قياسات عظمة الساق:
أظهـرت النتـائج انخفـاض الـوزن النسـبي لعظمـة السـاق باسـتخدام (٠,٣٠,٠١٥ %) من الفسفور المتاح .
لا يوجد اختلاف معنوى بين إضافة كل من إنزيم الفيتيز الميكروبى و الفسفور المتاح عند الأعمار ٢ و ٦ اسابيع , بينما يوجد اختلاف معنوى ملحوظ لطول عظمة الساق النسبى عند المجاميع المغذاة على ٠,١٥% فسفور متاح (٨,٥٣ سم) مقارنة بالمجاميع المغذاة على (٠,٣٠,٠,٤٥ %) كانت (٧,٧٦,٧,٩٦سم) على التوالى.
- أظهرت النتائج انه مع التقدم فى العمر تزداد قوة كسر عظام الساق مع إضافة إنزيم الفيتيز الميكروبى, خصوصا عند ٦ أسابيع من العمر.

٦- الخارج من الفسفور فى الزرق:
أوضحت النتائج انه كان اقل نسبة من الفسفور المفرز فى الزرق للمجاميع المغذاة على ٠,١٥% فسفور متاح مع وجود الفيتيز (١٢,٠٧ ,١٣,٤٧ مليجرام/جرام مادة جافة) عند عمرى ٣ و ٦ اسابيع , بينما المجاميع المغذاة على ٠,٤٥% + ٧٥٠ وحدة دولية فيتيز كان (٢٥,٧٣ و, ٢١,٦٧ مليجرام/جرام مادة جافة) عند ٣ و ٦ أسابيع على التوالى .

٧- التركيب الهستولوجى لبعض الغدد و الكلى:
- أتضح من الفحص الهستولوجى لقطاعات الغدة الدرقية و جارات الدرقية وجود زيادة فى استجابتها الفسيولوجية لإضافة الإنزيم و كذلك نتيجة انخفاض مستوى الفسفور المتاح بالعليقة .
- هناك زيادة ملحوظة فى حجم الحويصلات الدرقية و فى شكل النسيج الطلائى المبطن لها و ذلك فى عمر ٦ اسابيع و بصفة خاصة فى تلك المجموعات المغذاة على عليقة منخفضة الفسفور و المضاف اليها الإنزيم باى مستوى .
- لم يتأثر التركيب الخلوى لنسيج الكلية بالمعاملات المختلفة و كان تاثير نقص الفسفور فى العليقة جيدا بالنسبة لزيادة عدد الأنابيب النخاعية المجمعة للبول فى الكلى .

و من خلال هذه الدراسة يتضح أن استخدام إنزيم الفيتيز الميكروبى فى علائق الدواجن النباتية التى تحتوى على مستوى منخفض من الفسفور مع ٥٠٠ وحدة دولية فيتيز يحسن معنويا كلا من وزن الجسم – الاستهلاك الغذائى – مقاييس الدم – قياسات العظم و تقليل الفسفور الخارج من الزرق للحد من التلوث البيئى .

و توصى الدراسة على ضوء النتائج التى تم الحصول عليها باستخدام إنزيم الفيتيز مع تقليل نسبة الفسفور فى العلائق (حتى ٠,١٥%) و هذا يتيح انخفاضا كبيرا فى كمية الفسفور المفرز فى زرق الدواجن كما يساعد على تقليل التلوث البيئى الناتج عن زيادته علما بان استخدام الإنزيم مع هذه النسبة من الفسفور لم تؤثر سلبا على الأداء الانتاجى او الحالة الفسيولوجية للطائر و ذلك من خلال دراستنا للتركيب الخلوى للغدة الدرقية و الجارات درقية و الكلية .

المستخلص

إيناس إبراهيم محمد إسماعيل ." تأثير إضافة إنزيم الفيتيز الميكروبي لعلائق الدواجن على بعض القياسات الفسيولوجية و البيئية". استكمال درجة الماجستير (قسم العلوم الزراعية) – معهد الدراسات و البحوث البيئية – جامعة عين شمس – ٢٠٠٦

أجريت هذه الدراسة بمعمل الفسيولوجى التابع لقسم إنتاج الدواجن – كلية الزراعة – جامعة عين شمس . و كان الهدف منها هو دراسةَ تأثيراتَ إنزيمِ الفيتيز الميكروبي المضاف لعلا يق الدواجن على بعض القياسات الفسيولوجية و البيئية .استخدم فى هذه الدراسة ٢٧٠ كتكوت لحم عمر يوم (سلالة الروس) , حيث قسمت عشوائيا إلى ٩ مجموعات تشمل كل مجموعة ٣٠ كتكوت فى ٣ مكررات بكل منها ١٠ كتاكيت استخدم ثلاث مستويات من الفسفور المتاح (٠,٤٥-٠,٣٠-٠,١٥ %)،و ثلاث مستويات من إنزيم الفيتيز الميكروبي (صفر-٥٠٠-٧٥٠) وحدة دولية فيتيز /كيلو جرام علف. تم تغذية الكتاكيت ورعايتها بطريقة موحدة لجميع المعاملات.

- كان هناك تاتير معنوى بالنسبة لوزن الجسم للطيور المغذاة على ٥٠٠ وحدة فيتيز و ٠,٣٠% فوسفور متاح.
- كلما زاد مستوى إنزيم الفيتيز كلما زاد الاستهلاك الغذائي .
- كان أفضل أداء لمعامل التحويل الغذائي فى المجاميع المغذاة على ٠,١٥% فوسفور متاح و ٥٠٠ وحدة فيتيز .
- الطيور المغذاة على ٠,١٥% فوسفور متاح أعطت زيادة معنوية فى الوزن النسبى للكلية مقارنة بالمجاميع المغذاة على ٠,٤٥ , ٠,٣٠ % فوسفور متاح.
- محتوى بلازما الدم من الكالسيوم زاد معنويا بزيادة إنزيم الفيتيز, بينما لم يكن البروتين الكلى معنويا طول فترة التجربة.
- انخفض محتوى الكوليسترول و بلازما الدم من الدهون الكلية بانخفاض محتوى العليقة من الفسفور المتاح و زيادة نسب إنزيم الفيتيز الميكروبى.

- لم يؤثر انزيم الفيتيز على وزن عظمة الساق بينما كان يلاحظ تاثير معنوى فى طولها للطيور المغذاة على ٠,١٥% فوسفور متاح.
- كانت الطيور المغذاة على٠,١٥% فوسفور متاح و بدون إضافة إنزيم الفيتيز أعطت اقل نسبة من الفوسفور الخارج فى الزرق .
- كانت هناك تغيرات كبيرة فى القطاعات الهستولوجية للغدة الدرقية , جارات الدرقية و الكلية بتأثير المستويات المختلفة من الفوسفور المتاح و إنزيم الفيتيز الميكروبى .

و توصى الدراسة على ضوء هذه النتائج بان إضافة إنزيم الفيتيز الميكروبى لعلائق الدواجن للمستويات المنخفضة من الفوسفور المتاح يحسن من الأداء الانتاجى للطيور و يقلل من تلوث البيئة بعنصر الفوسفور الخارج فى الزرق.

INTRODUCTION

It is well known that plant feedstuffs, specially cereals, legumes and oilseed meals, are considered the most little cost ingredients of poultry diets. Poultry feeds contain ingredients of plant origin from which 50-80% of the total phosphorus is present as phytate phosphorus. The inability of poultry to utilize phytate phosphorus, due to lack of endogenous phytase results in a substantial excretion of phosphorus into the environment. The disposal of animal waste leading to the accumulation of phosphorus in soils and subsequently its entry into surface and ground waters has sparked off major environmental concerns. In the absence of phytase, inorganic feed phosphates are often added to diets to meet the phosphorus needs of poultry, (**Chung, 2002).** The phosphorus of plant origin is present as phytic acid in the form of myoinositol phosphates (**Sebastian**, **et al. 1997)**. Phosphorus in phytic acid form (phytate-P) is poorly available to most monogastric animals such as poultry, because of the lack of endogenous phytase (the enzyme that hydrolysis phytic acid into inositol and orthophosphate, (**El-Medany, and El- Afifi, 2002).** The addition of phytase to poultry diets to release phosphorus from its phytic acid form was reported to improve dietary phosphorus and minerals utilization, **(Lan, et al. 2002, and Lim et al.2003**).

Phytase is one of the alternatives that help to reduce phosphorus excretion by poultry at least 30- 40% in order to fully explore the potential of phytase, (**Chung,2002).**

It is of paramount importance to identify factors that potentially influence its responses in practical diets, (**Yan et al. 2001**). The amount of phytate degraded by dietary phytase inclusion reduces the need for inorganic phosphorus addition by 1 to 102 g/kg in practical diets for pigs and poultry (**Yi et al.,1994**).

There has been a recent interest among poultry nutritionists in studying the effects of phytase on broiler performance. It was found that supplementing phytase to grower diets containing reduced levels of non-phytate phosphorus and calcium significantly improved performance and bone strength of broilers (**Sohail and Roland, 1999).**

Namkung and Leeson (1999) showed that growth rate and feed conversion of birds fed the low Pdiets containing microbial phytase were comparable with or even better than those for birds fed the control diets. They also found that phytase improved the digestibilities of essential amino acids, which was also confirmed by **Ravindran et al. (2001**). Additional studies showed the beneficial effects of phytase in terms of improving P utilization via increased plasma phosphate and tibia ash content (**Klunter and Steimle,2001).**

Recent studies have indicate that phytase supplementation in phosphorus deficient broiler diets improves phytate phosphorus utilization and broiler performance (**Desouky, 2001; Mohamed et al., 2001; El-Medany and El-Afifi, 2002; Salem et al.,2003**; **Sohail and Roland, 2002 and Rutherfured et al., 2002)**.

The aim of this study to determine the effect of dietary supplementation of microbial phytase on some physiological and environmental parameters of chickens.

REVIEW OF LITERATURE

1-Phosphorus and environmental pollution:

Pollution from animal manure has become one of the most challenging environmental problems. Therefore, the amount of nutrients (such as phosphorus and nitrogen contents) being excreted in animal manure can be reduced to minimize environmental pollution. The nutrients in manure can be utilized by crops as a source of fertilizers. Poultry manure, with their relatively high nutrient concentrations, may be a valuable asset, if correctly used in, new reclaimed areas in Egypt. Application of phytase in poultry diets in Egypt not only reduces the amounts of phosphorus and nitrogen excretion by poultry, but also, reduce environmental pollution (**Attia et al.,2001; Mohamed et al.,2001; Abd El-Samee,2002 and Attia,2003a,b**).

Chung, (2002) noted that the disposal of animal waste leading to the accumulation of phosphorus in soils and subsequently its entry into surface and ground waters has sparked off major environmental concerns. Poultry feed contain ingredients of plant origin from which 50-80% of the total phosphorus is present as phytate phosphorus. The inability of poultry to utilize phytate phosphorus due to lake of endogenous phytase results in a substantial excretion of phosphorus into the environment.

Continued pressure to reduce phosphorus excretion has investigated the search for alternatives that may help improve dietary phosphorus utilization.

Garcia et al. (2003) mentioned that only 30-40% of phytate phosphorus is available, therefore, it should be necessary to supplement birds diets with inorganic phosphorus (p) to satisfy the minimum requirement which may increases costs, p excretion and hence the environmental pollution.

Abd-Ellatif and Kamal, (2003) concluded that fecal calcium and phosphorus output was significantly reduced by adding phytase (750 U/kg) by 32.9% and 27.9% respectively in the phytase-supplemented groups. Results suggest that dietary phosphorus can be reduced without seriously affecting the skeleton of broilers and that phytase would have other benefits by reducing the cost of the diet as improving birds live weight gain, in addition to reducing environmental pollution as manifested by reduced fecal phosphorus output.

El Medany et al.,(1999) reported that dicalcium phosphate and bone meals could be replaced by the Cheap sources (raw rock phosphates or triple super phosphate) without adverse effect on growth rate, feed intake and feed conversion which did not differ significantly among various phosphate sources. Also, mortality rate, tibia ash, weight and length, was not affected by different sources of phosphate.

They concluded that further research is needed to investigate the possibility of fluorine accumulation in the body of the birds if they received raw rock phosphate for long periods.

Lima et al., (1995) found that addition of phytase can reduce phosphorus excreted in poultry manure which reduce phosphorus pollution in water and soil. Accumulation of phosphorus in the environment due to the disposal of animal waste has forced the livestock industry to better use the phosphorus available in feed ingredients. Phytase has proved able to release this phosphorus if it is used in the right way.

Zanini and Sazzad, (1999) reported that the addition of the exogenous enzyme phytase has been used to reduce the pollutant residues, through improving the utilization of phytate-bound minerals in pig and poultry diets and decreasing the use of inorganic sources.

An EMS need not comply with ISO standards; nevertheless, independent certification of a producer's commitment to environmental protection can be a valuable marketing tool that could help recoup all or part of the expense of developing and implementing an EMS. ISO 14000 is the only internationally recognized standard for environmental management systems. It seems prudent 2 that most, if not all, intensive pig and poultry producers requiring an IPC license should strive to implement an ISO 14000 compliant EMS. This means that the addition of expensive phosphorus sources like dicalcium phosphate can be reduced.

Furthermore, phytase can improve the growth rate, feed conversion and lower phosphorus excreted in poultry manure which reduce phosphorus pollution in water and soil **Magette, et al .,(2001).**

2-Chemical structure of phytate:

Phytic acid (phytate) is an organic complex generally regarded as the primary storage of both phosphate and inositol in plants. Phytic acid bears six phosphate groups on six-carbon molecule with a low molecular weight of 600. The structure of phytic acid proposed by **Anderson, (1914)** is now generally accepted because many of the physicochemical properties, interactions and nutritional effects can best be explained by this model (Plate a,b). On the basis of Anderson structure, the systemic name of phytic acid is myoinositol-1, 2, 3, 4, 5, 6-hexakis (dihydrogen phosphate).

Phytate not only considered as a nutrient because of its contents of phosphorus, but also as an anti-nutritional factor because it binds essential nutrients and reduce their availability. The alearone layer is the major site of phytate deposition in most cereals, whereas it is distributed uniformly through the kernels in dicotyledonous seeds, including oil seeds and grain legumes **Lott, (1984**).

In general, nonphytate phosphorus is a chemically defined entity, albeit it cannot be chemically analysed. It is derived mathematically by subtracting analysed phytate phosphorus from analysed total phosphorus **Chung, (2002**). .

Plate (a): Structure of phytic acid proposed by Anderson (1914)

Plate (b): Structure of the phytate molecule (Lott 1984)

Factors influencing phytate activity:

The hydrolysis and absorption of phytate phosphorus by poultry has been shown to be influenced by many factors including:

1- Dietary calcium and phosphorus levels:

Phytate phosphorus utilization by poultry has been shown to be influenced by both calcium and phosphorus levels in diet (**Mohamed et al.,1991**). At very high levels of calcium, phytate hydrolysis may be completely prevented **Taylor, (1965).** Under practical feeding conditions where calcium and phosphorus must be added to the diets for maximum performance and bone calcification, phytate phosphorus is probably utilized very little by poultry. However, **Mohamed et al., (199**1) reported that phytate phosphorus utilization was increased by 15% when dietary calcium levels were reduced from 1% to 0.5% in broiler diets. They added that the adverse impact of high phytate , low inorganic phosphorus diet on chicks were overcome by lowering the calcium level and / or increasing the vitamin of concentration.

Moreover, **Harms et al. (1962**) showed that widening the calcium: phosphorus ratio in diets from 1:1 to 2:1 decreased the availability of the phosphorus from phytic acid to a greater extent than from inorganic supplements such as phosphate.

2- Vitamin D_3 :

Vitamin D_3 primary function is the enhancement of intestinal absorption and mobilization, retention and bone deposition of calcium and phosphorus. Addition of vitamin D_3 markedly enhanced the amount of phytate phosphorus retained by chickens (**Edwards, 1991, 1993 and Mohamed et al., 199**1). In these studies the utilization of phytate phosphorus was increased from 31-50 % to 68-87% . This improvement may be attributed to one or more of the following mechanisms: 1- increased synthesis or activity of intestinal phytase (**Shafey et al., 1991**) 2- increased phytate hydrolysis (**Mohamed et al., 199**1) by stimulation of calcium absorption, thus rendering the phytate more soluble and available for utilization, and 3- enhanced absorption of phosphorus (**Wasserman and Taylor, 1973).**

3- Age of bird:

The ability of poultry to utilize phytate phosphorus increased with progress of age (**Edwards et al., 1989**). It is generally agreed that older birds hydrolyze phytate phosphorus to a greater extent than chicks (**Peeler, 1972**), the basis of this being that there is more phytase activity present in the gastrointestinal tract of older birds.

4- Type of dietary ingredients:

Difference in solubility of phytate from different sources have been reported by **De Boland et al. (1975**), who found that the phytate, in soybean meal, was more soluble than that in sesame meal. This would suggest differences in the extent of hydrolysis of phytates from different feedstuffs withen the gastrointestinal tract, if one assumes that soluble phytate is better substrate for enzyme.

5- Source of dietary fiber:

Source of dietary fiber have a significant influence on phytate phosphorus utilization. **Ballam et al., (1984**) reported that the hydrolysis of phytate was reduced by cotton seed hulls but unaffected by wheat bran. At a low calcium levels, alfalfa meal cellulose significantly increased phytate hydrolysis, whereas it was reduced by rice bran. These differences in phytate hydrolysis between fibrous feedstuffs may be related to the chemical variation within the fiber fraction.

6- Genotype of birds:

Limited evidence indicate that there may be breed and strain effect on the utilization of phytate phosphorus. **Edwards, (1983)** reported that the average retention of phytate phosphorus by Leghorn chickens was greater than that by meat-type broilers (56 vs. 37 %).

7-Processing of feed:

In general, phytic acid is relatively heat stable. However, its treatment in autoclave at higher temperatures can cause significant destruction of phytic acid and reduced phytate content in soybean protein (**Kratzer et al., 1959**), rice bran by 50% (**Takemasa and Hijikuro,1991)** and sesame meal by 20% (**Lease, 1966).**

3-Phytase mode of action:

Phytase (myo-inositol hexakisphosphate phosphohydrolase) is an enzyme that hydrolyses phytate to inositol and inorganic phosphate. Phytase is present in most cereals, but its activity varies widely amongst cereals (**Bartnik and Szajranska, 1987**).

Intrinsic phytase presented in seeds of higher plants (e.g. wheat) is recognized as 6-phytase. Phytase (microbial phytase) is also known to be produced by produced by fungi (Saccharomyces cervisiae) and Aspergillus (Aspergillus ficuum and Aspergillus niger), bacteria (Pseudomonas and Bacillus subtillus) and rumen microorganisms. Microbial phytase is recognized as 3-phytase. . Microbial phytase has a broader pH activity range than plant phytase (**Eeckhout and De Paepe, 1991**), and therefore it is more effective within the gastro-intestinal environment . .

Phytase activity (FTU) is assayed by measuring the amount of inorganic phosphate released from the hydrolysis of sodium phytate by phytase (**Engelen et al., 1994**).

Phytase activity is expressed as FTU, FYT, PU or U, all are same and defined as the amount of enzyme that liberates 1µmol inorganic orthophosphate / min. from 0.0051 mol sodium phytate / 1 min. at 37 ˚C and pH 5.5. Microbial phytase is considered as a raw material containing a high phosphorus value and can be used in linear programming for feed formation. The rate of incorporation of phytase to diet depends on feed costs, total phosphorus content in diet and processing of feed. Phytase is available in two forms , as a powder or a liquid **Mohamed, (2004)**

The action of these phytases differ in that the 3-phytase first hydrolyses the phosphate group at position number 3 on the phytate molecule, followed by the other phosphate groups, while the 6-phytase first hydrolyses the phosphate group at position number 6, and then the other groups (**Kies et al., 2001**).

Tamim et al. (2004) studied, in vitro, phytate phosphorus (PP) hydrolysis by a 3-phytase (0,500 U of phytase A /kg of diet), 6-phytase (500 U of phytase B) at pH 2.5 and 6.5 with added Ca levels equivalent to 0,0.1,0.2,0.4,0.7 or 0.9% of the diet, then test these effects in vivo using 2 levels of Ca (0,0.5% of $CaCO_3$) to corn-soybean meal. They included that 3-phytase improved (($p<0.05$) ileal PP disappearance from 25.4 to 58.9 % in diets containing 0 and 0.5% added Ca, but

The improvement was less pronounced with a 6-phytase.

Onyango et al. (2004) studied the efficacy of three E. coli-derived phytase preparations on the performance of broilers using all 3-phytase from different yeast production. A was produced in Pichia oastoris, B in Schizosaccharomyces pombe, and C in Saccharomyces cerevisiae all A,B, an C at 1000 U/kg of feed . They reported that all 3 preparations increased ($p<0.05$) Ca retention with phytase B or C showing a better retention of Ca than phytase A. All 3 phytase preparation showed similar P use as indicated by BW gain and tibia bone characteristics.

The enzyme is sensitive to a combination of factors-namely temperature, moisture and time. Today, new techniques are used and contribute to products that are sufficiently stable under most practical condition such as 1- selection of phytase producing micro-organisms from that water pools or geysers, 2- alter the enzyme by protein engineering-for instance, by replacing certain amino acids in the phytase molecule, 3- coating the enzyme product with certain substances (**Kies et al.,2001)**. In addition, phytase activity will be reduced if diets containing phytase –rich ingredients are subjected to temperatures above 60˚C during processing such as pelleting (**Desouky, 2001).**

4-Effect of phytase supplementation on:

4.1.Growth performance:

A number of studies have indicated that supplementing poultry diets with microbial phytase results in improved performance (**Farrell and Martin**, **1988 and Waldroup, et al.,2000**), particularly when dietary levels of nonphytate-p (npp) are low (**Denbow et al., 1995 and Gordon and Roland, 1997).** The improvements in growth of broiler chicks fed on a low-npp diet with phytase supplementation may be due to an increase in absorbed phosphorus (**Waldroup, 1999**), or the release of other minerals from the phytate-mineral complex (**Sebastian et al . 1996a)**, with an increase in digestibility of protein and amino acids (**Van der Klis and Versteegh, 1991).**

Abou El-Wafa, et al. (2005) used two levels of microbial phytase (0 and 750 FTU/kg diet**),** three different energy levels (2800, 3000 and 3200 kcal ME/kg diet) and two dietary phosphorus sources (bone meal BM, and di-calcium phosphate DCP). They observed that phytase supplementation to low-nPP diets improved growth performance. Diets formulated based on recommended energy level (3200 kcal ME/kg) significantly increased live body weight gain.

Viveros et al, (2002) studied the effect of the inclution of 500 U phytase /kg diet on performance of broiler chicks (0 to 6 weeks of age) fed different dietary levels of npp (0.35 and 0.22%) from 0 to 3 weeks of age and (0.27 and 0.14% from 3 to 6 weeks of age).

A positive control, adequate in npp (0.45% starter diet and 0.37% grower-finisher diets) and Ca without phytase was used. They found that phytase supplementation improved weight gain at 3 and 6 wk of age by 6.7% and 6.1% , respectively, and for the entire period 6.3%. However, feed efficiency was not affected at any stage by addition of phytase. Performance of chicks fed supplemental phytase with 0.35% (0 to 3 wks) and 0.27% (3 to 6 wks) npp were comparable to those fed the control diet.

Atia et al, (2003a) found that body weight at 28 days of age therefore of chicks fed the diet containing 20/18% crude protein mixed protein source CP MPS or phytase supplemented 18/16% CP MPS was heavier than that of those fed 20/18 CP SB or unsupplemented 18/16 CP MPS. There were insignificant decrease (4.8%) in growth of broiler when low protein level was compared to its counterpart groups fed the high level of CP. Phytase addition increased feed intake compared to its counterpart control group up to 56 days of age. Also, phytase significantly increased feed intake compared to 20/18 % CP SB diet during the most of the experimental period. However, phytase addition to low protein diet improved significantly feed conversion ratio compared to its negative control during 57-84 days of age.

Lan et al, (2002) noted that there were significant effects of dietary treatments on body weight, body weight gain, feed intake and feed conversion ratio. Chicks fed a low nonphytate phosphorus (npp) diet without active mitsuokella jalaudinii culture supplementation had significantly lower body weight at days 21 and 42 compared to those fed other dietary treatments.

Cheng et al, (2004) mentioned that addition of solanum glaucophyllum (SG) combination with phytase or phytase to the basel diet increased weight gain and no significant differences in feed efficiency among dietary treatment when broilers fed basal diet (0.41% nonphytate p) supplemented with phytase from 7 to 28 day of age.

Qota et al, (2002) studied the effect of adding soaked linseed cake (SLC) to the growing broiler diets at 0,2.5,5.0, and 10% with phytase or biogen supplementation during 1 to 42 days of age. Phytase and biogen addition improved growth by 2.6% and 1.9% compared to their counterpart control group, respectively. The amelioration of growth depression is a reflection of the increase in feed intake of broiler on these diets.

Sebastian et al. (1996a) studied the effect of two levels of microbial phytase supplementation(0 and 600 U/kg diet) to corn –soybean diets containing two dietary p – levels (0.46% and 0.33%) on the broiler performance from 0-21 days of age. They found that phytase supplementation of a low-p diet significantly ($p \leq 0.05$) increased the body weight and feed intake of male and female broiler chicks.

Sebastian et al. (1996b) determined the efficacy of supplemental microbial phytase (0 and 600 U/kg diet) at three dietary calcium levels (0.6, 1.5 and 1.25%) on growth performance of broiler chickens fed a low-p corn-soybean diet (0.31%) up to 21 days of age. They found that phytase supplementation significantly ($p \leq 0.0001$) increased the body weight and improved the feed efficiency. The optimum growth performance was achieved at the low (0.6%) dietary Ca level supplemented with phytase compared to recommended (1%) or high dietary Ca level (1.25%).

Sebastian, et al.(1997) showed the effect of microbial phytase supplementation on CP and amino acid (AA) digestibility at 28 days of age, using different levels of Ca and p and phytase : p and Ca levels were : normal p- normal Ca (0.45% available p(p_a) , 1.0% Ca) , low p- normal Ca (0.35% p_a , 0.6% Ca) ; and phytase at 0 and 600 U/kg diet. They found that phytase supplementation increased body weight gain ($p<0.014$) and feed intake ($p<0.004$) at 19 d in male chickens; in females, phytase increased ($p< 0.012$) only body weight at 19 d. The low p- normal Ca diet reduced ($p <0.05$) feed intake and body weight gain in both sexes at 7,14 and 19 d compared to the normal p- normal Ca diet; the reduction of Ca in the low p diet prevented the above depression, resulting in body weight and feed intake to a level comparable to that of normal p- normal Ca diet.

Amber and Abou-Zeid,(2005) studied the effect of substituting yellow corn (YC) by rice bran (RB) at different levels on male Muscovy ducklings performance with reference to enzyme supplementation. Diets were formulated in which RB replaced YC at levels of 50 and 100 %.

Birds were given grower diets (17% CP and 2900 Kcal. ME/kg) during the period from 6 to 77 weeks of age. Phytase enzyme were added in three diets by 800 FTU/ kg. They noted that phytase supplementation to control diets resulted in significant increase in growth rate by 3.7% and improved ($p<0.001$) FCR by 4.5%. the body weight grain (BWG), growth rate(GR), feed intake (FI) and feed conversion ratio (FCR) were significantly reduced with increasing level of rice bran (RB) from 0 to 66%. While it is improved significantly by adding phytase.

Yan et al. (2001) fed broiler chicks (from 3 to 6 weeks) diets with non-phytate p (npp) levels of 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 and 0.45% with or without phytase supplementation (800 FTU). In the absence of phytase , npp levels of 0.33,0.186 and 0.163% were required to optimize body weight gain and feed conversion ratio. In the presence of 800 FTU , levels of 0.24,0.151 and 0.109% were needed to optimize body weight gain and feed conversion ratio.

Abdo, (2003) added 3 levels of Eruca sativa seed meal as a substitute for soybean meal protein in broiler rations, they were (0, 25 and 50%) and 3 levels of microbial phytase (0,500 and 1000 FTU phytase/ kg dait). They found that increasing phytase level improved both body weight (BW)and body weight gain(BWG) significantly ($p\leq0.05$), where the total BWG valyes from 1-6 weeks of age 1811, 1856 and 1874 g for 0, 500 and 1000 FTU /kg diet. The interaction between Eruca sativa seed mesl level and phytase level showed that the best BW and BWG values were for 0% Eruca sativa seed meal + 500 FTU phytase /kg (2019 and 1965 g), while the lowest valus for 50% Eruca sativa seed meal without phytase (1666 and 1612 g). However, there were no significant differences between the values due to the interaction.

Salem et al. (2003) used broiler diets containing 4 levels of available phosphorus (Av.p) (0.30, 0.35, 0.40 and 0.45%) and 2 levels of microbial phytase (0 and 600 U/kg) . They indicated that adding phytase to low phosphorus diets (0.30 and 0.35%) improved weight gain and feed intake however, this improvement was decreased with increasing dietary p levels. Birds fed the diet containing 0.30% available p supplemented with phytase gave similar results of weight gain, feed intake as those fed 0.40 and 0.45% Av.p with phytase and better than these birds fed 0.40 and 0.45% without phytase. It showed be mentioned that supplementation phytase decrease the requirement of inorganic phosphorus sources to about 50%.

Wu et al. (2003)formulated a wheat-soybean basal diet to contain an adequate concentration of nonphytate p(0.45%) and supplemented with 500 U/kg phytase fed to broilers from 1 to 21 d old. They found that phytase supplementation significantly ($p<0.05$) improved body weight gain (17.5%) and feed efficiency (2.9%).

Akyurek et al. (2005) investigated the effect of three levels of available phosphorus (AP): 1-positive control (0.45%) , 2- negative control (0.35%AP) 3- negative control +phytase 0.5 g/kg, on the growth performance of Ross broiler chicks. They found that phytase supplementation did not have significantly (p>0.05) affect bird performance with respect to body weight gain , feed intake and feed conversion ratio.

Dilgar et al. (2004) compared between 2 trials, the first one 1used 8 d old male broilers in a 14 d trial to assess growth and nutrient utilization. Dietary treatments included a positive control 5.0g/kg nonpyhtate p (npp), negative control 1.2g/kg npp,and negative control + 500 or 1000 phytase units/kg diet. Trial 2 used 1 d male broilers over a 42 d period to evaluate growth performance. They noted that there was a linear increase in weight gain, feed intake, and feed efficiency (p<0.01) due to phytase addition over the 14 d period in trail 1.The addition of microbial phytase to the negative control diet improved each growth criterion over the first 21 d in trial 2 (linear, p<0.05). Only weight gain (linear, p<0.05) and feed intake (linear and quadratic, p<0.05) increased due to phytase inclusion from 22 to 43 and over the entire 42 d period of trial 2. weight gain, feed intake, and feed efficiency reached a plateau with phytase supplementation at 750 FTU/kg diet in trial 2, regardless of feeding period.

Banks et al. (2004) used male chicks fed 0, 62.5, 125, 250, or 370 ppm Cupper from Cupper sulfate in combination with 600 phytase FTU/kg from 9 to 22 d of age.

Nonphytate phosphorus (nPP) and Ca were formulated to be 0.2 and 0.7% of the diet, respectively. Three additional control diets were formulated to contain 0.27, 0.34 , and 0.40% nPP, each with 0.7% Ca. they concluded that birds fed increasing concentration of Cu plus 600 FTU of phytase/kg had linear reductions in all performance characteristics measured ($p \leq 0.0001$).

Zyla et al. (2004) studied in vitro and in a feeding trial with broilers fed corn-soybean meal the rate of phytate p removal from feed (level of dephosphorylation, DL) and the extent to which the molecule of phytic acid is deprived of phosphate moieties (conversion degree,CD). In the in vitro model Phtase A (0 or 250 phytase activity units (FTU) /kg) , enhance CD. in the feeding trial , 3-phytase A and 6-phtase A(at 750 FTU/kg) exerted similar effects on broiler performance. Phytase B (6.400 acid phosphates activity units (ACPU) /kg) enhanced feed intake, body weight gain. They showed that 3-phytase A was more effective than 6-phytase in enhancing feed intake, BWG, and FCR. Up to 3.200 ACPU /kg, phytase B did not influence performance but enhanced Ca retention. Higher levels of phytase B (4.800 to 6.400 ACPU /kg) decreased feed intake and BWG but improved p and Ca retention.

El-Sherbiny et al. (2005b) used 4 diets that contain 0.14, 0.25, 0.35 and 0.45% nPP from 1 to 21 days of age, and 0.13, 0.20, 0.30 and 0.35% nPP from 21 to 35 days of age.

Phytase enzyme was added to all diets at 2 levels 0 and 500 U/kg diet. They showed that adding phytase enzyme significantly improve (P<0.001) weight gain from 1118 to 1272 g (14 % increase). Increasing dietary nPP level from 0.14/0.13 up to 0.35/0.30 % gave significant increases (P<0.001) in feed intake. Addition of phytase enzyme significantly (P<0.001) improved feed to gain ratio for birds fed 0.35/0.30% nPP diet (1.58) while birds fed 0.14/0.13% npP diet without phytase supplementation (1.77).

Hammad, et al.(2005) studied the effect of two levels of available phosphorus (0.35 and 0.25% in grower diets with 0.25 and 0.15 in finisher diet respectively), and four levels of microbial phytase (0, 250, 500 and 750 U/Kg in grower diets and 0, 500, 750 and 1000, U/Kg in finisher diets respectively). They found that the decrease in available phosphorus,(AP) content in the diet depressed body weight gain (BG) from 1684g(0.45 AP) to 1551 and 1436g(0.35 and 0.25 AP, respectively) during 0-6wk. The addition of 250, 500 and 750 U phytase/Kg diet increased BG to 1485, 1454 and 1584 gm, respectively during 0 - 6 wk of age. Feed intake (FI) decreased linearly by reducing AP levels during 0-5 wk of age. Phytase addition to the low AP diets increased FI, but still under FI of the positive control.

Abd El-Hakim, (2005) concluded that the chicks could be fed on 0.40% phosphorus level with any level of phytase (500,750, and 1000 FTU/kg diet) during starter-grower period (2-3 weeks of age) and then switched to 0.35% phosphorus level with high level of phytase supplementation (1000 FTU) during the finisher period without any adverse effect on growth performance.

4.2.Mortality rate :

Yan, et al. (2001) indicated that chicks fed low-npp recorded higher mortality rate and poor growth performance compared to those fed low-npp plus phytase. Similar results were reported by **El-Medany et al. (1999**) who fed corn-soybean diet containing 24% crude protein, 3000 Kcal ME/kg, 0.45% available phosphorus and 0.8% calcium to 360one day old Japanese quail. They found that mortality rate was not affected by different treatments. Only five birds out of 360 quail chicks or 1.4 percent died during the experiment in all treatments **.**

Mohamed et al.(2001) used a basal diet(A) contained 23%CP, 3200 Kcal ME/kg, 0.37 total and 0.15%, non-phytate P with no inorganic P supplements. Three levels of dicalcium phosphate were added to the basal diet of B,C and D contained 0.52, 0.62 and 0.72% total and 0.30, 0.40 and 0.50% available P, respectively. Phytase enzyme (Alltech®) was added to diets A,B and C (1g/Kg diet i.e. 1000 U/kg) for 28 day. They found that the addition of phytase to diets B & C containing 0.52 and 0.62% P, respectively, exceeded mortality rate to be 40%.

Mortality rate declined to the normal levels with increasing the dietary phosphorus content or addition of phytase. The high mortality of chicks fed on diet A was mainly due to the fact that diet A was deficient in P, since phytate-P was not available to their chicks and the requirement of P is much higher than the amount offered.

Abd-Elsamee, (2002) fed White Loghorn laying hens three levels of AP (0.45% positive control), (0.30% medium control), and (0.15% negative control) and three levels of FTU (300,400 and 500 FTU/kg diet). Mortality rate values averaged between 2-3 hen/treatment, not due to the treatments but could be attributed to natural causes. Data concluded that mortality rate of laying hens was not affected by supplemental phytase to their diets.

Abd-Ellatif and Kamal ,(2003) used, for 39 days, a control diet adequate in non-phytate phosphorus (0.5%) and low in non-phytate phosphorus (0.4%). They reported that mortality rate was not influenced by dietary treatment as should be expected and they did not have such low dietary non-phytate phosphorus to seriously harm the birds health.

Garcia et al.(2003) concluded 2 experiments 1- using available phosphorus (AvP) levels of 0.12, 0.25, 0.35 and 0.45% with and without phytase (600 FTU/KG), 2- two AvP levels 0.08 and 0.39% and two levels of phytase (0 and 600 FTU / kg of feed). They showed that AvP levels affects the mortality rate ($P<0.05$), being lower at the 0.39% AvP level throughout the experiment.

The same result was seen at AvP 0.08% + 600 PTU treatments, since mortality diminished (P<0.05).

Akyurek ,et al.(2005) found that mortality rate during their trial was within acceptable levels (less than 2%) and was not related to dietary treatments.

Amber and Abou-Zeid,(2005) reported that mortality rate was 0.0% for dietary rice bran (RB) treatments but were 7.41 and 3.70% for negative and positive control diets.

4.3.Carcass characteristics:

El-Medany and El-Afifi, (2002) found that neither phytase, CP nor nPP affect carcass yield significantly.

Viveros, et al. (2002) found that decreasing nPP content of broiler diet caused an increase in relative liver weight. Phytase supplemented to low P diet reduced the relative liver weight by 6.3%. Spleen relative weight did not affected by phytase supplementation.

Hammad (2005a) indicated that the decrease in AP caused significant (P<0.05) increases in relative liver weight by about 12.5%. Also, the phytase addition (250-500 and 750-1000 U/kg diet) increases (P<0.05) relative liver weight by about 13.3 and 14.2%, respectively. The interaction between AP and phytase results in a significant (P<0.05) effect on relative spleen weight.

El-Sherbiny, et al. (2005a) used three starter diets contained 1.00, 0.85 and 0.65% Ca, and three grower diets contained 0.90, 0.75 and 0.55% Ca. The nonphytate-P (nPP) content was 0.35% for the starter and 0.26% for the grower diets.

Phytase enzyme was added to all diets at 2 levels, 0 and 500 U/kg diet. Results showed that decreasing dietary Ca level from 1.00/0.90 to 0.85/0.75 or 0.65/0.55% significantly decreased abdominal fat (P<0.001) and liver weight (P<0.01).Addition of phytase significantly (P<0.01) decreased abdominal fat only.

Mohamed, et al.(2005) used, at 35 days of age, starter diet contained 0.85%Ca and 0.35% nPP, while the grower diet contained 0.75% Ca and 0.26% nPP. Phytase added to diet at 500 U phytase or (Avizyme 1500) enzyme preparation containing amylase, protease, and xylanase added to diet at 0.75 g. They concluded that the dressing and abdominal fat (%) significantly (P<0.001) increased with avizyme while addition of phytase enzyme did not affect dressing % , abdominal fat, liver, and heart relative weights percentage.

4.4.Blood plasma constituents:

Phytase supplementation to diets had no significant effect in plasma (**Stebastian et al. 1996a ; Lan et al. 2002 and Attia, 2003).** No significant interactions were observed between dietary energy levels, dietary phosphorus sources, and phytase supplementation for studied plasma minerals. Chicks fed low-nPP plus phytase recorded higher (P<0.05) plasma P concentration as compared to those fed normal nPP without phytase(**Abou El-Wafa, et al. 2005)**. This effect had been reported in broiler chicks **(El-Medany and El-Afifi, 2002)** However, phytase supplementation had no effect on plasma Ca.

Viveros et al, (2002) showed that phytase supplementation 500 FTU/kg diet to a low-nPP diets increased (P<0.0001) plasma P level by 8%. Decreasing nPP levels in the diet increased plasma Ca (P<0.0001) at 6 wk, by 11.6 and 27.5% decrease (P<0.0001) in plasma P level.

Cheng, et al. (2004) mentioned that addition of phytase to basal diet (0.41 % nonphytate P) fed to broilers from 7 to 28 d of age significantly increased plasma P concentrations.

Abdo, (2003) found that increasing phytase level from 500 to 1000 FTU/kg diet increased serum calcium values and decreased both total protein and total lipids values. The interaction between Eruca sativa seed meal level and phytase level had no significant effect on blood parameters which were ranged between 2.19-12.03 (mg/dl), 0.73-3.14 (g/dl) and 7.93-20.75 (g/l) for calcium, total protein and total lipids, respectively.

Salem, et al. (2003) noticed that broiler chick groups fed 0.35% AP had higher Ca and lower P than other levels which reduced similar levels of plasma Ca values, in spite of, plasma P increased with increasing dietary AP. The lower plasma P level for birds fed diet containing 0.35% AP may be due to plasma Ca in this group. This result agree with **(Perney, et al.1993 ; Mitchell and Edwards 1996 and Murai et al. 2002)** who found that plasma P was increased by increasing dietary available phosphorus (AP).

Qota ,et al.(2002) reported that plasma total protein and total lipids were insignifictly affected by feeding soaked linseed cake (SLC) up to 10%. On the other hand, formulating broiler diets based on available amino acids decreased significantly plasma cholesterol of 10% SLC containing diet as compared to their counterpart control group without phytase or Biogen .

4.5.Tibia measurements:

Phytase addition significantly (P<0.05) increased the concentration of minerals in tibia (**Mitchell and Edwards 1996; Zanini and Sazzad 1999; and Lan et al. 2002).**

Pereny, et al.(1993) designed 2 experiments 1- 0.21, 0.29, 0.37 and 0.44 AvP + 0.10% phytase at 3 days of age, 2- 0.32, 0.38 and 0.44 % AvP + 0.5, 1.0 and 1.5 % (250,500 and 750 units/kg). They found that tibia breaking strength was affected by dietary phytase in experiment 2, whereas it was not influenced by phytase in experiment 1.

Hammad, (2005a) studied the effect of 2 levels of available phosphorus (0.35 and 0.25% in grower diets or 0.25 and 0.15 in finisher diets) and 4 levels of phytase enzyme (0,250,500and 750 U/kg in grower diets or 0, 500,750 and 1000 U/kg in finisher diets, respectively). Data shows that reducing dietary AP to 0.35-0.025 and 0.25- 0.15% resulted in decrease (P<0.05) breaking strength for tibia by 22.6 and 19.9% respectively.

Added phytase by 500-750 and 750-1000U/kg diet increased (P<0.05) tibia breaking strength by 26.0 and 41.9% respectively.

Hammad ,(2005b) used 2 levels of available phosphorus AP (0.23-0.13%) and 4 levels of phytase enzyme (0,100,300 and 500 FTU/kg diet). The main effects showed that reduced dietary AP to 0.23 and 0.13% resulted in decreased tibia breaking strength by 13.5 and 15.6%, respectively. Birds fed low AP-diet without phytase gave tibia breaking strength lower by 22.4%. supplemenental phytase by 100, 300 and 500 FTU/kg diet did improve tibia breaking strength by 17.9, 6.0 and 15.8%, respectively. On the other hand, AP and phytase regimens had no significant (P>0.05) effect on femoral breaking strength. Hens fed the lowest AP-diet without phytase showed decreased in tibia wet weight by 10.79, while adding phytase up to 500 FTU/kg diet showed significant (P>0.05) improvement.

El-Sherbiny, et al. (2005a) found that decreasing dietary Ca level to 0.65/0.55% or added phytase to broiler diets from 1 to 35 days of age significantly (P<0.05) improve bone mineralization.

Atia, et al (2003b) noted that supplementing phytase in grower diets containing reduced levels of nPP and Ca significantly improved bone strength of broiler chickens.

El-Sherbiny, et al. (2005b) reported that increasing dietary nPP level from 0.14/0.13 to 0.35/0.30% without or with added phytase enzyme significantly (P<0.05) increased length of tibia. No significant differences in tibia length were detected among birds fed 0.35/0.30%

nPP wit phytase and birds fed 0.45/0.35% nPP with or without phytase enzyme.

4.6.Fecal phosphorus output:

The amount of Nitrogen (N) and Phosphorus (P) that is excreted by pigs and poultry is affected by three main factors:

(1) the amount of dietary N (i.e., protein) and P that is consumed.

(2) the efficiency with which they are utilised by the animal for growth and other functions.

(3) the amount of endogenous secretions (gases excreted by the animal) **(Magette,2001).**

Perney, et al.(1993) noted that dietary phytase significantly decreased P excretion, when added to diets containing the two higher levels of Av.P, but did not significantly increase P retention.

Zanini and Sazzad, (1999) used ,for 3 weeks, sexed broiler chickens fed 2 levels of metaboliable energy (11.72 and 12.55 MJ ME/kg) and 2 additions of microbial phytase(Natuphus® 5000), (0 and 500 U of microbial phytase /kg). They showed a reduction of 17% in P excretion compared with its controls. The reduction of P excretion as a result of phytase supplementation indicates that the P contained in phytate has been hydrolysed, and became available for absorption and use.

Hammad, (2005a) reported that fecal phosphorus level was not significantly affected($P<0.05$) by dietary AP levels. Phytase addition can reduce fecal phosphorus significantly ($P<0.05$) by about 21%.

Hammad, (2005b) indicated that lowering dietary AP levels decreased fecal phosphorus by 15.61 and 19.60% for groups fed 0.23 and 0.13% AP, respectively. Regardless of dietary AP levels, phytase supplementation by 500 FTU/kg diet decreased fecal phosphorus by about 27.15%.

Keshavarz, (2000) observed that the reduced total phosphorus P excretion is due to lowering dietary AP($P<0.05$) and phytase did not have any effect on P excretion.

Yan ,et al. (2001) reported that at the lower phosphorus levels (0.10%) birds fed diets with phytase –supplements excreted less P than those fed diets without phytase, and at higher P levels (0.45%), for which P needs of the chick were met and a dietary surfeit occurred, little difference in fecal P was noted between birds fed diets with or without phytase. Thus, addition of phytase alone, without a commensurate reduction in dietary P, will not ameliorate P excretion.

Yan, et al. (2003) designed two trails from 42 to 63 d of age with or without phytase supplementation.

Two levels of phytase (0 and 800 U/kg) and six levels of nPP (0.10 to 0.35% in 0.05% increments). They found that the reduced levels of dietary phosphorus in conjunction with phytase supplementation should allow for markedly reduced excretion of phosphorus in the litter with no reduction in live performance or in bone development.

Applegate, et al.(2003) stated that phytase supplementation did not affect solubility of P in the litter regard less of P feeding program.

Garcia, et al.(2003) reported that excreted phosphorus was less (P<0.05) on d 20 and 21 of age in diets with lower P content, although, when phytase was added, no effects on excreted phosphorus was found.

Abd-Ellatif and Kamal, (2003) suggested that fecal calcium and phosphorus were significantly (P<0.01) reduced by 32.9% and 27.9% respectively in phytase-supplemented group.

Wu, et al. (2003) found that increasing dietary P contents, by the addition of inorganic phosphate, increased (P<0.001) the excreta P content from 9.7 to 14.7g/kg dry matter. Phytase addition 500 FTU/kg diet tended (P<0.10) to reduce the excreta P content, but the effect were greater in birds fed on low P diets, as shown by a non-phytate P × phytase interaction (P<0.01). similar results was reprted by **Ravindran, et al. (2001)** who fed wheat-sorghum-soybean meal aseddiets containing phytic acid (10.4, 13.2 and 15.7 g/kg ; equivalent to 2.9, 3.7 and 4.4 g/kg phytate P) , available phosphorus 2.3 and 4.5 g/kg , microbial phytase (0,400 and 800 FTU/kg) from day 7 to 25 post-hatch.

Akyurek, et al.(2005) showed that the effect of phytase supplementation on phosphorus excretion was significant (P<0.05). phytase enzyme hydrolyzed phosphate groups bound to phytate molecules, thereby the amount of retained phosphorus was increased, while the excreted amount of phosphorus was decreased.

MATERIALS AND METHOD

The experimental work of the present study was carried out at, the Poultry Physiology Laboratory, Department of Poultry Production, Faculty of Agriculture, Ain Shams University.

This study was designed to asses the effect of dietary supplementation of microbial phytase (M.Ph) basal diets containing different levels of available phosphorus (Av.P) on some physiological and environmental parameters of chickens.

1. Birds and management :

A total number of 270 unsexed one-day old broiler chicks(Ross) were used. These chicks were randomly and equally distributed into nine groups of 30 chicks each, in three replicates (10 chicks each). A 3×3 factorial arrangement of treatments was used with three levels of available phosphorus (0.45-0.30-0.15%), and three levels of microbial phytase (0-500-750 FTU/kg diet).All birds were housed in woody cages, and the brooding temperature was set at 33˚C during the first week post hatching and then gradually decreased to reach the room temperature (26-28 ˚C). A continuous lighting programme was provided throughout the whole experimental period. Feed and water were supplied ad libitum and chicks of all groups were vaccinated against Newcastle and Infectious Bursal disease by using eye drops or in drinking water according to the ordinary managerial outlines of the laboratory. The basal diet (Table 1) were formulated to meet or exceeds the recommended requirements of **NRC, 1994.**

1.1. Experimental design :

The basal diets were supplemented with different levels of microbial phytase (Natuphos 5000, BASF Corp. Mt. Olive, NJ) , from the first day to the end of the experimental period (6 wk of age).

*** The nine experimental treatments were as follows:**

Treatment 1 (T1): Birds fed on a diet containing 0.45% AvP with no added M.Ph 0 FTU/kg diet.

Treatment 2 (T2): Birds fed on a diet containing 0.45% AvP+500 FTU/kg diet.

Treatment 3 (T3): Birds fed on a diet containing 0.45% AvP+750 FTU/kg diet.

Treatment 4 (T4): Birds fed on a diet containing 0.30% AvP+0 FTU/kg diet.

Treatment5 (T5): Birds fed on a diet containing 0.30% AvP+500 FTU/kg diet.

Treatment 6 (T6): Birds fed on a diet containing 0.30% AvP+750 FTU/kg diet.

Treatment 7 (T7): Birds fed on a diet containing 0.15% AvP+0 FTU/kg diet.

Treatment 8 (T8): Birds fed on a diet containing 0.15% AvP+500 FTU/kg diet.

Treatment 9 (T9): Birds fed on a diet containing 0.15% AvP+750 FTU/kg diet.

1.2.The studies traits:

1.2.1.Body weight (BW):

Body weight and weight gain were recorded for each treatment weekly intervals until 6 weeks of age.

1.2.2Weight gain (∆Wt):

The average live body weight gain was calculated by subtracting the average initial live weight of chicks for a

Table (1) Composition and calculated analysis of the basal diets.

Ingredients %	Phosphorus levels		
	0.45%	**0.30%**	**0.15%**
Yellow corn	60.00	60.00	60.00
Soybean meal (44%)	24.35	24.35	24.35
Gluten (60%)	8.50	8.50	8.50
Wheat bran	0.00	1.20	2.46
Molasses	3.05	3.05	3.05
Limestone	1.40	1.00	0.58
Di-calcium phosphate	1.75	0.95	0.11
Lysine	0.20	0.20	0.20
DL-Methionine	0.20	0.20	0.20
Premix*	0.30	0.30	0.30
Salt	0.25	0.25	0.25
Anti fungi	0.10	0.10	0.10
Total	**100**	**100**	**100**
Calculated analysis			
Crude protein %	21.10	21.29	21.48
ME Keal/kg diet	2940.02	2955.62	2972.00
Calcium %	0.90	0.60	0.30

* vitamins and minerals mixture, each kg contains the following: vitamin A, 12 miu, vitamin D3, 2 miu, vitamin E. 10g, vitamin K, 2g, vitamin B1, 1g vitamin B2, 4g, vitamin B2, 1g, vitamin B2, 4g, vitamin B6, Nicotinic acid, 20g, Folic 1.5g, vitamin B12, 10g, Pantothenic acid, 10g, acid, 1000mg, Choline, 300 g, Cooper, 10g, 10 dine, 1g, 30g, Manganese, 55g, Zinc, 55g, Slenium, 0.1g.

certain period from average final live body weight at the same period.

1.2.3. Feed intake and feed conversion(F.I & F.C):

Average weight gain and feed consumption were recorded at weekly intervals from post hatching till six weeks of age. Also, cumulative weight gain and feed consumption were calculated.

Feed intake (F.I) = feed consumed during one week.

Feed conversion ratio = Average intake (g) / Body weight gain (g).

1.3. Mortality rate:

Cumulative mortality rate was recorded from 0 till 6 weeks of age for each treatment. It was calculated by subtracting the number of live birds at the end of the experimental period from the initial total number of birds.

1.4. Carcass characteristics:

Birds were individually weighed before slaughter. They were killed by severing the carotid artery and jugular veins, and reweighed to calculate blood weight by difference. Feather were manually removed and the birds were reweighed to obtain the feather weight then they were processed by removing the heads and shanks . The internal organs (liver, kidney, bursa of Fabricias, thyroid, thymus and spleen) were removed and their absolute and relative weights were recorded and calculated .

1.5. Blood parameters:

At 2, 4 and 6 weeks of age, a total of 27 chicks (3 chicks/ treatment) were selected randomly and slaughtered . The blood samples were collected into dry and clean centrifuge tubes containing few drops of heparin solution. Plasma was separated by centrifugation at 4000 (r.p.m) for 15 minutes and then stored at -20˚C till biochemical assay. Plasma total protein, total lipids and cholesterol were determined by using commercial kits, following the same steps as described by manufactures, at Animal Health Research Institute, Ministry of Agriculture, Cairo.

1.6. Bone measurements:

At autopsy the two tibiae were dissected, removed, cleaned of flesh and all soft tissues. The left tibia was taken to determine tibia weight and tibia length. Tibia breaking strength was measured by using an universal testing machine, Tinuls Olsen Toting Machine Co., Materials Department, Faculty of Engineering, Ain Shams University.

1.7.Fecal phosphorus:

At 3 and 6 weeks of age excreta were collected and dried by leaving it at open air for 3 days, to determine fecal phosphorus by using commercial kit at Faculty of Agriculture, Ain Shams University.

1.8. Histological observations:

The left kidney, the left thyroid and parathyroid glands were dissected from three birds per treatment group and immediately fixed in 10 % formalin-saline solution. A small spicemen from the middle lope of the kidney, and the thyroid gland with its adherent parathyroids were then dehydrated in ascending grades of ethanol, cleared in zylene and then embedded in paraffin was. Transverse sections (4-5 micron thick) were taken, mounted and stained with the ordinary haematoxyline and eosin technique (H+E) according to the method of **Campbell, (1952)**.

1.9. Statistical analysis:

The data were subjected to the analysis of variance procedure using linear model of SAS® software statistical analysis **SAS User's Guide, (1994)**. Significant means were separated by Duncan's Multiple Range Test **Duncan, (1955**).

RESULTS AND DISCUSSION

Effect of supplemental phytase on :

1.Growth performance:

1.1.Body weight (B.W):

Body weight (B.W) of broiler chicks fed different levels of available phosphorus (Av.P) and microbial phytase (M.Ph) is presented in Table (2).

Regardless of Av.P level, birds fed phytase (500 &750 FTU) –supplemented diets had higher body weight as compared with those fed phytase-unsupplemented diet (the control diet) from the second week up to six weeks of age. However, this difference in B.W between the treated –groups and the control one was not significant during the whole experimental period. Also, regardless of M.Ph levels B.W of birds fed medium (0.30%) and low (0.15%) Av.P diets was significantly ($P \leq 0.05$) increased when compared with that of birds fed normal (0.45%) Av.P diet from two up to six weeks of age. The elevation in B.W was more pronounced with the significant effect of the interaction between M.Ph and Av.P levels where the percentage of increased B.W of the groups fed 0.15% Av.p with 500 and 750 FTU (computed on the basis of B.W of those fed 0.45% Av.P with phytase-supplemented levels) was higher than that of groups fed 0.30% Av.P with the same supplemented phytase levels. For example, B.W of birds fed 0.15% Av.P with 500 or 750 FTU was higher by about 71 or 34% versus 25% or 19% of these fed 0.30% Av.P with the same phytase levels when compared with that of birds fed 0.45% Av.P with 500 or 750 FTU at six weeks of age, respectively. It is clear to detect from the previous example that the highest percentage of increased BW achieved with feeding 0.15% Av.P with 500FTU. This trend was also noticed from two weeks up to six weeks of age. It is of interest to notice that B.W increased as Av.P decreased with no added phytase level where the groups fed 0.15% Av.P had higher B.W than those fed 0.30% Av.P by about (13, 37, 40, 46, 51 and 54% versus 11, 11, 7, 0.7, 0.1 and 0.1 at 1, 2, 3, 4, 5 and 6 weeks of age respectively).

In general, the present results may indicate that B.W increased as Av.P deceased and M.Ph increased up to 750 FTU and the highest increase in BW was induced by feeding 0.15% Av.P with 500 FTU. These results were confirmed with **Lan, (2002**) who noted that chicks fed a low nonphytate phosphorus (npP) without active mitsuokella jalaudinii culture (AMJC) supplementation had significantly lower body weight at 21 and 42 days compared to those fed other dietary treatments. Also, **Sebastian et al. (1996a)** who found that phytase supplementation (600 FTU) of a low-P diet (0.33%) significantly ($P \leq 0.05$) increased body weight of male and female broiler chicks.

1.2. Body weight gain (BWG):

Body weight gain (BWG) of broiler chicks as affected by different levels of Av.P and M.Ph from one to six weeks of age is showed in Table (3).

Regardless of M.Ph level, birds fed diet containing 0.30 or 0.15% Av.P gained more significant ($P \leq 0.05$) weight when compared with those fed the recommended level of Av.P (0.45%) at the first week of age, while those fed 0.15% Av.P had significant ($P \leq 0.05$) higher BWG than those fed 0.45% or 0.30% Av.P at 2, 3, 4, 5 and 6 week of age and when the BWG was computed for the whole experimental period. The group fed 0.30% Av.P gained more significant ($P \leq 0.05$) weight comparing to that fed 0.45% Av.P at all periods of estimation. This trend for the pronounced increases in BWG of birds that fed on low phosphorus diet (0.15%) was noticed from the first week till six weeks of age.

On the other hand, weight gain was insignificantly increased as affected by M.Ph level regardless of Av.P level, where birds fed diet supplemented with 500 or 750 FTU gained more weight as compared with those fed the control diet without added phytase, except during the period from 5-6 weeks of age where the higher level of M.Ph was significantly better than the control one.

In general, it appears that weight gain increased as Av.P decreased and M.Ph level increased. The highest increase in BWG was showed by feeding 0.15% Av.P with 500 FTU when the comparison was subjected on the basis of the whole experiment period.

The present results are contrary to those reported by **Sebastian et al., (1997)** who reported that low-phosphorus diets reduced ($P \leq 0.05$)body weight gain compared with normal-P: normal-Ca diets. However, our results confirmed with the findings of **Ravindran, et al. (2001) ; Abdo, (2003);** and **Akyurek et al.(2005)** who found that increasing phytase level improved body weight gain.

1.3. Feed intake (FI):

Feed intake (FI) of broiler chicks as influenced by different levels of Av.P and M.Ph from one up to six weeks of age is presented in Table (4).

Regardless of M.Ph level, birds generally consumed more feed as Av.P level decreased. The groups fed 0.30% Av.P had significant ($P \leq 0.05$) and higher F.I than that of those fed 0.45%Av.P at all periods of estimation, while the group fed 0.15% Av.P had significantly higher F.I than that of those fed the control diet (0.45% Av.P) through all experimental weeks of age and as computed for the whole experimental period (0-6 wks). There is a significant difference in F.I between 0.30% Av.P- fed group and 0.15% Av.P- fed one only at 2 and 4 weeks of age. Regardless of Av.P level, F.I increased as M.Ph level increased from two till six weeks of age, however these values were not significant. The difference in F.I between phytase-supplemented groups and control one was insignificant during the whole experimental period except at 2-3 weeks of age where 750 FTU-supplemented group significantly consumed more feed than the control one.

These results were agreed with **Atia, (2003a)** who mentioned that phytase addition increased feed intake of meat type ducks compared to its counterpart control group.

1.4. Feed conversion (F.C):

Feed conversion (F.C) of broiler chicks fed diets containing different levels of Av.P and supplemented with different levels of M.Ph is presented in Table(5).

Regardless of M.Ph level, birds fed diet containing 0.15% Av.P showed a significant ($P \leq 0.05$) improvement in F.C comparing to those fed 0.45% Av.P- containing diet from 1-2 up to 5-6 weeks of age also on the basis of the whole experimental period (0-6 wks). The group fed 0.30% Av.P-diet also showed insignificant improvement in F.C when compared with 0.45% Av.P-fed group from 1-2 up to 5-6 weeks of age.

Regardless of Av.P level, there was no significant difference in F.C between the groups fed 500 or 750 FTU- supplemented diet and that fed the control diet during the whole experimental period except at 5-6 weeks of age and the period of 0-6 wks where 750 FTU-fed group revealed a significant and a nonsignificant improvement comparing to the control group and 500 FTU-fed group, respectively. Again, the best improvement in F.C was achieved by the group fed diet containing 0.15% Av.P and supplemented with 500 FTU which may reflect the enhanced body weight gain of this group of chicken. It is likely that phytase enzyme addition improved amino acid and hence protein metabolism in birds which in turn causes improvement of body weight.

These results were confirmed with **El-Medany and El-Afifi, (2002)** and **El-Sherbiny et al.,(2005b)** who showed that increasing dietary npP level from 0.14/0.13 to 0.35/0.30% significantly (P≤0.001) improved feed/gain ratio.

2. Some related organs:

2.1.Thyroid gland:

Thyroid gland relative weight of broiler chicks as influenced by different dietary levels of available phosphorus (Av.P) and microbial phytase (M.Ph) at 2, 4, and 6 weeks of age is presented in Table (6).

Thyroid relative weight of birds fed 500 FTU-supplemented diet were positively influenced. The difference between this group and the other two groups fed 0 and 750 FTU-added diet was significant (P≤0.05) at two weeks of age, while it was not at four and six weeks of age. No significant effect was noticed between the different dietary Av.P levels (0.45, 0.30, and 0.15%) at all tested ages. It is clear from the present results that both Av.P and M.Ph levels showed a non significant role on thyroid gland weight as the birds become older. However, the increased thyroid relative weight of birds fed 500 FTU of M.Ph is difficult to be explained, although the increased metabolic activity of birds during this period seems to be a good response. **Sebastian, et al.(1997)** and **Viveros et al, (2002)** Suggest that same biological effects of phytase are due to its indirect role in stimulating some endocrine gland and improving the physiological response via cycle-AMP mechanisme. These findings are in close agreement with the results obtained in our study.

2.2. Liver :

Liver relative weight of broiler chicks fed different levels of available phosphorus (Av.P) and microbial phytase (M.Ph) at 2,4,and 6 weeks of age is shown in Table (7).

No significant ($P \leq 0.05$) effect of the tested Av.P or M.Ph levels as well as the interaction between both of them on liver relative weight was noticed at all tested ages. Birds fed 500 FTU-supplementated diet had nonsignificant increase in liver relative weight when compared with those fed 0 or 750 FTU-supplemented diet at two, and four weeks of age, while this trend was reversed at six weeks of age where liver relative weight of 500 FTU-fed group was insignificantly decreased comparing to 0 or 750 FTU-fed one. The reduction that observed at 6 weeks of age in the liver weight may be due to the low fat content of liver as influenced by phytase phosphorylation activity and inhancement of lipids metabolism. Our results showed the plasm total lipids and cholesterol (discussed latter) to be reduced in phytase-supplementated groups, indicative of decreased lipogenic activity of liver. These results were agreed with **Hammad, (2005a)** and **Viveros et al.,(2002)** who showed that phytase supplementation to low-P diet reduced the relative liver weight by 6.3% .However, **Mohamed et al.(2005)** reported that phytase addition did not significantly affect liver relative weight, which confirm the present results.

2.3. Kidney :

Kidney Relative weight of broiler chicks as affected by different dietary levels of available phosphorus (Av.P) and microbial phytase (M.Ph) at two, four and six weeks of age is presented in Table (8).

Supplementation of M.Ph didn't significantly affect kidney relative weight at any age. Although, birds fed 500 FTU-added diet had lower kidney relative weight comparing to 0 and 750 FTU-fed groups at two and six weeks of age, it had a higher value than both of them at 4 weeks of age. Feeding 0.15% Av.P diet resulted in a significant ($P \leq 0.05$) decrease in kidney relative weight when compared to that resulted from feeding 0.45 and 0.30 % Av.P-containing diets or 0.30 % Av.P only at six and four weeks of age, respectively.

Same trend was noticed but it lost its significancy at two weeks of age. It appears that long-term feeding of low Av.P diet supplemented with M.Ph is associated with minor changes in the structure of kidney as the filtration and reabsorption load of nephrons decreased, due mainly to the improved Ca and P retention and protein utilization. These observations are in agreement with the previous studies by **Biehl and Baker, (1997) ; El-Medany and El-Afifi, 2002; Namkung and leeson, (1999) ; Sebastian, et al.(1997)** and **Onyango et al. (2004)** who generally reported that mineral retention and protein utilization were improved by phytase addition to basal diets.

2. Immune-related glands:

2.4.Thymus gland:

Thymus relative weight of broiler chicks as affected by different dietary levels of available phosphorus (Av.P) and microbial phytase (M.Ph) at two, four, and six weeks of age is shown in Table (9).

Birds fed 750 FTU-supplemented diet had significant ($P \leq 0.05$) higher thymus relative weight than that of those fed 0 or 500 FTU-supplemented diet, only at four weeks of age. Moreover, an insignificant increase in thymus relative weight was noticed at six weeks of age with feeding 750 FTU-supplemented diet or 0.15% Av.P-containing diet . Thymus relative weight as affected by M.Ph or Av.P levels was fluctuated at two weeks of age.

2.5. Bursa of fabricius gland:

Effect of feeding different levels of available phosphorus (Av.P) and microbial phytase (M.Ph) on bursa relative weight of broiler chicks at two, four, and six weeks of age is presented in Table (10).

Statistical analysis of bursa relative weight data revealed no significant differences between treatments whether the M.Ph or Av.P levels as well as the interaction between both of them. However, a non significant decrease in bursa relative weight was noticed with feeding 500 or 750 FTU-supplemented diet when compared with feeding phytase-unsupplemented diet only at two weeks of age, regardless of dietary Av.P levels.

The opposite trend was noticed as Av.P level decreased where bursa relative weight was insignificantly increased as Av.P levels decreased, at two weeks of age only regardless of M.Ph level.

2.6. Spleen:

Spleen relative weight of broiler chicks as affected by different levels of available phosphorus (Av.P) and microbial phytase (M.Ph) at two, four, and six weeks of age is presented in Table (11).

Birds fed 500 or 750 FTU-supplemented diet showed a significant ($P \leq 0.05$) decrease in spleen relative weight when compared with those fed the control diet at four weeks of age .The difference between phytase-treated groups and the control one was nonsignificant at two and six weeks of age. No significant differences between dietary levels of Av.P were detected at two, four and six weeks of age. These results were confirmed with **Viveros et al. (2002**) who noted that spleen relative weight did not affected by phytase addition. Based on the previous results of the immune-related organs weight (%) as indicators of liveability and both humoral and cell-mediated immunity of chicks, it would appear that phytase addition improved immunity. This holds trus as the mortality rate in our study was very low to be neglecated, in the phytase-supplemented groups. These results are in close agreement with the findings of **Abd-Elsamee, (2002) ; Amber and Abou-Zeid,(2005**); **Garcia, et al.(2003); and Yan ,et al. (2001)** who

generally reported decreased mortality rate in phytase-fed chicks.

3. Blood parameters:

3.1. Plasma calcium concentration:

Effect of different dietary levels of Av.P and M.Ph on the plasma calcium level at 2,4, and 6 weeks of age is presented in Table (12).

Plasma calcium level of birds fed 500 or 750 FTU-supplemented diet was significantly ($P \leq 0.05$) increased as compared with that of birds fed the control diet regardless of Av.P level at 2,4,and 6 weeks of age. There was a significant ($P \leq 0.05$) difference in plasma calcium level between 500 FTU- fed group and 750 FTU fed one only at 2 weeks of age. There was a significant interaction effect between the tested levels of Av.P and M.Ph particularly at 6 weeks of age where the calcium level in plasma of birds fed diet containing 0.15% Av.P and supplemented with 500 or 750 FTU was significantly ($P \leq 0.05$) higher than that of birds fed the other two tested levels of Av.P and M.Ph. It is of interest to notice that plasma calcium concentration was not significantly changed with age as affected by the dietary level of phytase or Av.P.

In general, the present results indicate that plasma calcium level was significantly ($P \leq 0.05$) increased as phytase–supplementation level increased at all tested ages. On the other hand, it was insignificantly decreased as Av.P level decreased at 2 and 4 weeks of age while it was insignificantly increased at 6 weeks of age. These results are in agreement with **Salem et al.,(2003)** who noticed that broiler groups fed 0.35% Av.P showed higher plasma Ca and lower P than other levels studed. However, some other studies reprted that dietary phytase addition had no significant effect on plasma calcium level (**Atia, (2003a,b); Lan, et al. (2002**).

3.2.Plasma phosphorus concentration:

Plasma phosphorus concentrations of broiler chicks as affected by different dietary levels of Av.P and M.Ph at 2,4, and 6 weeks of age are presented in Table (13).

Regardless of Av.P level, phosphorus level in plasma of groups fed phytase-supplemented diets was significantly ($P \leq 0.05$) increased comparing with that of group fed phytase-unsupplemented diet. There was a significant ($P \leq 0.05$) difference between the group fed 500 FTU-added diet (6.14 mg/dl) and the other fed 750 FTU-added diet (9.44 mg/dl) at 4 weeks of age only .

Also, the effect of different Av.P treatments was significant only at 4 weeks of age , where birds fed diet containing 0.45% Av.P had the highest plasma phosphorus level followed By those fed 0.15% Av.P and then birds fed 0.30% Av.P. It is interesting to notice that plasma P was increased with age and this trend was opposite to that noticed with plasma calcium concentration.

Regardless of M.Ph level, plasma P didn't significantly affected by the dietary level of Av.P at 2 and 6 weeks of age, while it was significantly ($P \leq 0.05$) increased with feeding the lower levels of Av.P (0.30 and 0.15%). These results are in agreement with **Cheng et al.,(2004**) who reported that addition of phytase to npP-diets fed to broilers from 7 to 28 day of age significantly increased plasma P. The increase in plasma P gives further evidence of phytase P utilization by M.Ph supplementation to broiler diets, especially in the low-phosphorus ones. These results concur with other studies in chickens (**Salem et al., 2003; Mitchell and Edwards 1996).**

3.3.Plasma cholesterol concentration:

Plasma cholesterol concentration of broiler chicks as affected by different levels of Av.P and M.Ph at 2,4,and 6 weeks of age is presented in Table (14).

Regardless of dietary levels of Av.P, birds fed 750 FTU-supplemented diet had significantly ($P \leq 0.05$) lower concentration of plasma cholesterol as compared with those fed the control diet (0 FTU) at all tested ages.

While feeding of 500 FTU resulted in a decreased plasma cholesterol level comparing with non phytase supplemented diets at all tested ages and this decrease was significant ($P \leq 0.05$) only at 4 weeks of age. The plasma cholesterol level of groups fed 0.15 % Av.P + 500 or 750 FTU and 0.30% Av.P + 500 or 750 FTU-diets was significantly decreased as compared with those fed 0.45% Av.P + 500 or 750 FTU-diets.

Regardless of M.Ph-supplemented level, birds fed low and medium -phosphorus diets (0.15 and 0.30% Av.P) had lower plasma cholesterol level than that of those fed normal-phosphorus diets (0.45% Av.P). There was a significant difference between birds fed 0.15% Av.P containing –diet and those fed 0.45% Av.P- containing diet only at 4 and 6 wks of age. It is interesting to notice that plasma cholesterol level decreased as dietary level of Av.P and M.Ph decreased and increased, respectively. These results are in agreement with **Ravindran, et al. (2001)** who found that phyrase addition to broiler diets improved the digestibility of fat which may contribute to the decreased plasma cholesterol level.

3.4.Total plasma lipids:

Total plasma lipids concentrations of broiler chicks as affected by different dietary levels of Av.P and M.Ph at 2,4 and 6 weeks of age are presented in Table (15).

Regardless of Av.P levels, TPL as affected by feeding of 750 FTU-supplemented diet was significantly ($P \leq 0.05$) decreased when compared with that of groups fed the control diet (0 FTU) at 4 and 6 weeks of age. However, TPL were insignificantly increased for the same previous group when compared with that of the control group at 2 weeks of age. Groups fed 500 FTU-supplemented diet had a lower TPL comparing with those fed the control diet and this decrease was significant and non significant at 2 and 6 , and at 4 weeks of age, respectively. Regardless of M.Ph levels, TPL of birds fed 0.15% Av.P-containing diet was significantly decreased as compared with that of those fed 0.45 or 0.30% Av.P-containing diet only at 2 weeks of age. This decreased was more pronounced as the birds aged. It is clear from the present results that phytase addition decreased plasma total lipids which may be due in part to the formation of inosoluble metallic soaps in digestive tract which is a constraint on lipid utilization. **Akyurek et al.(2005); Chung, (2002) Ravindran, et al. (2001) and Wu, et al. (2003**) proposed that by preventing the formation of mineral-phytase complexes, phytase may reduce the degree of soap formation and enhance the utilization of energy derived from lipids.

3.5.Total plasma proteins:

Total plasma proteins concentration of broiler chicks fed on different dietary levels of Av.P and M.Ph at 2,4,and 6 weeks of age is shown in Table (16).

Both M.Ph and Av.P levels have a nonsignificant effect on the TPP of broiler chicks at 2,4,and 6 weeks of age. It is likely that supplemental phytase improved the utilization of dietary protein by increasing its digestibility. Several studies confirm the present results which showed that both nitrogen retention and amino acids metabolism improved by phytase addition **Akyurek et al. (2005).**

4. Tibia measurements:

4.1 Tibia relative weight :

Tibia relative weight of broiler chicks as influenced by different levels of available phosphorus (Av.P) and microbial phytase (M.Ph) at two, four, and six weeks of age is presented in Table (17).

Phytase addition to the diet did not significantly affect the tibia relative weight at all tested ages. Also, no significant effect of different dietary levels of Av.P on tibia relative weight was noticed at two and six weeks of age. However, tibia relative weight of birds fed 0.30 or 0.15 % Av.P-containing diet was significantly ($P \leq 0.05$) decreased at 4 weeks of age only (0.85 or 0.83 , respectively) when compared with those fed the control diet (0.97).

4.2. Tibia length :

Effect of different dietary levels of available phosphorus (Av.P) and microbial phytase (M.Ph) on tibia length of broiler chicks at two, four and six weeks of age is presented in Table (18).

There were nosignificant differences between different dietary levels of M.Ph and Av.P in tibia length at two and six weeks of age, However, significant difference was found between the group fed 750 FTU added diet (8.34 cm) and supplemented diet (7.90 cm), at 4 weeks of age, also, a positive significant ($P \leq 0.05$) difference was noticed between tibia length of birds fed 0.15 % Av.P-treated diet (8.53 cm) and that of other groups fed 0.45 or 0.30 % Av.P-treated diet (7.96 or 7.76 cm, respectively) at 4 weeks of age.

4.3. Tibia breaking strength :

Tibia breaking strength of broiler chicks fed different levels of available phosphorus (Av.P) and microbial phytase (M.ph) at two, four, and six weeks of age is shown in Table (19).

Tibia breaking strength of birds fed 500 or 750 FTU-included diet was significantly ($P \leq 0.05$) increased (17.07 or 16.71 kg, respectively) comparing to that of those fed phytase-unsupplemented diet (10.37 kg) at six weeks of age. At four weeks of age, broiler group fed 750 FTU-added diet had a significantly ($P \leq 0.05$) and higher tibia breaking strength comparing to those fed 0 or 500 FTU-added diet (11.41 verses 6.82 or 8.40 kg, respectively).

No significant differences were detected between the tested dietary M.Ph levels at two weeks of age. Also, no significancy was noticed between the different Av.P levels at two and four weeks of age, while a significant ($P \leq 0.05$) difference was noticed between the effect of feeding 0.15% Av.P and feeding 0.45 or 0.30 % Av.P (17.68 versus 12.13 or 14.33 kg) at six weeks of age. There was a significant ($P \leq 0.05$) interaction between the tested dietary levels of Av.P and M.Ph only at six weeks of age.

It is of interest to notice that 0.15 % Av.P + 500 FTU-fed group had the strongest tibia (the highest value of tibia breaking strength, 23.2 kg) at sex weeks of age. In general, tibia breaking strength increased as birds grow up and as added phytase level increased particularly at six weeks of age. It appears that the improvement of all tibia measurements of the phytase-supplemented groups of chicks is due mainly to the increased tibia contents. The release of inorganic P and Ca from phytate and the subsequent deposition of these minerals in bone may support the results of the present study.

The results of the present study are in close agreement with several studies which conclude that phyatse supplementation to poultry diets can improve bone quality and breaking strength (**El-Sherbiny, et al. 2005a,b ; Hammad 2005a; Lan, et al. 2002;Salem et al. 2003 and Sebastian et al. 1996a).**

5. Excreta phosphorus level :

Excreta phosphorus level of broiler chicks fed different levels of Av.P and M.Ph at 3 and 6 weeks of age is presented in Table (20).

Regardless of M.Ph level, excreta phosphorus level decreased as the percentage of Av.P decreased. Birds fed 0.15% Av.P–containing diet showed decrease in fecal phosphorus level compared with droppings of those fed 0.45% or 0.30% Av.P–containing diet, at 3 and 6 weeks of age. It is of interest to notice that feeding of 0.15% Av.P resulted in a reduction in phosphorus level in droppings by about 23.7% and 29.3% than that of 0.45% Av.P feeding at 3 and 6 weeks of age, respectively. In spite of the affect of supplemented phytase level on the phosphorus level of droppings, the latter insignificantly increased as the former increased at both two ages. This reduction was decreased by feeding 0.30% Av.P (13.4 and 20.8% at 3 and 6 weeks of age, respectively).

In general, the lowest phosphorus level in droppings was detected with feeding 0.15% Av.P with 0 FTU (12.07 and 13.47 mg/g DM at 3 and 6 wks of age, respectively), while the highest level was showed by 0.45% Av.P + 750 FTU- fed birds (23.75 and 21.67 mg/g DM at 3 and 6 wks of age respectively).

These results are in agreement with **Pereny et al.,(1993**) who reported that dietary phytase significantly decreased P excretion. However**, Zhang et al.,(2000)** mentioned that several phytase levels were tested in corn-soy bean meal diets with 0.21% Av.P, it was found that the phytase addition reduced quadraticaly phosphorus excretion from 18 to 8.3% in 1 to 6 wks old chickens. It is important to conclude that fecal phosphorus levels can be significantly reduced while maintaining optimum live performance of birds, by judicial choice of dietary P level and phytase addition or both. This will decrease pollution from the Poultry manure.

6. Histological observations

6.1. Thyroid and Parathyroid glands:

The histological structure of thyroid and parathyroid glands of chicks fed different levels of Av.P and M.Ph at 2 and 6 weeks of age are illustrated in plates 1 to 18.

After 2 weeks of feeding broiler chicks the phytase-supplemented diets, results reveal that when M.Ph was added to an adequate (0.45%) Av.P diet (as recommended by, **NRC, (1994)**, the histological appearance of both thyroid and parathyroid glands was normal in the control-unsupplemented birds (Plate – 1). Thyroid follicles are large rounded or spherical to oval in shape. They contain considerable amounts of colloid (C) indicative for thyroid activity. In the same plate, parathyroid gland section appears also normal with its irregular anatomizing cords of chief cells (Ch).

Abbreviation key for the histology sections:

B=blood vessels ; C= colloid ; Ch = chief cells

CT = connective tissue capsule ; F = thyroid follicle ;

H + E = Haemotoxlin and eosin stain ; K = Kidney ;

MD = Medullary collecting duct ; S = segments of the medullary loops

X 200 = magnification power ; T.S. = transverse section

Plates 2 and 3 illustrate, however, that the thyroid follicles of phytase treated chicks are slightly larger and tented to be polyhedral in shape. The linning epithelium is low cuboidal or flattened in appearance. Also, the amount of colloid seems smaller compared by (plate -1). Parathyroid chief cells are likely larger and dark-stained, which may indicate higher activity of the gland especially in the high phytase-fed chicks (Plate – 3). Concerning the effect of decreasing the Av.P level , histological sections of both thyroid and parathyroid glands show that thyroid follicles of M.Ph-treated chicks were more abundant , polyhedral and contained less amounts of colloid (Plate 5,6) compared with control (Plate 4) unsupplemented chicks. No observed changes in the structure of parathyroid gland cells (Ch) , except the irregular cords of chief cells with many connective tissue fibers in the paranchema of the high-phytase fed chicks (Plate – 6) compared with those fed the low-phytase diet (Plate – 5). It is of great interest to observe the dramatic changes in histological feature of glands from birds fed the low-phosphorus diets (Plates 7, 8 and 9). In the control section (Plate - 7) , thyroid follicles are seen to have an irregular or elongated shape with a high columnar epithelium linning and more abundant connective tissue fiber especially in the control and high-phytase (Plate – 9) fed chicks. It is also important to observe that the parathyroid chief cells are more abundant and compactly-arranged in a solid-like masses of dark stained cells especially in T7 (control) and T9 (high-phytase) fed groups.

At six weeks of age, an enlarged thyroid follicles are seen in all treated groups (Plates 10, 11, 12) . Also, chief cells of the parathyroid are seen larger with little connective tissue fibers between the irregularly-arranged cords. These cells are stained pale which indicate their physiological activity. A similar trend was observed in the histological sections of birds fed the intermediate-phosphorus diet (0.30), where both thyroid follicles and parathyroid chief cells were hypertrophied, indicative of higher activity (Plats 13, 14 and 15). However, in the high-phytase supplemented group the incidence of hyperplasia of chief cells was clearly observed with a narrow paranchymal storms between the unobvious cell cords (Plate – 15). Plates (16 , 17 and 18) clearly show the effect of low-P diet and M.Ph levels on the histological section of thyroid and parathyroid glands at 6 weeks of age. In all sections thyroid follicle are larger and their epithelial linning was squamous in the control (Plate – 16) ; high columnar in plates 17 and 18. These follicles are filled with colloid, however a dense connective tissue septum was seen between thyroid and parathyroid glands in all sections. From the previous results it seems that the available phosphorus level and phytase addition are effective in stimulating the secretion of thyroid hormones (T3, T4) which are known to influence most of the metabolic pathways. Consequently the parathyroid hormone is secreted due to the stimulation responses of both calcium and phosphorus levels in the diets and (or) in blood.

Unfortunately, the published data concerning the effect of Av.P and (or) M.Ph on thyroid and parathyroid activity are scanty. However, the role of parathyroid gland in Ca and P metabolism are well known **Sturkie, (1986)**. The shape , number and size of chief cells along with their staining ability are reported to be an indicators of the gland activity **Gould and Hodges, (1971**). In the present study it is likely that Av.P level had the most obvious effect on parathyroid histology than the effect of M.Ph level.

6.2. Kidney histology:

Histological examination of kidney section as influenced by Av.P and M.Ph levels are shown in Plates (19 to 36).

At 2 weeks of age the kidney sections of birds fed the recommended Av.P level diet (0.45%) are illustrated in Plate – 19 (control) ; Plate – 20 (500 FTU) and Plate – 21 (750 FTU).

It is clear that normal appearance of renal tissue are seen. A pale medullary layer is seen intermingling with the cortical (dark) layer. Many medullary collecting ducts along with cortical tubules were present in all sections. Connective tissue fiber containing some lymphoid nodules are send specially. In control (Plate – 19) section. Moreover, a small segments of the fine collecting ducts (S) coming from the medullary (Henle`s) loops are also observed. On the other hand, great changes are detected in the histological sections of kidneys from birds fed the intermediate Av.P (0.30%) diets (Plates 22, 23, 24).

The medullary ducts (MD) are larger in diameter with highly columnar epithelium linning and many cortical (T) and thin segmental loops of tubules. This may be due to hyper activity of these ducts to reabsorb more Ca and P because of their low levels in diets. A similar observation is also noticed for kidney sections of birds fed the low-Av.P (0.15%) diet (Plates 25, 26, 27). However, more collecting ducts of different types are seen with more interstitial connective tissue septa between the collecting ducts. The epithelial cell lining of these ducts are will defined and darkly stained, indicative of their normal functions. At six weeks of age, and as the birds became older, the changes in the structure of kidney was more obvious (Plates – 28, 29, 30). It is clear that the medullary collecting ducts and tubular ducts diameter became larger and the demarcation between the medullary and cortical layers are difficult. However, no abnormal changes in all sections could be seen. It is of interest to observe that the Av.P level (0.30%) with or without phytase addition has a pronounced effect on the structure of the kidney (Plates 31, 32, 33). Numerous tubular and medullary ducts are found in the control (plate – 31) and in the 500 FTU-fed (Plate – 32) groups. The same was observed in the kidney section of birds fed the high phytase (750 FTU) level, but the tubular diameters are larger (Plate – 33) than the other sections of the same treatment.

Finally, the low-P (0.15%) diets exert a considerable effect on the structure of the kidney (Plates – 34, 35, 36). The diameter and number of the collecting ducts are larger in all sections regardless to the phytase level. It appears from the previous results that phytase addition my enhance the physiological responses of both thyroid and parathyroid gland to dietary levels of Av.P . This response was supported by the previous data concerning the improvements in plasma metabolites (cholesterol, total lipids, total protein) which reflect thyroid status. Moreover, the response of parathyroid gland in regulating Ca and P metabolism, especially in groups of chicks that fed low-P diets, was more obvious, in the different tibia measurements. In summary, based on productive performance of birds, blood metabolites, bone characteristics and the histological observation, the addition of phytase to broiler diets are safe and would improve the utilization of phytate phosphorus, especially in low-P diets. It is suggested that low-P diet with 500 FTU of phytase / kg of diet can allow for markedly reduced excretion of phosphorus in the litter with no reduction in live performance or in bone development. These results confirm many studies dealing with phytase as a safe feed additive and for reducing the pollution of excreta phosphorus (**Zhang, et al., 2000; Desouky, 2001; Hocking, et al., 2002; Yan, et al., 2003, and others).**

SUMMARY AND CONCLUSION

This study was conducted at the Poultry Physiology laboratory, Poultry Production Department, Faculty of Agriculture, Ain Shams University.

The main objectives of the present study was to study the effects of dietary microbial phytase enzyme supplementation on some environmental and physiological parameters. Therefore, 270 one-day old broiler chicks (Ross) were randomly distributed into nine groups of 30 birds each, which subsequently were subdivided into 3 replicates, 10 birds each. A 3×3 factorial arrangement of treatments was used with three levels of available phosphorus (0.45-0.30 and 0.15 %), and three levels of microbial phytase (0-500 and 750 FTU/kg diet). Birds were fed the experimental diets from one-day old till six weeks of age.

Results obtained can be summarized as follows:

1- Microbial phytase supplementation (750 FTU/kg) to low-phosphorus diet cases a significantly increase in live body weight of broiler chicks at the end of the experimental period.

2- Body weight gain was significantly increased in birds fed the low-P with 500 and 750 FTU/kg, respectively during the whole experimental period.

3- Feed intake of the whole experimental period was significantly increased in birds fed on the low-phosphorus diets (0.30 and 0.15% Av.P) and also for the two M.Ph-supplemented chicks.

4- Feed conversation ratio during the experimental period was significantly improved by phytase addition, however, the best value was reduced for birds which fed the low-P diet (0.15%).

5- Thyroid gland relative weights of broiler chicks fed on phytase-supplemented diets were nearly similar, but higher values were obtained for the chick group which fed on 500 FTU/kg at 2 and 4 weeks of age only.

6- No significant effect of the tested Av.P or M.Ph levels as well as the interaction between both of them on liver and kidney relative weight was noticed at two , four and six weeks of age.

7- Thymus glands relative weight was significantly affected by the level of added phytase at 4 weeks of age, where birds fed 750 FTU/kg-supplemented diet showed higher value than those fed on the control or 500 FTU/kg-M.Ph added diet.

8- A non significant increase in the relative weight of bursa of fabricius and spleen was noticed as the Av.P level decreased.

9- Plasma calcium level was significantly ($P \leq 0.05$) increased as phytase -supplemented level increased at all tested ages.

10- Plasma phosphorus concentration didn't significantly affected by the dietary level of Av.P at 2 and 4 weeks of age while it was significantly ($P \leq 0.05$) decreased with feeding the lower levels of Av.P (0.30 and 0.15%) particularly with feeding 0.30% Av.P.

11- Plasma cholesterol level decreased as dietary level of Av.P and M.Ph decreased and increased, respectively.

12- Tested levels of M.Ph or Av.P had no effect on total plasma protein of broiler chicks at 2,4 and 6 weeks of age.

13- Total plasma lipids concentrations decreased as M.Ph -supplemented level increased starting at 4 weeks of age and as Av.P levels decreased.

14- Tibia relative weight of birds fed 0.30 or 0.15 % Av.P-contained diet was significantly ($P \leq 0.05$) decreased when compared with those fed the control diet .

15- There were no significant difference between the different dietary level of whether M.Ph or Av.P at two and six weeks of age.

16- Tibia breaking strength (kg) increased as birds grow up and as added phytase level increased particularly at six weeks of age.

17- The lowest phosphorus level in droppings was with feeding 0.15% Av.P diet-supplemented detected with both M.ph levels.

18- The highest level was showed by 0.45% Av.P + 750 FTU- fed birds at 3 and 6 weeks of age.

19- After two weeks of age, thyroid gland histology of the treated and untreated broiler chicks were nearly similar, the effect of Av.P and M.Ph levels on the size and shape of thyroid follicles was not obvious.

20- At six weeks of age, an enlarged thyroid follicles filled with colloid can be seen in the histological sections of all treated groups.

21- Parathyroid gland section of the low-phosphorus fed groups showed dark-stained cells cords with large chief cells, indicative of hyper activity especially in phytase-supplemented diets.

22- An enlarged chief cells withen irregularly-arranged cords can be seen in the parathyroid gland sections of birds fed low-P high-M.Ph diets at 6 weeks of age.

23- An obvious histological changes were observed in the histological sections of kidney from birds that fed on the low Av.P diets either supplemented by M.Ph or unsupplemented diets.

24- The number, diameter and epithelial linning of the different types of renal collecting tubules were dramatically changed as the birds aged (6 wk) and with decreasing the Av.P level to 0.30% with or without phytase addition.

It is suggested that phytase addition to broiler diets are safe and would improve the utilization of phytate-phosphorus especially in the low-P diet.

Based on the results of the productive performance of broiler chickens, and on the histological observation, it could be concluded that phytase addition to the low-P diets may be used as an important tool for reducing the excreta phosphorus to alleviate soil and aquatic live pollution.

REFERENCES

Abd El-Hakim, A. S (2005). Effect of using different levels of available phosphorus and microbial phytase on the performance of broiler ckicks. 3rd International Poultry Conference. 4-7 Apr. Hurghada -Egypt.

Abd-Ellatif, H.A. and Azza M. Kamal, (2003).Effect of phytase supplementation on the performance of broilers grown to market weights. Vet .Med. J. Giza. 51: 343-354.

Abd-Elsamee, M.O., (2002).Effect of different levels of available phosphorus and microbial phytase on laying hen performance and egg quality. Egyptian Poult. Sci. 22: 407-425.

Abdo, Z. M. A. (2003). Using egyption eruca sativa seed meal in broiler rations with or without microbial phytase. Egyptian J. Nutrition and Feeds 6: 97-114.

Abou El-Wafa, S.; El-Husseiny, O.M. and Shabaan, M. (2005). Influence of microbial phytase and energy levels on broiler performance fed low-phosphorus diets. 3rd International Poultry Conference. 4-7 Apr. Hurghada -Egypt.

Akyurek, H. ; Senkoylu, N. and Ozduven, M. L. (2005). Effect of microbial phytse on growth performance and nutrients digestibility in broilers. Pakistan J. of Nutr. 4 (1): 22-26.

Amber, Kh. and Abou_Zeid A. E. (2005).The use of rice bran in growing male muscovy duck diets with reference to microbial phytase supplementation. 3rd International Poultry Conference. 4-7 Apr. Hurghada –Egypt.

Anderson, R. J. (1914). A contribution to the chemistry of phytin. Journal of Biological Chemistry, 17:171-190. (C. F. Word`s Poult. Sci. J. , 54:27-47).

Applegate,T.J. ; **Joern,** B.**C. ; Nssbaum-Wgler, D.L. and Ragel**.**(2003)**.Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poult. Sci. 82: 1024-1029.

Atia, Y.A. (2003a). performance, carcass characteristic, meat quality and plasma constituents of meat type ducks fed diets containing different levels of lysine with or without a microbial phytase. Arch. Anim. Nutr. 57:39-48.

Atia, Y. A. (2003b). Responses of growth performance, carcass characteristics, meat quality and plasma constituents of male Campbell ducks to dietary levels of methionine and phytase and interactions. Egyption Poult. Sci. J. Vol. 23(III): 557-580.

Atia,Y.A.; Abd El-Rhaman, S.A. and Qota E.M. (2001). Effects of microbial phytase without or with cell-wall splitting enzymes on the performance of broiler fed marginal levels of dietary protein and metabolizable energy. Egyption Poult. Sci. J. Vol. 21(II): 521-547.

Ballam, G. C.; Nelson, T.S. and Kirby, L. K. (1984). Effect of fiber and phytate source and of calcium and phosphorus level on phytate hydrolysis in the chick. Poult. Sci. 63: 333.

Banks, K.M.; Thopson, K.L. ; Janes, P. and Applegate T.J.(2004). The effects of copper on The efficacy of phytase , growth, and phosphorus retention in broiler chicks Poult. Sci. 83: 1335-1341.

Bartnik, M. and szajranska, I. (1987). Changes in phytate content and phytase activity during germination of some cereals. J. Cereal Sci. 5: 23-28.

Biehl, R. R. , and Baker, D. H. (1997). Microbial phytase improves amino acid utilization in young chicks fed diets based on soybean meal but not diets based on peanut meal. Poult. Sci. , 76: 355-360.

Campbell, R. A. (1952). Histological techniques for normal and pathobgical tissue and indentification of parasites. Fifth addition, Oxford, University Pressi. U. K.

Cheng, Y.H.; Goff, J.P. ; Sell, J.L. ; Dallorso, M.E. ; Gil, S. ; Pawlak, S.E. and R.L.Horst. (2004). utilization solanum glacophyllum alone or with phytase to improve phosphorus utilization in broiler. Poult. Sci. 83: 406-413.

Chung , T. K. (2002). Maximizing the response to phytase. World Poult. 18: 17-19.

De Boland, A. R.; Garner, G. B. and O'Dell, B.L. (1975). Identification and properities of phytate in cereal grains and oilseed products. J. Agric. Food Chem. 23: 1186.

Denbow, D. M.; Ravindran, V.; Kornegay, E. T.; Yi, Z. and Hulet, R. M. (1995). Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poult. Sci. 74:1831-1842.

Desouky, Zeinb, M.A. (2001). Effect of supplemental microbial phytase on productive performance of broiler chickens. Ph.D. Thesis, Faculty of Agric., Ain Shams University, Egypt.

Dilgar, R. N.; Onyango, E. M. ; Sands, J. S. and Adeola, (2004). Evaluation of microbial phytase in broiler diets. Poult. Sci. 83:962-970.

Duncan, D.B. (1955). Multiple range and multiple F tests. Biometrics 11: 1-42.

Edwards, H. M. Jr. (1991). Effects of phytase utilization by monogastric animals. Proceedings, the Georgia Nutrition Conference for Feed Manufacturers, Atlanta, Pp. 1-6.

Edwards, H. M., Jr. (1993). Dietary 1,25_dihydroxycholecalciferol supplementation increases natural phytate phosphorus utilization in chickens. J. Nutr. 123: 567-577.

Edwards, H. M. Jr. and Vellmann, J. R. Jr. (1983). The role of calcium and phosphorus in the aetiology of tibia dyschondroplasia in young chicks. J. Nutr. 113: 1568-1575.

Edwards, H. M. Jr. ; Palo, P. ; Soonchaerenying, S. and Elliot, M. A. (1989). Factors influencing the bioavailability of phytate phosphorus to chickens. In: Nutrient Availability: Chemical and Biological Aspects (Southgate, D., Johnson, I. and Fenwick, CR., Eds) , The Royal Society of Chemistry, Cambridge, Pp. 271-276.

Eeckhout, .W. and De Paepe, M. (1991). The quantitative effects of an industrial microbial phytase and wheat phytase on the apparent phosphorus absorbability of a mixed feed by piglets. Proceedings of fifth forum of applied bio-technology. Part II , University of Gent, Belgium, Pp.1643-1646.

El-Medany, N.M. ; Afifi ,M.A. and Shehata, M.A. (1999). Evaluation of some phosphorus sources in Japanese quail diets. Egyptian J. Nutrition and Feeds. (Special Issue): 719-727.

El-Medany, N. M. and El- Afifi, S. F. (2002). The effect of microbial phytase on utilization of protein and phosphorus in broiler rations. Egyption Poult. Sci. Vol.22 (II): (427-443).

El-Sherbiny A.E ; Mohamed, M. A. ; Hammuda, Y. A. F. and Hassan, H. M. A. (2005a). Influence of adding phytase to low calcium broiler diets on performance, bone and carcass characteristics. Egyption Poult. Sci. Vol 25: 653-668.

El-Sherbiny A.E ; Hammuda, Y. A. F. ; Hassan, H. M. A. and Mohamed, M. A. (2005b). Performance, bone mineralization and carcass characteristics of broilers fed low phosphorus diets supplemented with phytase. Egyption Poult. Sci. Vol 25: 669-688.

Englen, A.J. ; Van Der Heeft, F.E. ; Ransdorp, P.H.G. and Smith, E. Le. (1994). Simple and rapid determination of phytase activity. J. of the Association of Analytical Chemists International. 77: 760-764.

Farrell D. J. and Martin, **E**. **(1988).** Strategies of improve nutritive value of rice bran in poultry diets. III. The addition of inorganic phosphorus and phytase to duck diets. Br. Poult. Sci. 39: 601-611.

Garcia, M. C. ; Guadalupe, R. ; De La Rosa Carrillo, Arturo Pro Martinez, and Jorge Baeza Lopez. (2003). Phosphorus availability in soybean meal and sorghum-corn gluten meal, supplemented with phytase for starter broilers. Tec Pecu Mex 41:295-306.

Gordon, R. W. and Roland, D. A. (1997). Performance of commercial laying hens fed various phosphorus levels with supplemental phytase. Poult. Sci. 76: 1172-1177.

Gould, R. P. and Hodges, R. D. (1971). Studies on the fine structure of the avian parathyroid glands and utimobranchial bodies. Endocr. , 19: 567-604.

Hammad A.M.S. (2005a). Effect of phytase enzyme in broiler plant protein diets contain descending levels of available phosphorus. 2- carcass characteristics, bone quality and mineral retention. Egyption Poult. Sci. Vol 25: 533-552.

Hammad A.M.S. (2005b). Effect of phytase enzyme on layers fed plant protein diets containing low levels of available phosphorus 1- bone characteristics, mineral retention and nutrients digestibility. Egyption Poult. Sci. Vol 25: 971-985.

Hammad, M. S. , Abd El-Maksoud, A. A. A. and Khidr, R. E. (2005). Effect of phytase enzyme in broiler plant protein diets contain descending levels of available phosphorus, 1- Growth performance. 3rd International PoultryConference.4-7 Apr. Hurghada –Egypt.

Harms, R. H. ; Waldroup, P. W. ; Shirley, R. I. and Anderson, C.B. (1962). Availability of phytic acid phosphorus for chicks. Poult. Sci. 41: 1189-1191.

Hocking, P.M.; Wilson, S. ; Dick, L. ; Dunn, L.N. ; Robertson, G.W. and Nixey, G. (2002). Role of dietary calcium and available phosphorus in the aetiology of tibia dyschonderoplasia in growing turkeys. Br. Poult. Sci. 43: 432-441.

Keshavarz, K. (2000). Reevaluation of nonpytate phosphorus requiermet of growing pullets with and without phytase. Poult. Sci. 79:1143-1153.

Kies, A. K.; Van Hemert, K. H. F. and Sauer, W. C. (2001). Effect of phytase on protein and amino acid digestibility and energy utilization. World's Poult. Sci. J. 57: 109-125.

Klunter, A. M. and Steimle, C. (2001). Effect of peniophora lycii phytase in broiler chickens fed a low phosphorus diet. 13th Euro. Symp. Poult. Nutr. Oct. 2001, Landenberg, Belgium.

Kratzer, F. H. ; Alfred, J. B. ; Davis, P. N. ; Marshall, B. J. and Vohra, V. (1959). The effect of autoclaving soybean protein and the addition of ethlenediaminetetraacetic acid on biological activity of dietary zinc for turkey poults. J. of Nutr. 68: 313-323.

Lan, G.Q. ; Abdullah, N. ; Jalaludin, S. and Ho, Y.W. (2002). Efficacy of supplementation of a phytase producing bacterial culture on the performance and nutrient use of broiler chickens fed corn-soybean diets. Poult. Sci. 81:1522-1532.

Lease, J. G. (1966). The effect of autoclaving sesame meal on its phytic acid content and on the availability of its zinc to the chick. Poult. Sci. 45: 237-241.

Lim, H. S. ; Namkung, H. and Paik, I.K. (2003). Effects of phytase supplementation on the performance, egg quality and phosphorus excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorus. Poult. Sci. 82: 92-99.

Lima. F.R. ; Mendonca, C.X. JR. ; Alvarez, J. C. ; Ratti, G. ; Lenhro, S. L.R. ; Kahn, H. and Garzillo, J.M.F. (1995). Chemical and physical evaluations of commercial dicalcium phosphorus as sources of phosphorus in animal nutrition. Poult. Sci., 74: 1659-1670.

Lott, J.N.A. (1984). Accumulation of seed reserves of phosphorus and other minerals. In Seed Physiology, vol. 1, Development, Pp.139-166 (DR Murray, editor). Academic press.

Magette, W.L. ; Curran, T. ; Dodd, V. A. ; Provolo , G. ; Glennon, C. and Quirke, A. (2001). Development and testing of an environmental management system for wastes from intensive pig and poultry units. The management of organic wastes from intensive pig and poultry units. Environmental Protection Agency, An Ghniomhaireacht um Chaomhu Comhshaoil, PO Box 3000, Johnstown Castle Estate, County Wexford, Ireland.

Mitchell, R.D.; and Edwards, H.M.E. JR. (1996). Effects of phytase and 1,25-dihydroxycholecalciferol on phytase utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci. 75:95-110.

Mohamed, S. H. M. (2004). Response of broiler performance to dietary microbial phytase, energy level, amino acid and phosphorus sources. Ph.D. Thesis, Faculty of Agric., Cairo, University, Egypt.

Mohamed, M.A.; El-Sherbiny, A.E. ; **Hammouda, Y.A.F. ; Eman, F. ; El-Daly and Hassan, H.M.A. (2001**). Effect of supplemental phytase on performance , bone characterstics and protein digestibility of broiler chicks. Egyptian J. Anim. Prod. 38(2): 143-156.

Mohamed, M. A. ; El-Sherbiny, A.E ; Hammuda, Y. A. F. and Hassan, **H. M. A. (2005**). Addition of phytase and other enzyme preparation to low phosphorus- low calcium broiler diets. Egyption Poult. Sci. Vol 25: 689-702.

Mohamed, A. ; Gibney, M. J. and Taylor, T.C. (1991). The effect of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the digestibility of phytate-P by the chick. Br. J. of Nutr. 66: 251-259.

Murai, A. ; Kobayashi , T. ; Okada, T. and Okumura, J. (2002). Improvement of growth and nutritive value in chicks with non- genetically modified phytase product from aspergillus niger. Br. Poult. Sci. 43: 687-695.

Namkung, H. and Leeson, S. (1999). ERffect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78: 1317-1319.

National Research Council, NRC. (1994). Nutrient requirements of poultry. 9th Rev. ed. National Academy Press, Washington, DC.

Onyango, E.M. ; Bedford, M. R. and Adeola ,O. (2004). The yeast production system in which Escherichia Coli phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. Poult. Sci. 83:421-427.

Peeler, H. T. (1972). Biological availability of nutrients in feeds availability of major mineral inos. J. Anim. Sci. 35:696-706.

Perney, K.M. ; Cantor, A.H. ; Straw, M.L. and Herkelman, K.L. (1993). The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. Poult. Sci. 72:2106-2114.

Qian, H. ; Veit, H.P. ; Kornegay, E.T. ; Ravindran, V. and Denbow, D.M. (1996a). Effects of supplemental phytase and phosphorus on histological and other tibia bone characteristics and performances of broilers fed semi-purified diets. Poult. Sci. 75:618-626.

Qota, E.M.A. ; El-Ghamry, A.A. and El-Mallah. G.M. (2002). Nutritive value of soaked linsed cake as affected by phytase, biogen supplementation or formulating diets based on available amino acid on broilers performance. Egyptian Poult.Sci. Vol.22:461-475.

Ravindran,V., Selle, P.H. ; Ravindren, G.; Morel, P.C.H.; Kies, A.K. and Bryden, W.L. (2001). Microbial phytase improves performance, apparent metabolizable energy, and lleal amino acid digestibility of broilers fed a lysine-deficient diet. Poult.Sci. 80: 338-344.

Rutherfurd, S.M. ; Chung, T.K. and Moughan, P.J. (2002). The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. British Poult. Sci. 44: 598-606.

Salem ,F. M.; El-Alaily , H. A. ; El-Medany, N. M. and Abd El-Galil, K. (2003). improving phosphorus utilization in broiler chick diets to minimize phosphorus pollution. Egyptian Poult.ci.vol.23: 201-218.

SAS Institute (1994) SAS/STAT Users Guide: Statistics. Ver. 6.04, Fourth Edition SAS Institute Inc., Cary, NC.

Sebastian,S. ; Touchburn, S. P. and Chavez, E.R. (1998). Implications of phytic acid supplemental microbial phytase in poultry nutrition, a review. World's Poult. Sci. J. 54:28-47.

Sebastian, S. ; Touchburn, S.P. ; Chavez, E.R. and Laguf, P.C.(1996a). The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed a corn-soybean diets. Poult. Sci. 75: 729-736.

Sebastian, S. ; Touchburn, S.P. ; Chavez, E.R. and Laguf,P.C.(1996b). Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci. 75: 1516-1523.

Sebastian, S. ; Touchburn, S.P. ; Chavez, E.R. and Laguf,P.C.(1997). Apparent digestibility of protein and amino acids in broiler chickens fed a corn- soybean diet supplemented with microbial phytase. Poult. Sci. 76: 1760-1769.

Shafey, L M. ; Mcdonald, M. W. and Dincle, J. C. (1991). Effects of dietary calcium and available phosphorus concentration on digesta pH and on the avilabilty of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. Br. Poult. Sci. 32: 185-194.

Sohail, S.S. and Roland, D.A. SR. (2002). Influence of dietary phosphorus on performance of high-line w36 hens. Poult. Sci. 81: 75-83.

Sohail, S.S. and Roland, D.A. SR. (1999). Influence of Supplemental phytase on performance of broilers four to six weeks of age. Poult. Sci. 78: 550-555.

Sturkie, P. D. (1986). Avian Physiology. Fourth ed. Springer- verlag, New York, Berlin, PP:466-478.

Takemasa , M. and Hijikuro, S. (1991). Effect of autoclaving of defatted Rice bran on the phosphorus utilization for chicks. Jpn Poult. Sci 28: 284.

Tamim, N. M. ; Angel, R. and Christman, M. (2004). Influence of dietary calcium and phytase on phytate phosphorus hydrolsis in broiler chickens. Poult. Sci. 83: 1358-1367.

Taylor, T. G. (1965). The availability of the calcium and phosphorus of plant materials for animals. Proc. Nutr. Soc. 24: 105-112.

Van der Klis, J. D. and Versteegh, H.A. J. (1991). Ileal absorption of phosphorus in light weight white laying hens using microbial phytase and various calcium contents in laying hen. Feed Spelderholt Publication No. 563, Spelderholt, Beekbergen, The Netherlands.

Viveros, A. ; Brenes, A. ; Arija, I. and Centeno, C. (2002). Effects of Microbia phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci. 81: 1172-1183.

Waldroup, P. W. (1999). Nutritional approaches to reduce phosphorus excretion by poultry. Poult. Sci. 78: 683-691.

Waldroup, P.W.; Kersey, J.H. ; Saleh, E.A. ; Fritis, C.A. ; **Yan, F. and Stillborn, H.L. (2000)**.Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci. 79: 1451-1459.

Wasserman, R. H. and Taylor, A. N. (1973). Intestinal absorption of phosphate in the chicks. Effect of vitamin D_3 and other parameters J. of Nutr. 103: 586-599.

WU, Y.B. ; Ravindran, V. and Hendriks, W.H. (2003).Effects of microbial phytase , produced by solid-state fermentation, on the performance and nutrient utilization of broilers fed maize- and wheat-based diets. Br. Poult. Sci. 44:710-718.

Yan, F. ; Kersey, J.H and Waldroup, P.W. (2001). Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poult. Sci. 80: 455-459.

Yan, F. ; Kersey , J.H. ; Fritts, C.A. and Waldroup, P.W. (2003). Phosphorus requirements of broiler chicks six to nine weeks of age as influenced by phytase supplementation. Poult. Sci. 82: 294-300.

Yi, Z. ; Kornegay, E. T. and McGuik, A. (1994). Replacement values of inorganic phosphorus by microbial phytase for pigs and poultry. J. Anim. Sci. 72: 330 (Abstract).

Zanini, S.F. and Sazzad, M.H. (1999). effects of microbial phytase on growth and mineral utilization in broilers fed on maize soybean-based diets. Br. Poult. Sci. 40: 348-352.

Zhang, Z.B. ; Koruegay, E.T. ; Radeliffe, J.S. ; Denbow, M. ; Veit, H.P. and Larson, C.T. (2000). Comparison of genetically engineered microbial and plant phytase for young broilers. Poult. Sci. 79: 709- 719.

Zyla, K. ; Mika, M. ; Stodolak, B. ; Wikiera, A. and Swiatkiewicz, S. (2004). Towards complete dephosphorylation and total conversion of phytates in poultry feeds. Poult. Sci. 83: 1175-1186.

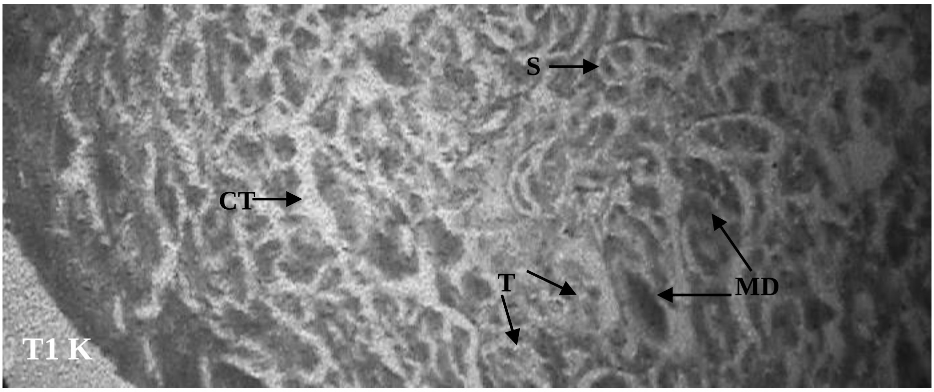


Plate (19): T.S. in the kidney of T1 broiler chicks at 2 weeks of age (H&E×200).

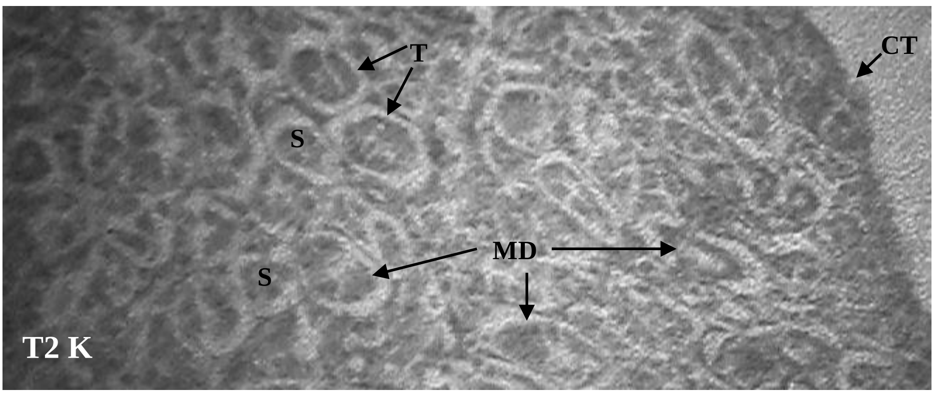


Plate (20): T.S. in the kidney of T2 broiler chicks at 2 weeks of age (H&E×200).

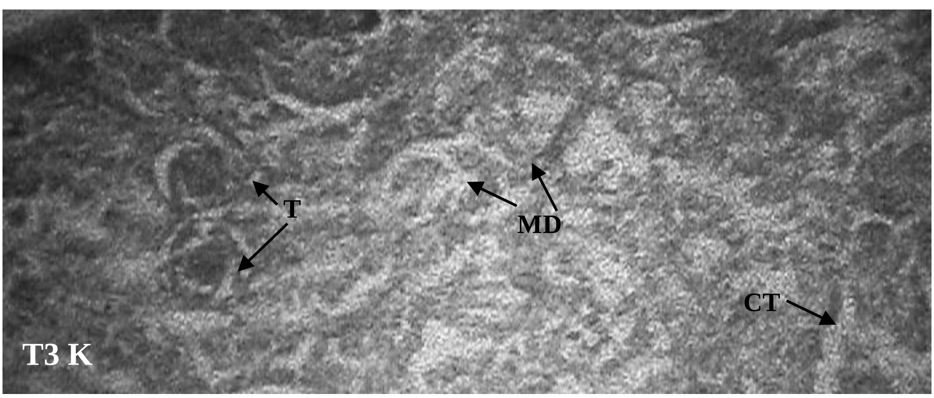


Plate (21): T.S. in the kidney of T3 broiler chicks at 2 weeks of age (H&E×200).

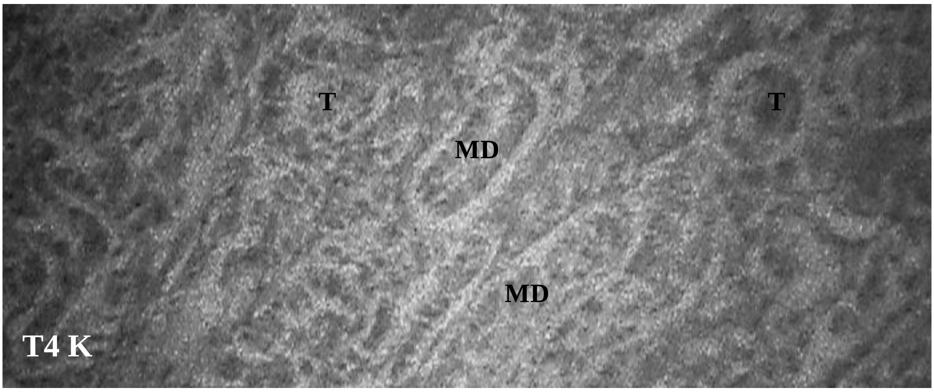


Plate (22): T.S. in the kidney of T4 broiler chicks at 2 weeks of age (H&E×200).

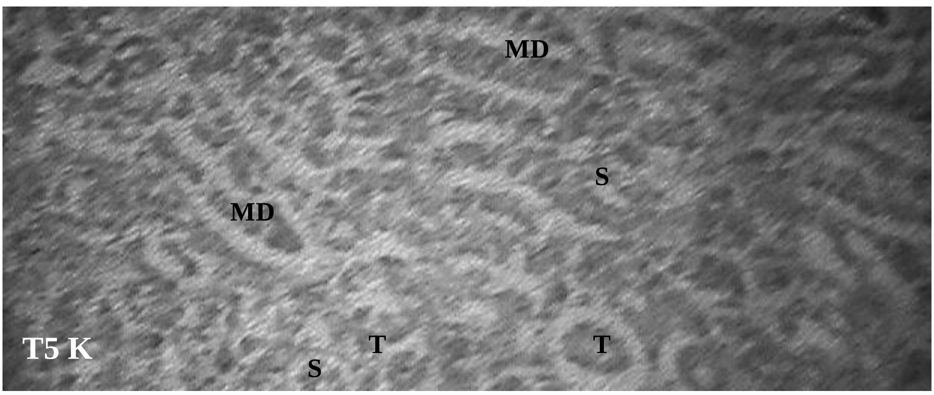


Plate (23): T.S. in the kidney of T5 broiler chicks at 2 weeks of age (H&E×200).

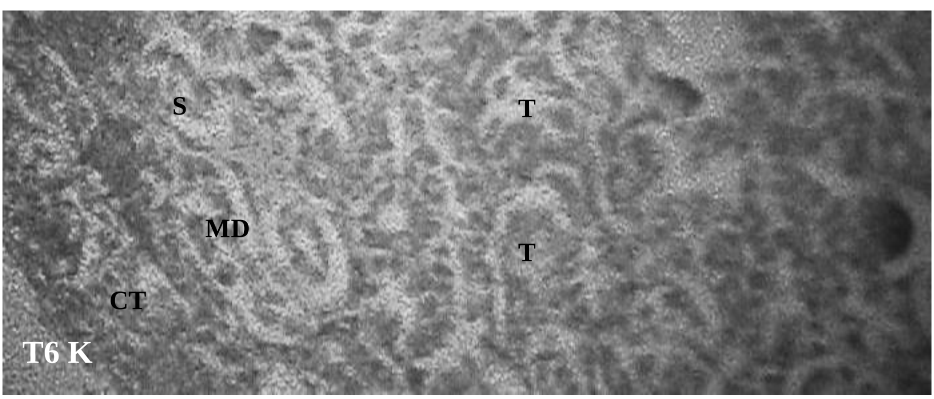


Plate (24): T.S. in the kidney of T6 broiler chicks at 2 weeks of age (H&E×200).

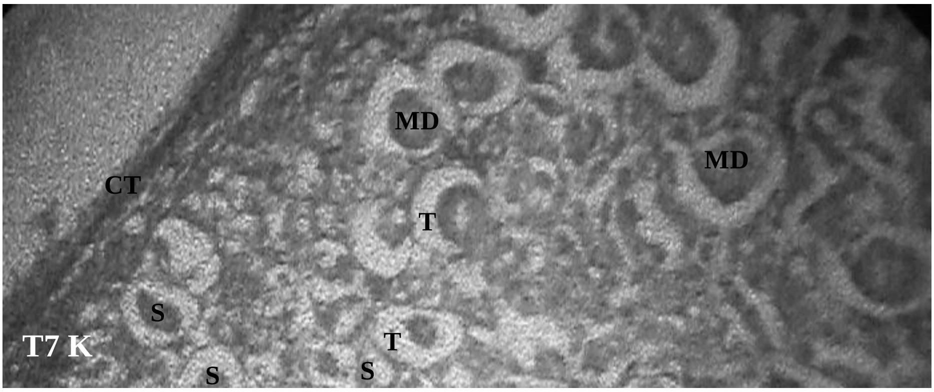


Plate (25): T.S. in the kidney of T7 broiler chicks at 2 weeks of age (H&E×200).

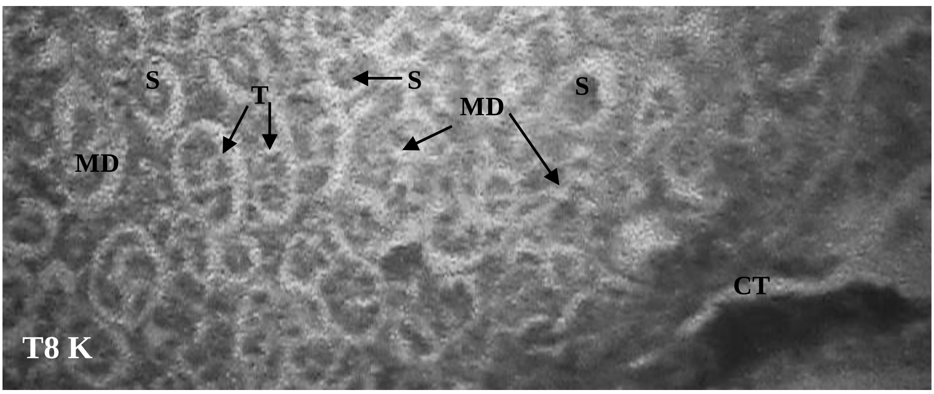


Plate (26): T.S. in the kidney of T8 broiler chicks at 2 weeks of age (H&E×200).

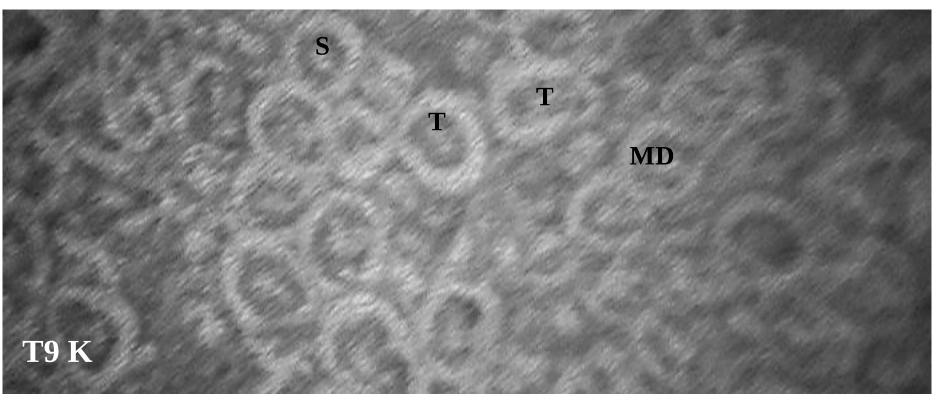


Plate (27): T.S. in the kidney of T9 broiler chicks at 2 weeks of age (H&E×200).

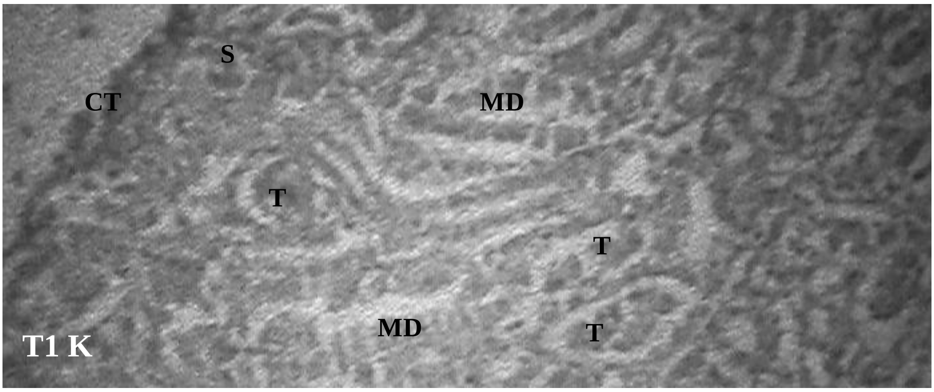


Plate (28): T.S. in the kidney of T1 broiler chicks at 6 weeks of age (H&E×200).

Plate (29): T.S. in the kidney of T2 broiler chicks at 6 weeks of age (H&E×200).

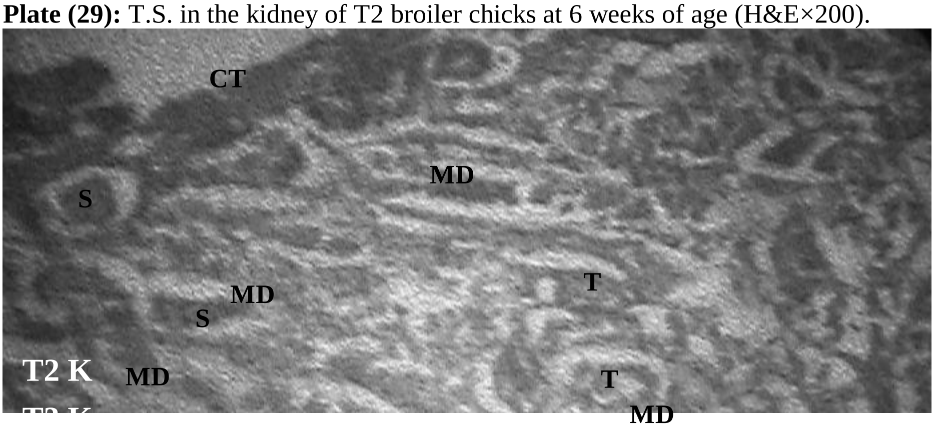
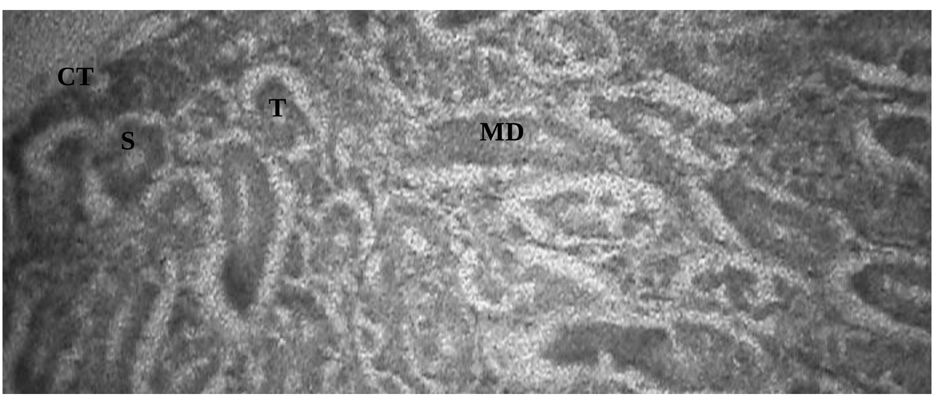


Plate (30): T.S. in the kidney of T3 broiler chicks at 6 weeks of age (H&E×200).



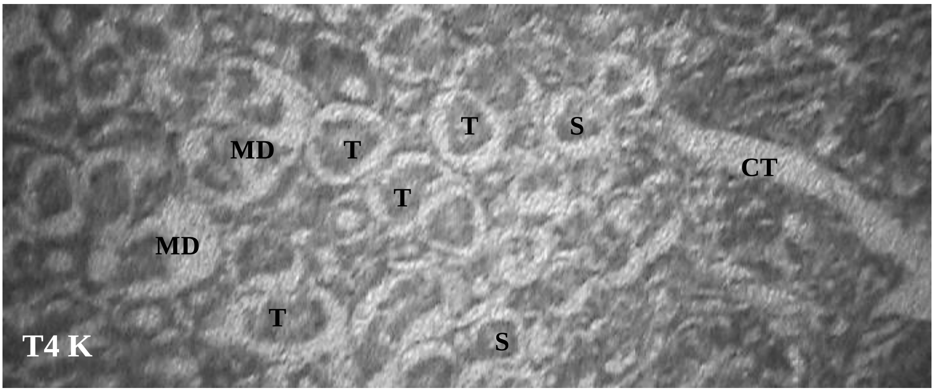
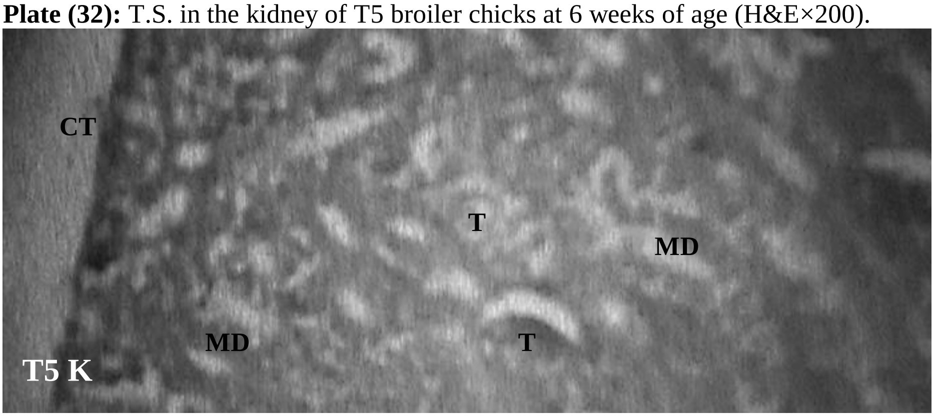


Plate (31): T.S. in the kidney of T4 broiler chicks at 6 weeks of age (H&E×200).

Plate (32): T.S. in the kidney of T5 broiler chicks at 6 weeks of age (H&E×200).



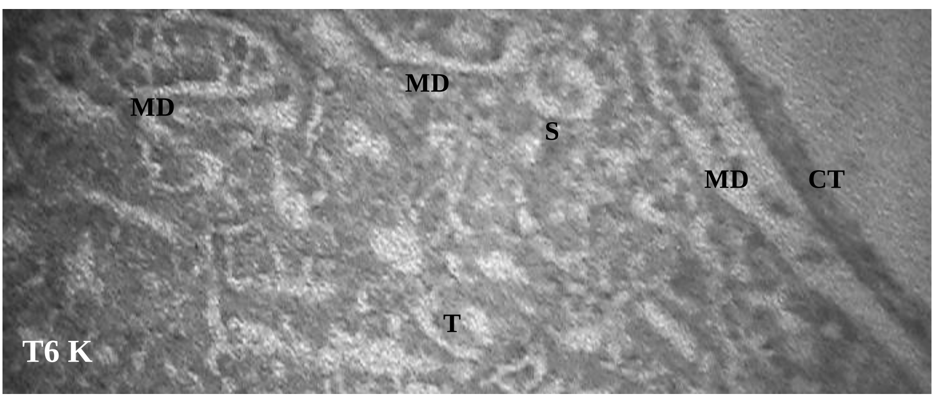


Plate (33): T.S. in the kidney of T6 broiler chicks at 6 weeks of age (H&E×200).

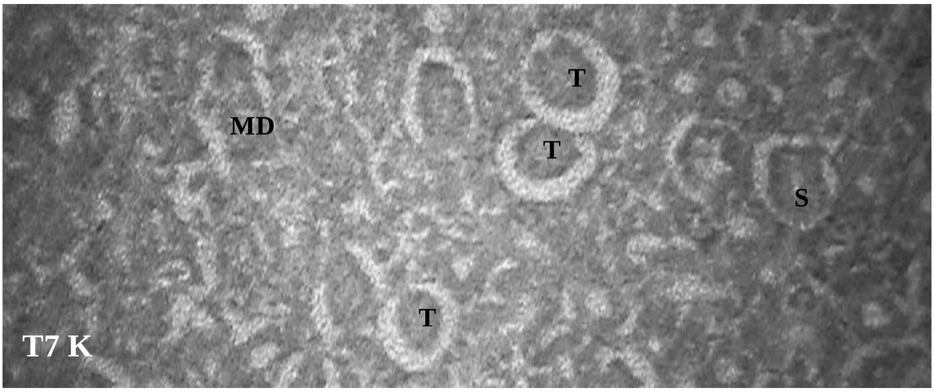


Plate (34): T.S. in the kidney of T7 broiler chicks at 6 weeks of age (H&E×200).

Plate (35): T.S. in the kidney of T8 broiler chicks at 6 weeks of age (H&E×200).

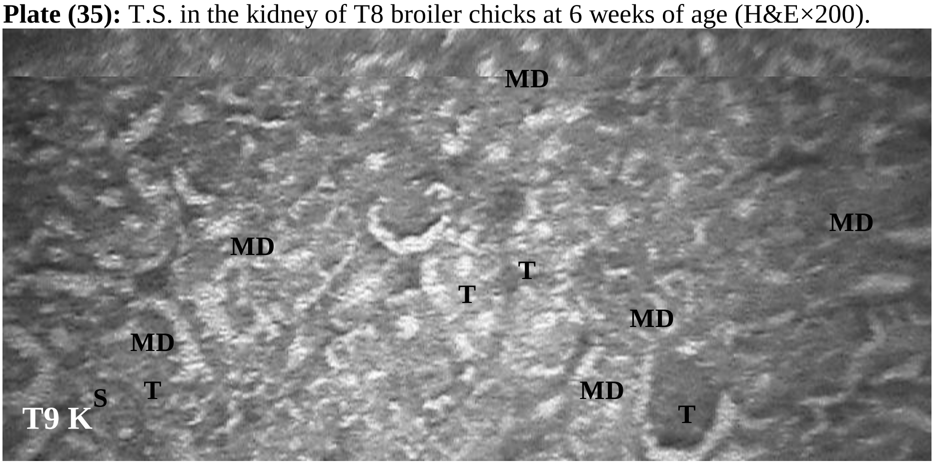


Plate (36): T.S. in the kidney of T9 broiler chicks at 6 weeks of age (H&E×200).

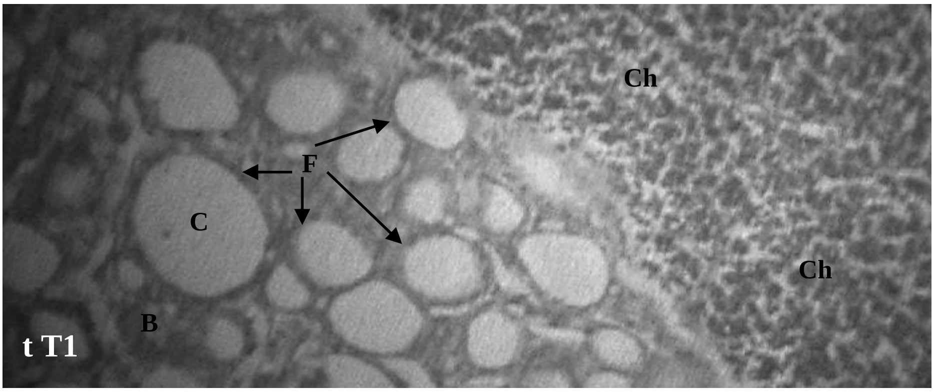


Plate (1): T.S. in the thyroid and parathyroid glands of T1 broiler chicks at 2 weeks of age (H&E×200).

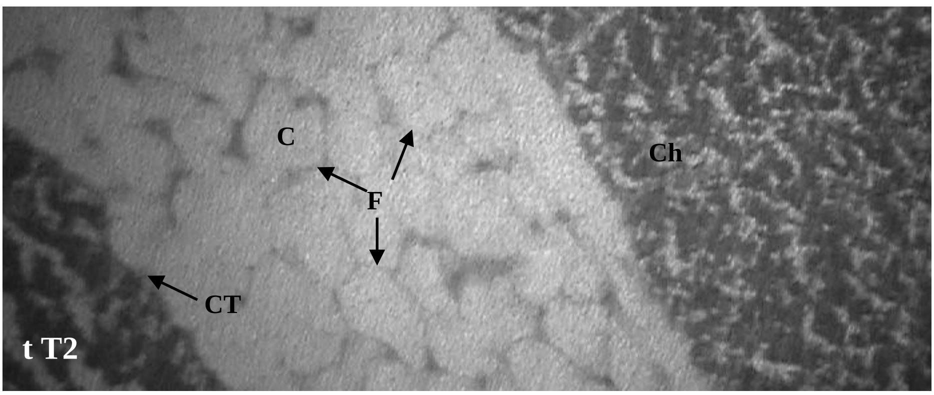


Plate (2): T.S. in the thyroid and parathyroid glands of T2 broiler chicks at 2 weeks of age (H&E×200).

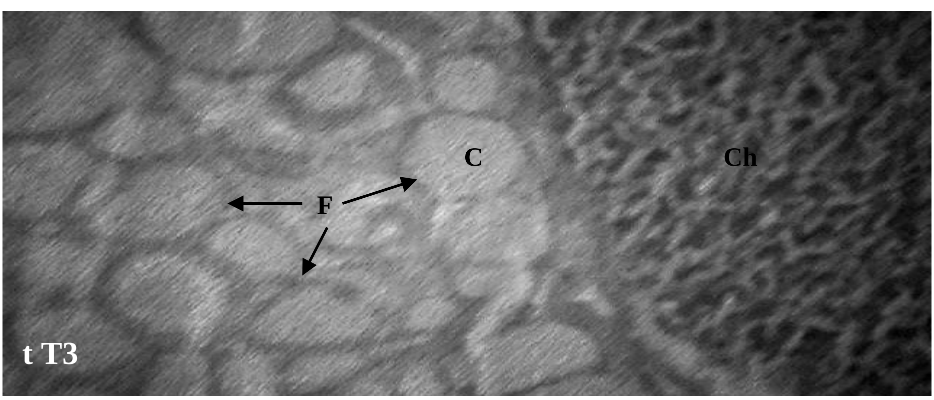


Plate (3): T.S. in the thyroid and parathyroid glands of T3 broiler chicks at 2 weeks of age (H&E×200).

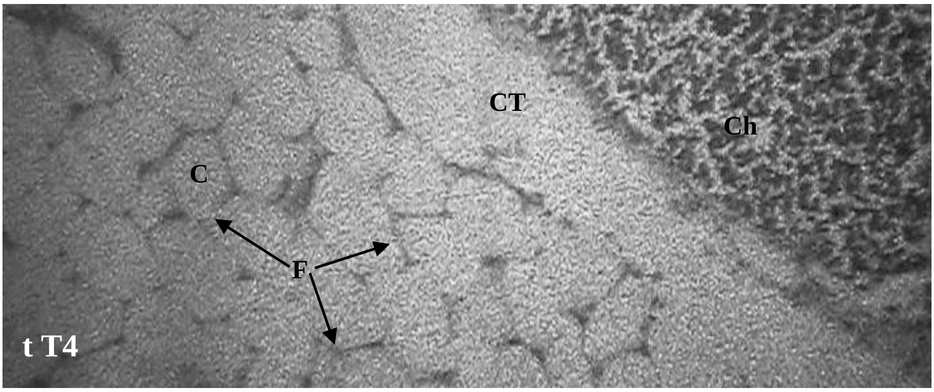


Plate (4): T.S. in the thyroid and parathyroid glands of T4 broiler chicks at 2 weeks of age (H&E×200).

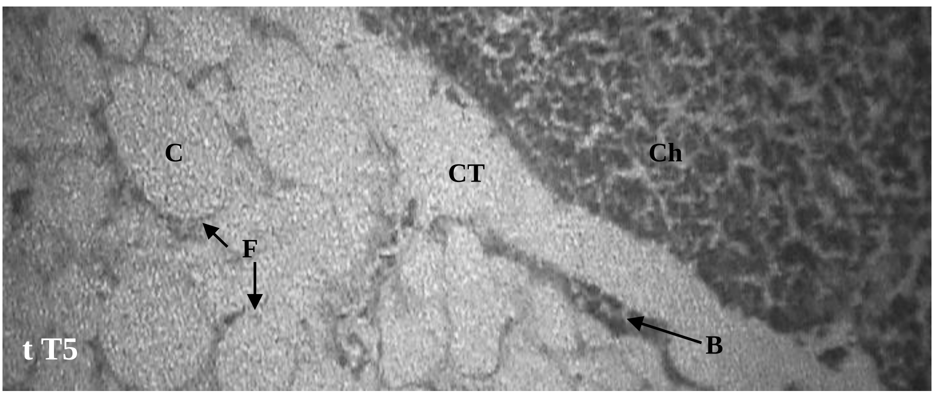


Plate (5): T.S. in the thyroid and parathyroid glands of T5 broiler chicks at 2 weeks of age (H&E×200).

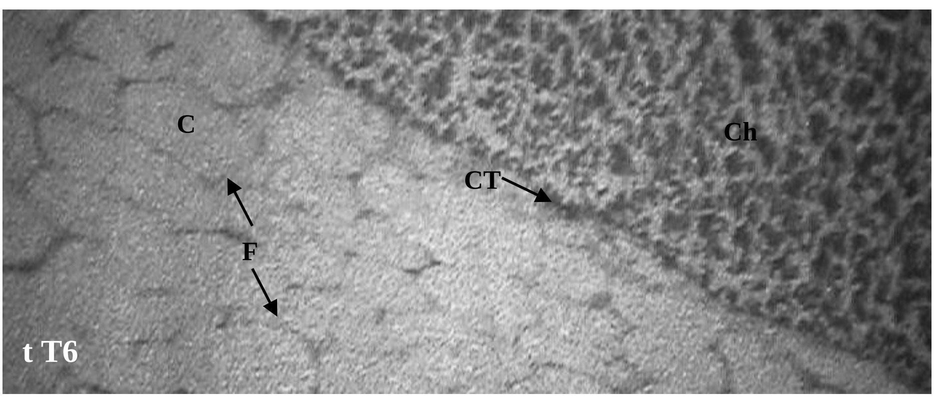


Plate (6): T.S. in the thyroid and parathyroid glands of T6 broiler chicks at 2 weeks of age (H&E×200).

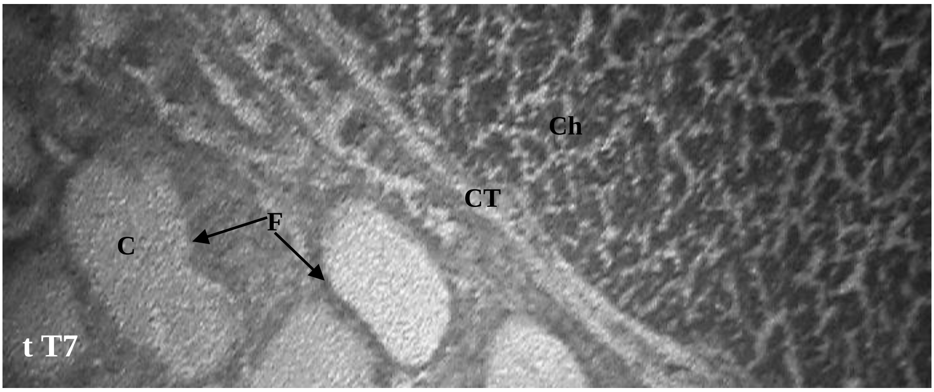


Plate (7): T.S. in the thyroid and parathyroid glands of T7 broiler chicks at 2 weeks of age (H&E×200).

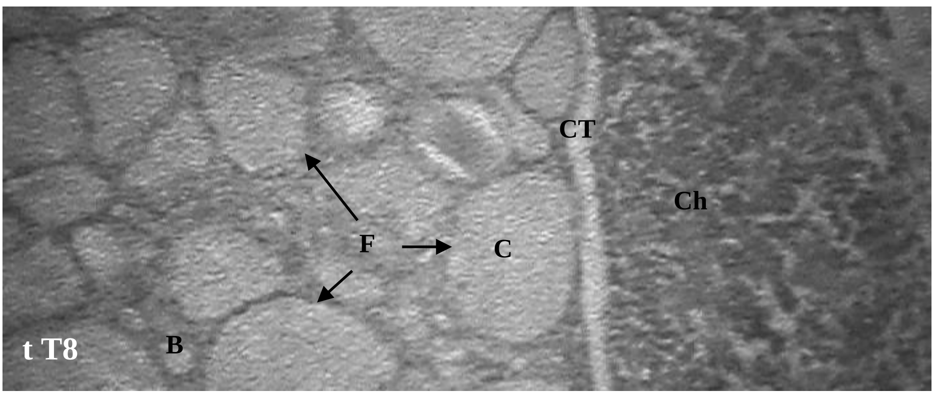


Plate (8): T.S. in the thyroid and parathyroid glands of T8 broiler chicks at 2 weeks of age (H&E×200).

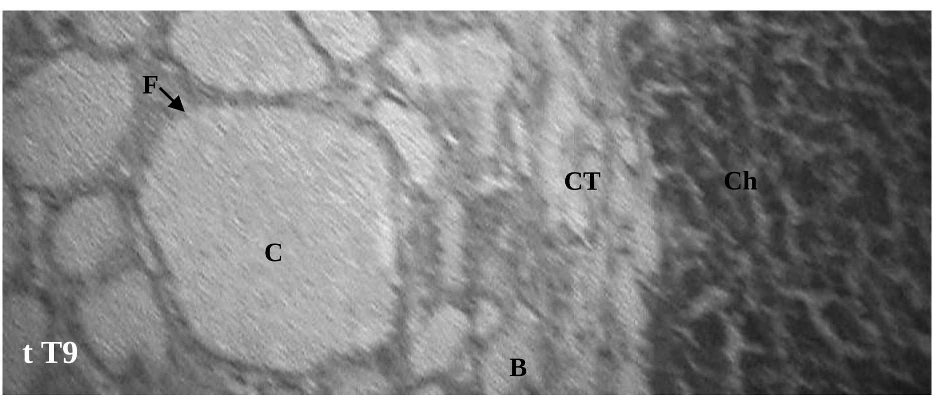


Plate (9): T.S. in the thyroid and parathyroid glands of T9 broiler chicks at 2 weeks of age (H&E×200).

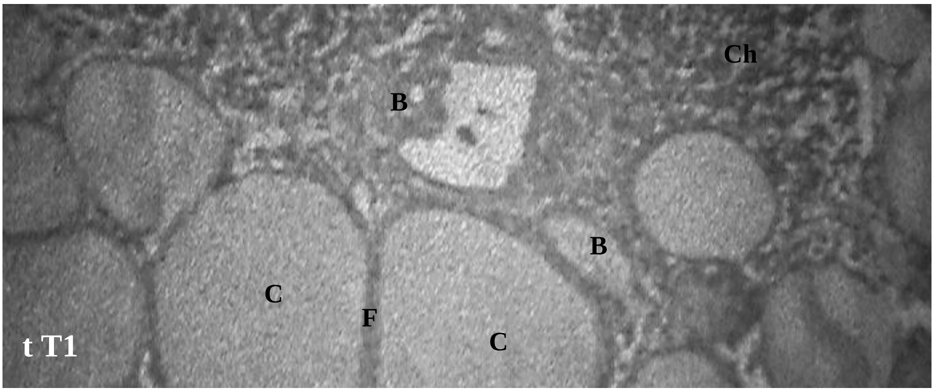


Plate (10): T.S. in the thyroid and parathyroid glands of T1 broiler chicks at 6 weeks of age (H&E×200).

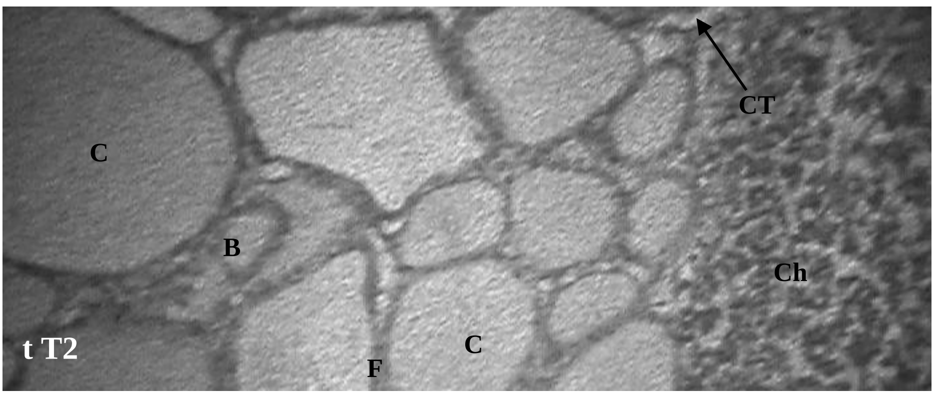


Plate (11): T.S. in the thyroid and parathyroid glands of T2 broiler chicks at 6 weeks of age (H&E×200).

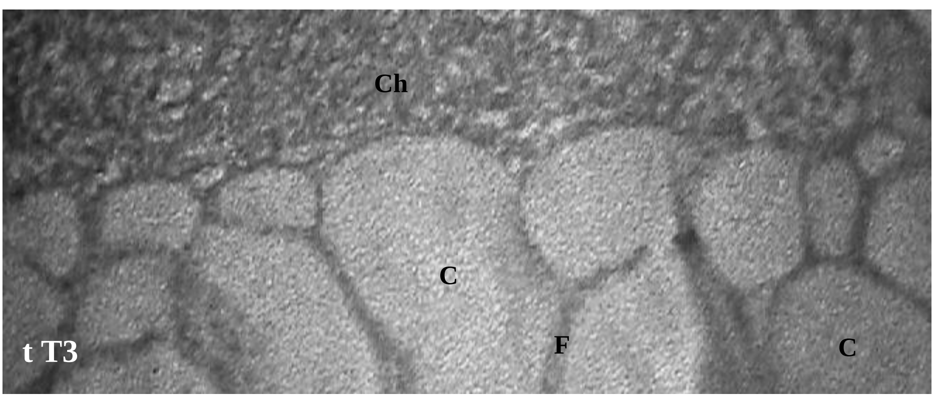


Plate (12): T.S. in the thyroid and parathyroid glands of T3 broiler chicks at 6 weeks of age (H&E×200).

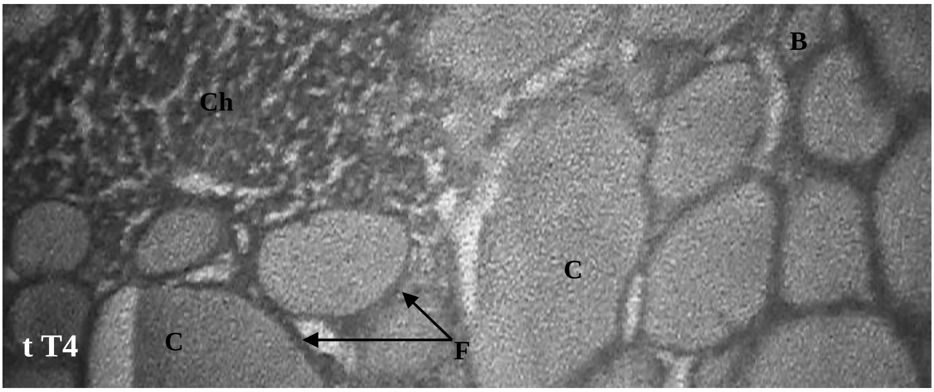


Plate (13): T.S. in the thyroid and parathyroid glands of T4 broiler chicks at 6 weeks of age (H&E×200).

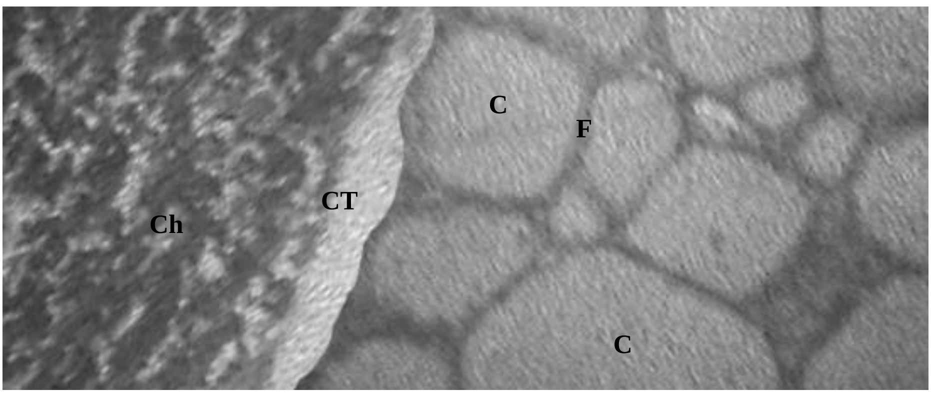


Plate (14): T.S. in the thyroid and parathyroid glands of T5 broiler chicks at 6 weeks of age (H&E×200).

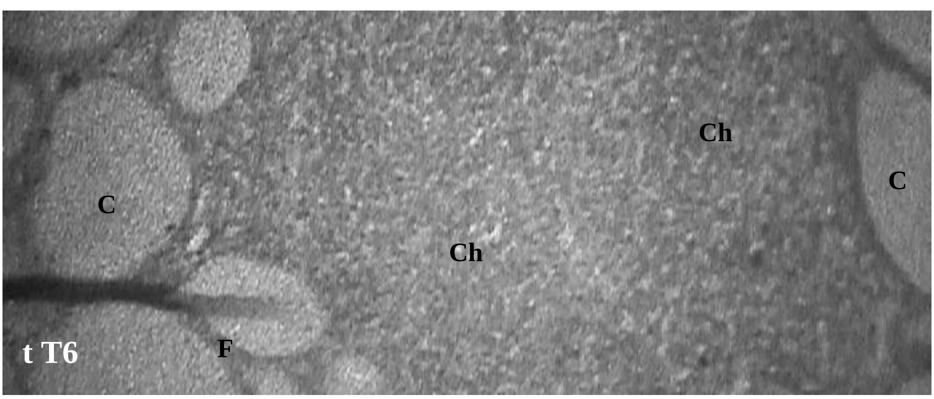


Plate (15): T.S. in the thyroid and parathyroid glands of T6 broiler chicks at 6 weeks of age (H&E×200).

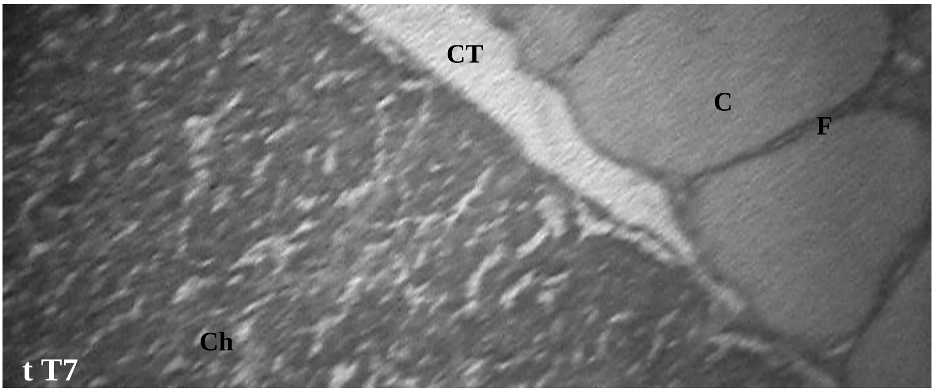


Plate (16): T.S. in the thyroid and parathyroid glands of T6 broiler chicks at 6 weeks of age (H&E×200).

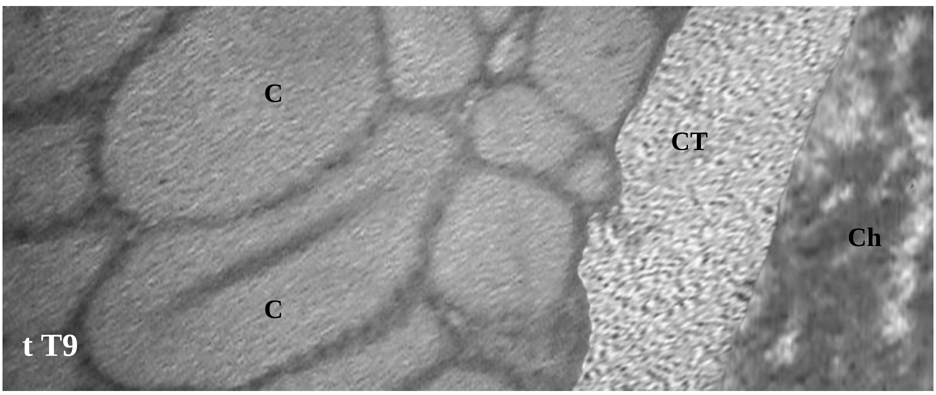


Plate (17): T.S. in the thyroid and parathyroid glands of T8 broiler chicks at 6 weeks of age (H&E×200).

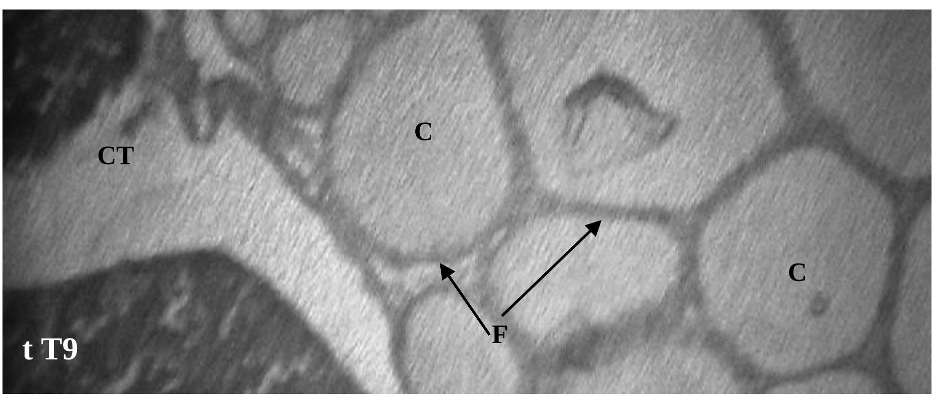


Plate (18): T.S. in the thyroid and parathyroid glands of T9 broiler chicks at 6 weeks of age (H&E×200).