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Effect of plant maturity stage on digestibility and distance walked for diet selection by goat at north Kordofan State, Sudan

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ABSTRACT

The main objective was to study grazing behavior of goats; diet selection, nutritive value, digestibility of range plant and body gained at flowering and seed setting stage at September and November 2010 respectively in El-khuwei locality (El Rosa). A completely randomized design was used (CRD). Sampling was done by two stage flowering and seed sating stage were selected diets and feed intake locating a 2000 x 2000 m plots. The average weights gains during the flowering and seed setting stage were 17 and 18.28kg respectively. Goats during the flowering stage was preference on bite counts of the different species, however highly ($P < 0.0001$) at the flowering and least during the seed setting stage. Goat preference ranked Bano *(Eragrostis tremula)*, Huskneet (*Cenchrus biflorus)*, Difra (*Echinocloa colonum)*, leflef (*Luffa aegyptiaca)*, Gaw (*Aristida spp.*), Fisiya (*Fimbristyls hispidula)*, Himeira (*Hymenocardia acida)*, Nuida (*Sida cordofolia)*, Tmrfar (*Oldenlandia senegalensis)* and Aboelrakhus (*Andropogon gayanus*), while Gadgad (*Geigeria alata)*, Buid (*Commelinia subulata)*, Simeima (*Sesamum alatum)*, Abodaib (*Ceraotheca sesamoid)* and Rabaa (*Zalea* spp) least than that. A significant higher ($P < 0.001$) goats selective feed intake, *in vitro* dry matter digestibility, dry matter, organic matter and crude protein higher at flowering stage and lowers during the seed setting stage. However; ash contents and crude fiber of plants were significantly higher at the seed setting stage. Body weight gain was significantly highest during the flowering stage, while the distance walked by goats for diet search was significantly longest during the seed setting stage. It was concluded that flowering stage beneficially goats highly preference and selectivity different species, feed intake and inviter dry matter digestibility and body weight gained. The seed setting stage was highly ash contents, crude fiber distant walked.

Key words: Availability, palatability, selectivity, digestibility, distant walked and body weight gains.

INTRODUCTION

Sudan is the largest country in Africa, with an area of 1.88 million Km2. It has a population of 33.42 million (CBS, 2011) and has the second largest animal population in Africa. In 2006, there were 138.2 million livestock, of which 50.39 million sheep, 42.76 million goats, (Ministry of Animal Wealth and Fisheries, 2006). Western Sudan has the most livestock (40%), followed by southern Sudan (27%) and central Sudan (23%). The majority of breeds is raised within tribal groups and often carries the name of the tribe. They are well adapted to the harsh environment and often trek long distances in search of feed and water. Productivity is low but can be improved with good management in more favorable conditions. Cattle are mainly descended from boss Taurus, or zebu. In central Sudan they are generally kept for milk, and in western Sudan for meat production. Sheep are of the Sudan Desert type, with live weights up to 70 kg and excellent meat and carcass characteristics. Goats, mostly of the large, black Nubian type, are found in central Sudan and are kept for milk. There are two types of the single-humped camel, one kept for riding and the other as a pack or baggage animal. Camels are exported mainly for meat. Forage produced from natural pastures represents 86.6% of national animal feed requirements, and about 14% of the population is involved in livestock production activities on the rangelands (MAW, 2005). North Kordofan amounts to almost 25 million ha, out of this area; 14.5 million ha are rangeland (AFRICOVER, 2004). The State is considered among the leading regions of Sudan in terms of animal and range resources, where more than 13 million heads of sheep, goats, camels and cattle are present (RPA, 2005). Animal production in the State is mainly practiced under traditional extensive systems, depending on natural rangeland (Cook and Fadlalla, 1987). Cattle dominate the southern part of the State, while sheep, goats and camels are present in larger numbers in the northern and drier part (El-Hag, 1993). The main objective of this paper was to investigate effects of grazing behavior on the various distances walked and digestibility from a diet selection by goats.

MATERIALS AND METHODS

This study was conducted at El-khuwei locality (El Rosa). It lies between longitudes 28˚:33' to 28˚:30'N and latitudes 12˚:14' to 14˚:12'E, about 105 Km west of El Obeid town, North Kordofan State lies between latitudes 11˚:20' to 16˚:36'N and longitudes 27˚:13' to 32˚:24'E. The close range system was established in 2007 in an area of about 500 ha, El-khuwei locality own large export market of animals (Hammer sheep) in west Sudan according to (MAWF, 2009). The long term average annual rainfall is about 300-mm, consisting of storms of short duration between July and September with the highest rainfall generally occurring in August. The soil of the site lies within the sand dune area locally known as “Goz” soil. The site is naturally dominated main grasses include namely Huskneet (*Cenchrus biflorus),* Shuleny (*Zornia glochidiata*) and Bigual (*Blepharis linarifolia*). The trees Humied (*Sclerocarya birrea*), Higlig (*Balanites*), Arad (*Acacia etbaica*) and Sider (*Zizuphus spina).* The Shrubs include Kursan (*Boscia senegalensis*), Usher (*Calotropis*), Mereikh (*Polygala eriotera*) and Aborakhus (*Andropogon gayanus*) according to (MAWF, 2009).

Sampling and experimental animals:

Sampling was done on two stages of plant maturity at flowering and seed setting in selected locations (2 km^2 each). Within each stage twenty goats randomly selected, their average weights gains were 17.00 and 18.28kg, respectively (Fadlalla and Cook, 1985).

Nutritional value of range quality:

Feed or diet selection:

The parameters measured diet botanical composition was estimated using the bite-count techniques, (Fadlalla and Cook 1985). The parameters measured included diet botanical composition and voluntary intake of dry matter. Within each season twenty goats was kept for this study. The first goat was followed for five times, and then the second one followed for another five minutes and so on for all goats. The procedure was repeated tine times, thus each goats followed for one hour in the first day, was also followed by observer for three days and 600 bites, and species of plant ingested and bite were recorded.

Voluntary feed intake and *in vitro* dry matter digestibility:

The total fecal collection and *in vitro* dry matter digestibility techniques were used to measure voluntary intake. In this technique, the total faces produced by grazing goat were collected into appropriately designed collection bags attached to animals. Collection bags attached to goats were emptied at least twice a day and weighted. *In vitro* DM and *In vitro* dry matter digestibility determined (Tilly and Terry, 1963). The sample for flowering and seed setting stages was obtained by observing plant species and plant parts selected by goats during grazing and then collecting similar material for analysis by Tilley and Terry (1963). I*n vitro* dry matter digestibility *INVDMD* was calculated according to the following formula:

$$INVDMD\% = \frac{\text{Sample DM} - (\text{Residue sample} - \text{Mean.resid DM inoc.blank})}{\text{sample DM}} \times 100$$

The voluntary intake of DM was determined according to Fadalla and Cook, (1985).from the following formula:

$$\text{Dry matter intake (DM)} = \frac{\text{Total fecal output /24 hr}}{100 - \text{DM digestibility } (in\ vitro)} \times 100$$

Measuring Parameters

Average distance walked

Distance from the goats search to voluntary feed intake. The first goat was followed for five minutes, and then the second one followed for another five minutes and so on for all goats. The procedure were repeated distance walked at five minutes of bite count by matter, thus each goat followed for one hour in the first day, ware also followed by observer for the total of distance walked under four days. However measured distance refers to the distance grazing area as measured by meter per hour according to Fadlalla and Cook (1985).

Average body weight gains

Two stages flowering and seed setting were measuring body weight gains (2 km^2 each). Within each season twenty goats was kept for this study. The procedure was repeated initial and final body weight of goats before and after grazing. The weight between initial and final equal weight change gram/day were recorded at three weeks on two stages Fadlalla and Cook (1985). Weight and body condition, for instance, provide a measure of the nutritional response, integrated over weeks or months (Lambourne *et al*, 1983).

Laboratory Analyses

Dry-matter weight (DM) is determined by drying the feed in the oven at 105°C for 12-15 hours and weighing. Organic matter (OM), crude protein (CP) was determined by (AOAC, 1980). Crude fiber (CF) was determined by (Van Soest, 1982). *In vitro* dry matter digestibility (*INVDMD*) was determined (Tilley and Terry, 1963).

Statistical Analysis

Completely Randomized Design (CRD) was used in this experiment. Data were subjected to analysis of variance and means were estimated. Chi Square test was used to compare diet selection (Steel and Torrie, 1960). SPSS (Statistical Package for Social Sciences) computer software was used for the statistical analysis.

RESULT AND DISCUSSION

Bite Counts

Table 1 shows the bite counts of range species by goats during the flowering and seed setting stages. Goats during the flowering stage was preference on bite counts of the different species, however highly ($P < 0.0001$) at the flowering stage and least during the seed setting stage. Laca *et al* (2001) indicated that rates of nutrient intake are reduced at too low or too high levels of plant biomass. Intake is influenced by bite size; bite

rate, and grazing time. Goat and sheep differed significantly (P<0.001) in selection of different range plants supporting the findings of (Hodgson, 1979).

Table 1. Bite counts (%) ♀ **of the different range species by goat during the flowering and seed setting stages at El-Khuwei locality, north Kordofan, Sudan.**

Plant species		**Bite count** (%)	
Latin names	**Local name**	**Flowering stage**	**Seed stage**
Eragrostis tremula	Bano	74.25	34.67
Cenchrus biflorus	Huskneet	48.46	30.56
Echinocloa colonum	Difra	33.85	29.99
Luffa aegyptiaca	Leflef	31.71	24.10
Aristida sp	Gaw	29.55	20.82
Fimbristyls hispidula	Fisiya	23.20	19.00
Hymenocardia acida	Himeira	21.09	14.07
Sida cordofolia	Nuida	18.18	13.89
Oldenlandia senegalensis	Tmrfar	17.99	11.21
Andropogon gayanus	Aborakhus	16.50	8.86
Geigeria alata	Gadgad	14.98	-
Commelinia subulata	Buid	10.14	-
Sesamum alatum	Simeima	4.00	-
Ceraotheca sesamoid	Abodaib	2.86	-
Zalea sp	Rabaa	2.86	-
Zornia glochidiata	Shuleny	2.50	-

Voluntary feed intake and digestibility:

Voluntary feed intake and *in vitro* dry matter digestibility were presented in table 2. Feed intake was significantly (P<0.001) higher at the flowering stage compared to the seed setting stage. The results revealed that the goats had significantly (P< 0.0001) better *in vitro* dry matter digestibility during the flowering stage compared to seed setting stage. The decreasing digestibility of dry matter during seed setting stage may be due to age of grasses. McDonald *et al* (1973) who reported 50-80% dry matter digestibility was higher for young grasses. The animals' feed preferences are influenced by feed availability, plant structure, nutrient deficiencies (e.g. salt) and appetite and, of course, different species of animals prefer different types of feed Chacon *et al* (1978). For instance, seasonal rainfall is often assumed to be an indicator of feed conditions while stocking rate has been used as a substitute for feed intake (Abel *et al*, 1987).

Table 2. Feed intake and *in vitro* dry matter digestibility during the flowering and seed setting stages at El

Parameters	**Flowering stage**	**Seed setting stage**	**SE±**
Feed intake **(**g/day)	0.54	0.36	0.04**
IVDMD **(%)**	67.46	60.15	1.05***

Means in the same column under the same factor with different letters are significantly different.
* = significant (P < 0.05), ** = high significant (P < 0.01) and *** = highly significant (P < 0.001)

Live weight gains

Table 3 shows the live weight gains of goats during two stages of plant maturity. During the flowering stage goats significantly (P<0.0001) gained more body weight (25.00 g/day), when compared to seed setting stage (0.47 g/day). Devendra *et al* (1970) reported the forage intake of the goat has been taken as 2.5% of body

weight, based on a range of 2.1 to 3.2%. When making comparisons between animals of different size to determine the importance of nutrition as a constraint, DM intake should be expressed in relation to the live weight (and preferably the metabolic weight, i.e. LW0.75) of the animal (Graham, 1972).

Table 3. Body weight gains of goats, during the flowering and seed setting stages at El-Khuwei locality, north Kordofan, Sudan.

Parameter	Flowering	Seed setting	SE ±
Body weight gains (g/day)	25	0.47	1.58***

Means in the same column under the same factor with different letters are significantly different.
* = significant (P < 0.05), ** = high significant (P < 0.01) and *** = highly significant(P < 0.001)

Distance walked

Table 4 shows the distance walked per hour (m/hr) by goats. Also the results revealed that the goats significantly (P<0.0001) walked more distance during the seed setting stage (92.63 m/h) compared to the flowering stage (44.50 m/h). De Leeuw and Chara (1985) used the technique to compare goat and sheep browse preferences in mixed Maasai flocks in Kenya. Range condition is based on density and production of native, palatable, perennial grasses. A better criterion might be the diversity of palatable forage species. It might be desirable if up to 20% of yearly forage production is composed of palatable annuals (Holechek, 1984).

Table 4. Distance walked (m/hr) by goats at the flowering and seed setting stages at El-Khuwei locality, north Kordofan, Sudan.

Parameter	Flowering	Seed setting	SE ±
Distance walked (m/hr)	44.50	92.63	2.34***

Means in the same column under the same factor with different letters are significantly different.
* = significant (P < 0.05), ** = high significant (P < 0.01) and *** = highly significant (P < 0.001)

Nutritive value of diet

Table 5 shows the nutritive value of diet intake. The nutritive values of diet such as dry matter (DM), organic matter (OM) and crude protein (CP) were significantly higher during the flowering stage compared to seed setting stage. However; ash content and crude fiber (CF) were significantly (P<0.0001) higher during the seed setting stage than the flowering stage. Leaves of grasses from forbs and shrubs are generally higher in protein, crude fiber, dry matter, organic matter and ash content than the grass leaves and stems at comparative stages of growth (Vansoest, 1982).

Table 5. Nutritive value of diet (%) during the flowering and seed setting stages at El-Khuwei locality, north Kordofan, Sudan

Parameter	Flowering	Seed setting	SE±
DM	0.96	0.95	0.10***
OM	0.86	0.83	0.18***
Ash	0.09	0.12	0.35***
CF	0.31	0.37	0.47***
CP	0.14	0.10	0.12***

Means in the same column under the same factor with different letters are significantly different.
* = significant (P < 0.05), ** = high significant (P < 0.01) and *** = highly significant (P < 0.001)

CONCLUSION

It was concluded that flowering stage beneficially goats highly preference and selectivity different species, feed intake and inviter dry matter digestibility and body weight gained. The seed setting stage was highly ash contents, crude fiber distance walked.

REFERENCE

Abel NO, Flint ME, Hunter ND, Chandler D and Make G (1987). Cattle keeping, Ecological change and communal management in Ngwaketse.Vol.1. Summary; Vol. 2. Main report. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia; Integrated Farming Pilot Project, Ministry of Agriculture, Gaborone, Botswana and Overseas Development Group, School of Development Studies, University of East Anglia, Norwich, UK Vol. 1, 45 pp; Vol. 2, 82 pp.

Africover (2004). Sudan Spatial Aggregated Map, Afrikaner Project, FAO, Rome, Italy.

AOAC (1980). Official method of analysis (13th Ed). Association of official. Analytical Chemists. Washington D.C.

CBS (2011). Central Bauru of Statistics; estimated population in country, Khartoum, Sudan.

Cook RH and Fadlalla (1987). Seasonal Variation in plasma phosphorus Level Of transhumant sheep in Kordofan, Sudan. Trop. H1th.prod. 19:57-62.

Chacon EA Stobbs TH and Dale MB (1978). Influence of sward characteristics on grazing behavior and growth of Hereford steers grazing tropical grass pastures. *Australian Journal of Agricultural Research* 29(1):89-102.

De Leeuw P and Chara P (1985). Species preferences in browse of sheep and goats in mixed Maasai flocks in eastern Kajiado, Kenya Paper presented to the fourth CRSP (Collaborative Resource Support Programmers) workshop on small ruminants, Kebete, Kenya, 5- 6 March 1984.

Devendra C and Burns (1970). Nutritional analysis by University of Hohenheim of dietary grab samples collected by CR. Field.

El-Hag FM (1993). Conserved range grasses for dry season feeding goats In shiekan province, North Kordofan, Sudan, El Obied Agricultural Research Station, 1:1-2.

Fadlella B and Cook RH (1985). Design and implementation of in-herd/on-range trials: use of sentinel herds. In: Research methodologies for livestock on-farm trials.

Graham NM (1972). Units of metabolic body size for comparisons amongst adult sheep and cattle. In: Proceedings of the Australian Society of Animal Production, Ninth Biennial Conference held in Canberra, Australia, Feb. 1972. Ramsay, Ware Publishing Pty Ltd. Australia. Vol. 9, pp. 352-355.

Holechek JL (1984). Comparative contribution of grasses, forbs, and shrubs to the nutrition of range ungulates. Rangelands 6:245-248.

Hodgson J (1979). Nomenclature and definitions in grazing studies. *Grass and Forage Science* 34, 11–18.

Laca E, Shipley LA and Reid ED (2001). Structural antiquality characteristics of range and pasture plants. *J. Range Manage*. 54:413-419.

Lambourne LJ, Dicko MS, Semenye P and Butterworth M (1983). Animal nutrition. In Pastoral systems research in sub-Saharan Africa. Proceedings of the IDRC/ILCA Workshop held at ILCA, Addis Ababa, Ethiopia, 21-24 March 1983. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia. pp. 183-208.

MARF (2009). Ministry of Animal Resource and Fisheries, report. North Kordofan, Sudan.

MAWF (2005). Ministry of Animal Wealth and Fisheries .Animal Population Report, Khartoum, Sudan.

MAWF (2006). Ministry of Animal Wealth and Fisheries. Animal Population Report, Khartoum, Sudan.

McDonald P and Whitten bury R (1973). The ensilage process. In G.W.Butler and R.W.Baily. (eds) Chemistry and biochemistry of herbage. Academic press, Inc., London.

RPA (2005). Range and Pasture Admin, North Kordofan State. Annual Range Survey Report.

Steel RCD and Torrie JH (1960). Principles and procedures of Statistics. Mc Graw, Hill Book Co. New York (Ends) 454 pp.

Tilly JM and Terry RA (1963). A two stage Techniques for in vitro digestion of forage crop J. Br Soc (England) 18, 104-111.

Van Soast PJ (1982). Forage conservation. In nutrition anal ecology of ruminant. O and B book, Inc. Corvallis, Oregon, U. S. A. P 139.

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Impact of the feed metabolizable energy on protein and amino acids demand of Japanese quails

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ABSTRACT

The production of Japanese quail has great importance in the production of foods and their nutritional needs are well established, yet still make feed based on recommendations misleading and can reduce the efficiency of production. The nutritional recommendations are presented in different countries and methodological ways to achieve levels, but an important detail is not often considered, the relationship between the nutrients, especially between metabolizable energy and other nutrients. On this basis, it is clear that there is a gap in this direction and this paper will discuss nutritional recommendations, compare levels and simulate diets practices to make an assessment of how best and most efficiently. Based on these data and discussion in this paper, it is concluded that Japanese quails have similar recommendations, but the metabolizable energy: nutrients ratio is different and not be considerate and this can adversely affect production and efficiency of quails.

Key words: Amino acids, energy, Japanese quail, requirements

INTRODUCTION

With the need to increase the production of quality food and high availability in the market, studies were performed for this purpose and the act of formulating diets became one of the most important steps in the process of global poultry production. Thus, to formulate diets commercially viable and possible to demonstrate all the genetic potential of the poultry, the diet formulator must understand the whole process complex and responsible for much of the efficiency of poultry. Such as balancing the diet properly so that the bird does not intake much or little amount of nutrients necessary for their higher yield potential, which it seems simple, it often does not occur satisfactorily.

Protein and energy are required for egg maintenance, growth and production, being the energy the main component in diet formulating for all animal species, because the energy regulates feed intake and as quails have

feed intake higher than the layers in the live weight proportion (Silva *et al*., 2007a), diets with inadequate energy level can influence to a bigger or lesser feed intake and quail performance. The prediction models are mathematical tools that help the animal nutritionist to make decisions, correct and set new feeding programs based on optimizing the performance of quails (Silva *et al*., 2004ab) and therefore can influence better performance during the egg production phase.

On this basis, especially concerning to the modification of nutritional recommendations of the birds in recent years, as well as the influence of metabolizable energy on the need for protein and amino acids, this material is intended to report the influence and impact of metabolizable energy of Japanese quail on the nutritional requirements of protein and amino acids for these birds.

Metabolized Energy

The energy value is a very important expressing base to the value of nutritional importance of feed used in the diets. The high energy relative to low protein in the diet, namely, a high metabolizable energy: protein ratio reduces feed intake (Page & Andrews, 1973; Bertechini, 2004), which promotes a lower intake of protein and other essential nutrients, also raising the accumulation of abdominal fat. Likewise a low metabolizable energy: protein ratio raises the use of protein as an energy source, causing an increased metabolic cost and, of course, economical, invalidating the efficient production.

The energy level is one of the most important components in the formulation of poultry feed, considering that the birds feed intake to meet their energy needs initially. According to Moura *et al*. (2010), the feed intake is regulated by the energy density of the diet and by nutritional requirements, which consequently affects the egg performance and egg quality. Thus according to Bertechini (2006), all nutrients must be related to the energy content of the diet. Excess energy in the diet in the form of carbohydrates, lipids and proteins (disequilibrium in the amino acid profile) causes a reduction in voluntary intake by birds (Baião & Lara, 2005) and fat deposition in the carcass. According Bertechini (2006) excess fat in the liver and ovary may occur due to the imbalance of energy in the diets of commercial laying hens, promoting a reduction in egg production.

The existing recommendations of metabolizable energy for Japanese quails are not, among many, widely divergent. So, the NRC (1994) recommends a diet with 2900 kcal/kg metabolizable energy (ME) for Japanese quails at the initial phase and egg production phase, while Silva & Costa (2009) recommend similar values, but differentiate the quails in three distinct phases, ie, 2900, 3050, 2800 and 2850 kcal/kg ME to quails from 1 to 21, 22 to 42 days of age and in the I and II egg production phases, respectively. Rostagno *et al*. (2011) recommend a level of 2900 and 2800 kcal / kg ME for Japanese quail I and II growth phases, and in egg production phase, when the quails have, at this stage, a weight of 177g body weight, respectively.

Although the levels recommended for metabolizable energy of the three recommendations listed above are not quite variable, which draws more attention is the metabolizable energy: protein ratio of them. To get an idea, the NRC (1994) recommends a metabolizable energy: protein ratio of 120.8 and 145 for initial and egg production phase, respectively. Similarly, Silva & Costa (2009), based on the recommendations of metabolizable energy and crude protein, recommend a ratio of 116, 138.6, 140 and 129.5 for initial phase, growth, egg production I and egg production II. Meanwhile, Rostagno *et al*, (2011) recommend a ratio of 131.8 and 148.9. Given this, we realize that we cannot formulate diets for quail just based on nutrition recommendations of metabolizable energy, but the important thing is to check what the ideal metabolizable energy: protein ratio is due, primarily not to promote changes in the metabolism of the Japanese quail that may cause loss in the efficiency of egg production. This effect on protein will influence, concomitant, the metabolizable energy: amino acids ratio and will affect in large scale the efficiency of the lot,because of the modified feed intake and amino acid disbalance that can be caused.

Moura *et al*. (2008) evaluated diets of different energy densities and metabolizable energy: nutrients ratios constantly in the feeding of Japanese quail and found an increase of 8.9% in feed intake when the energy density decreased from 2900 to 2500 kcal/kg. Freitas *et al*. (2005) also found a linear increase in feed intake by reducing the metabolizable energy level of Japanese quail. Thus, we can infer that the feeding behavior of Japanese quail is changed and that feed intake varies according to the level of dietary metabolizable energy directly influencing the quality of eggs produced.

Researches point the effect of different energy levels in the diet about the variables of egg production. Freitas *et al*. (2005) had observed a linear reduction in egg weight and egg mass with the increase in metabolizable energy level of the feed. Such authors have tested four levels of metabolizable energy (2585, 2685, 2785 and 2885 kcal/kg ME) in diets of Japanese quails and observed a 2.3% reduction in egg weight when compared to the egg weight of quails with feed diet containing 2585 and 2885 kcal/kg ME and 7.7% of reduction in the egg mass/hen/ day in these same metabolizable energy levels. Barreto *et al*. (2007) evaluating metabolizable energy levels (2650, 2750, 2850, 2950 and 3050 kcal/kg ME) in isonitrogenous diets (20% Crude Protein) for Japanese quails in initial phase of egg production observed linear reduction in feed intake, egg weight, yolk weight and albumen weight by the increasing of dietary metabolizable energy levels. It was further found that the increase in dietary metabolizable energy level has not resulted in an increase of yolk cholesterol concentration. Therefore, we can say that the low feed intake observed with the increasing metabolizable energy results in insufficient intake of nutrients for maintenance of egg weight and formation of their constituents, showing the adjustment of the bird between feed intake and energy in the detriment of reproductive processes, as the cholesterol present in the yolk of eggs is essential for embryo development.

The energy density of diet appear to exert little influence on the formation of the yolk, as Moura *et al*. (2010) evaluated the effect of the reduction in energy density (2900, 2800, 2700, 2600 and 2500 kcal/kg ME), keeping fixed the metabolizable energy: nutrients ratio from the diet on the characteristics of Japanese quail eggs, found no significant differences to the weight percentage of yolk, albumen and egg shell. Significant differences in weight and yolk percentage were not found by Costa *et al*. (2004) when evaluated diets with levels of 2700, 2800 and 2900 kcal/kg ME and 17.5% crude protein in laying hens. The results related to yolk suggest that Japanese quails and laying hens usually are not influenced by the energy density in the yolk formation, in other words, the percentage of egg yolk produced has values near to 30% of the egg weight.

Protein and Amino Acids to Japanese Quails

For many years, studies in poultry nutrition evaluated animal performance based on the levels of crude protein dietary. However, due to the increase in production costs associated with the rising prices of feed used as protein sources in the poultry diets, such as soybean meal, research began to evaluate the bird's nutritional requirements with respect to the levels of essential amino acids, what has become possible due to higher industrial production of amino acids by the feed industry. Thus, based on the protein structure knowledge, the poultry, in fact, do not have a specific requirement for crude protein, but for essential and non essential amino acids, the latter being synthesized from non-specific nitrogen, so that the energy: protein ratio ceases to be the first in importance, because what most affects the performance of Japanese quail is the energy: amino acids ratio.

Research conducted with laying hens show the relationship between the levels of methionine and egg weight, showing that the increasing levels of methionine promotes a higher egg weight (Pinto *et al*., 2003; Freitas et at., 2005). This occurs, possibly, by the fact that methionine is the initiator amino acid of protein synthesis and one of its main functions is to act on the weight and number of eggs. These values agree with the ones presented by Novak *et al*. (2004) who found that higher intake of methionine and lysine significantly influenced the weight of albumen.

According to Pinto *et al*. (2003) increased levels of methionine + cystine may cause amino acid disbalance, promoting reduction of protein synthesis and inhibiting the absorption of the limiting amino acids, along with an increase in the catabolism thereof. This is because, unlike what happens with carbohydrates and lipids, the birds present low ability to store protein. Thus, the bird may suffer from deficiency of vitamins, such as choline, which in these conditions can promote fat accumulation in the liver, generating a hepatose.

The estimate of its requirement in poultry feed provides adequate balance of diets based on the ideal protein concept, since lysine is considered the standard for establishing the protein requirements and other amino acids. This way, having lysine as the limiting amino acids standard, any changes to your requirement or recommendation, either by environmental factors, dietary or genetics, will not change the ratio of the ideal lysine and other amino acids, for it is always constant, but that does not mean that will be the same dietary level.

Costa *et al*. (2008) determined the digestible lysine requirements for Japanese quails and found that egg production was influenced and was higher in the level of 1.03% of digestible lysine. Similar results were observed by Ribeiro *et al*. (2003), who found higher egg production with 1.07 and 1.15% of total lysine in diets

containing 20 or 23% of crude protein, respectively. Similarly, Pinto *et al*. (2003) estimated for better egg production, requirement of 1.045% digestible lysine in the diet. Already Garcia *et al*. (2005) evaluated three levels of protein (16, 18 and 20%), three of methionine + cystine (0.700, 0.875 and 1.050%) and two levels of lysine (1.100 and 1.375%) during the egg production phase of Japanese quail verified increase in the percentage of egg yolk with the increasing levels of methionine + cystine, but the protein had no effect on this variable. No effect was observed in the protein, methionine + cystine and lysine on the percentage of albumen. The percentage of protein in the yolk was influenced by the levels of protein and methionine + cystine, where quails fed with 18% or 20% of crude protein had higher levels of protein in the yolk compared to quails fed with 16%. As for methionine + cystine, the level of 0.700% provided higher amount of protein in the egg yolk, compared to the 0.875% level.

The excess of methionine causes a reduction in the growth of poultry due to a defect generated around threonine, with a reduction in plasma of threonine. Another factor is about the lysine amino acid, its excess can cause a deficiency of threonine (Umigi *et al*. 2007). Thus, a way to prevent any excess or lack of any amino acid, particularly threonine, is the study and evaluation of the best threonine: lysine ratio.

Lima *et al*. (2013) evaluating the supplementation of threonine from 0.66 to 0.86% varying 0.04% in each of total threonine level in diets of Japanese quails, equivalent to ratios of 66, 70, 74, 78, 82 and 86 with the total lysine. The authors found no significant effect on feed intake, as observed previously by Umigi *et al*. (2007), but unlike the data from Umigi *et al*. (2007) they found a quadratic effect on the egg production, egg weight, egg mass, egg mass conversion and the egg dozen conversion obtaining optimal ratios of 77, 75, 78, 79 and 81, respectively. The performance of Japanese quail can be significantly improved by supplementation of threonine in the diets and the levels recommended by the NRC (1994) no longer appear to attend the daily needs of the Japanese quails, which makes the levels recommended by Silva & Costa (2009) and Rostagno *et al*. (2011) to be more befitting with reality and corroborate with the recent literature.

Therefore, the threonine appears to influence the performance of the quails, but not the quality of the eggs, although noticeable histological differences in the reproductive system of the birds, according to Lima *et al*. (2013) when reported that the highest threonine: lysine ratio in diets of laying Japanese quail, the tubular glands of the magnum were presented in greater numbers, more active functional stage and presenting higher amount of albumen, which, according to the authors these features allow that the egg production to be done in a shorter period of time, thereby increasing egg production, egg weight, egg mass, egg mass conversion and egg dozen conversion. Lima *et al* (2013) also found that increasing threonine: lysine ratio also increased the amount of secondary folds of these quails uterus. Such characteristics, according to the authors, allow the formation of the shell in a shorter time, increasing, thus, the egg production since the egg formation is located in the uterus for a variable time to the deposition of calcium carbonate, being this one accelerated by the increase of the internal surface of the organ.

According to the NRC (1994), the requirement of total tryptophan for Japanese quails in the initial phase and in reproduction is of 0.22 and 0.19%, with 24 and 20% CP and 2,900 kcal of ME/kg diet, respectively. Shim & Lee (1993) reported that, for optimum egg production and feed efficiency in the diets of laying quails shall contain, in total amino acids, 1.0% of lysine, 0.43% of methionine, 0.18% of tryptophan and 0.63% of threonine. However, studies performed by Shim (1984) determined the requirement of 0.25% of total tryptophan. Leeson & Summers (2005) disagreed with the previous authors, recommending 0.22% of total tryptophan in the diet of quail production phase.

Pinheiro *et al*. (2008) evaluated the levels of tryptophan in the Japanese quail's diet, using quails with 21-30 weeks of age, with weight and egg production averaging 158.5 g and 84.50%, respectively. The quails were fed with isocaloric and isoproteic diets, except for to the digestible tryptophan, which were, for each experimental diet, 0.120 to 0.280%, with a range of 0.04% for each level of digestible tryptophan, totalizing five levels, what corresponded to the digestible tryptophan: lysine digestible ratio at 12, 16, 20, 24 and 28%, respectively. After analyzing the data, the authors verified tryptophan influence on the tryptophan intake and in egg production, so that they had a linear increasing effect in relation to the tryptophan level in the diet, where for each 1% of the digestible tryptophan in the diet, the egg production increased 1.26%. The results obtained from Pinheiro *et al*. (2008) confirm those obtained by Harms & Russell (2000) and Deponti *et al*. (2007), that had worked with

laying hens and observed improvements in egg production, as increasing levels of tryptophan were added to the diets.

Pinheiro *et al*. (2008) recommended 0.21% of digestible tryptophan, corresponding to the intake 45.0 mg of digestible threonine/quail/day for better performance and egg quality of Japanese quail. Moreover, Rizzo *et al*. (2008), working with tryptophan levels between 0.23 and 0.98%, found no effect on performance or on the physiological parameters of Japanese quail, concluding that 0.23% of tryptophan in diets with 18% protein gross and 2800 kcal ME / kg, are sufficient for these quails.

According to studies mentioned and discussed above, it is noticed the variation on the tryptophan nutritional recommendations for Japanese quails, since that there are few researches about these amino acids. Therefore, when comparing the suggested recommendations to Japanese quail with the suggested for laying hens, it is perceived the similarity in the diet level, however, as the quails intake about 25% of the laying hen diet quantity, so what directly influences is the energy daily intake, that will modify and regulate the amino acids intake. So the energy really determines the amino acid intake in Japanese quail, because if we base on a diet with 2900 kcal ME, one laying hen consuming 100g/bird/day and Japanese quail consuming 25g/bird/day, would be consuming 290kcal and 72.5 kcal/kg daily.

Comparative of the Nutricional Recommentadions for Japanese Quails

Based on the previously commented, the protein and energy are prymordial factores in an efficient diet and need to be always correlated. The knowing of the metabolize energy: nutrients ratio is essencial for the success of the Japanese quail production, as in any other animal production, principally because in the poultry production, more specifically the Japanese quail production, the feed offer is at will and so, the nutritional recommendations must be balanced according to energetic content of the diets that quails are consuming, which requires a correction as it alters the energy level of poultry feed.

Based on this, we will go further to do a practical comparative on nutritional recommendations such as those suggested by NRC (1994), Silva & Costa (2009) and Rostagno *et al*. (2011).When comparing the recommendations, according to Table 1, we found out that the level of metabolizable energy practically does not differentiate more than 4 points, especially, in the initial stages. The NRC (1994) maintains the same recommendation for initial and laying phase, Silva & Costa (2009), in the other hand, recommend varying levels, similar to Rostagno *et al*. (2011). However, Rostagno *et al*. (2011) divides the rearing phases of the quails production in just two phases, like the NRC (1994), unlike Silva & Costa (2009) that divides the rearing phases into four distinct phases, recommending specific nutritional levels for each one of them.

Table1. Resume of the nutritional recommendations of amino acids to Japanese quails according to NRC (1994), Silva & Costa (2009) and Rostagno *et al*. (2011)

Items	NRC(1994)		Silva & Costa (2009)				Rostagno (2011)	
	Initial	Laying	Initial (1-21d)	Growth. (22-42d)	Laying I	Laying II	Growth I and II	Laying (177g/quail)
Crude Protein,%	24	20	25	22	20	22	22	18.8
Metabolized Energy, kcal/kg	2900	2900	2900	3050	2800	2850	2900	2800
Arginine, %	1.25	1.26	1.16	1.05	1.26	1.38	1.19	1.273
Isoleucine,%	0.98	0.9	0.89	0.74	0.87	0.96	0.8	0.713
Lysine,%	1.3	1.0	1.19	1.05	1.03	1.05	1.12	1.097
Methionine +Cystine, %	0.75	0.7	0.8	0.74	0.7	0.72	0.76	0.9
Threonine, %	1.02	0.74	0.87	0.82	0.67	0.73	0.79	0.658
Tryptophan,%	0.22	0.19	0.2	0.15	0.18	0.2	0.21	0.23
Valine, %	0.95	0.92	0.84	0.74	0.87	0.94	0.95	0.823

Even though we realize a similarity in data presented on Table 1 of the recommendations, especially, when it comes to metabolizable energy and crude protein, in Table 2, we realized that is not enough to compare these two factors, but relations between the the other nutrients, because in Table 2 we introduce the metabolizable energy: nutrients ratio diets.

Table 2. Metabolizable energy: nutrients ratio based on the recommendations suggested by NRC (1994), Silva & Costa (2009) and Rostagno *et al*. (2011)

Item, %	NRC(1994)		Silva & Costa (2009)				Rostagno (2011)	
	Initial	Laying	Initial (1-21d)	Growth (22-42d)	Laying I	Laying II	Growth I and II	Laynig I (177g/bird)
Crude Protein	120.8	145.0	116.0	138.6	140.0	129.5	131.8	148.9
Arginine	2320.0	2301.6	2500.0	2904.8	2222.2	2065.2	2437.0	2199.5
Isoleucine	2959.2	3222.2	3258.4	4121.6	3218.4	2968.8	3625.0	3927.1
Lysine	2230.8	2900.0	2437.0	2904.8	2718.4	2714.3	2589.3	2552.4
Methionine	5800.0	6444.4	6304.3	7439.0	7179.5	6785.7	6904.8	5668.0
Methionine + Cystine	3866.7	4142.9	3625.0	4121.6	4000.0	3958.3	3815.8	3111.1
Threonine	2843.1	3918.9	3333.3	3719.5	4179.1	3904.1	3670.9	4255.3
Tryptophan	13181.8	15263.2	14500.0	20333.3	15555.6	14250.0	13809.5	12173.9
Valine	3052.6	3152.2	3452.4	4121.6	3218.4	3031.9	3052.6	3402.2

As seen before, Table 1 shows some similarity. However, when we calculate the metabolizable energy: Nutrients ratio we realize that there are far more differences than we have observed, either among the recommendations or between phases of growthig quail for all nutrients. To have an idea, the crude protein recommended for Initial Growth, and of Growth I and Growth II in diets of Japanese quail according to NRC (1994), Silva & Costa (2009) and Rostagno *et al*. (2011) is very similar, since recommended levels of 24, 25, 22 and 22%, respectively. But when we calculate the ME:CP ratio, we find much difference, as can be seen in Figure 1.

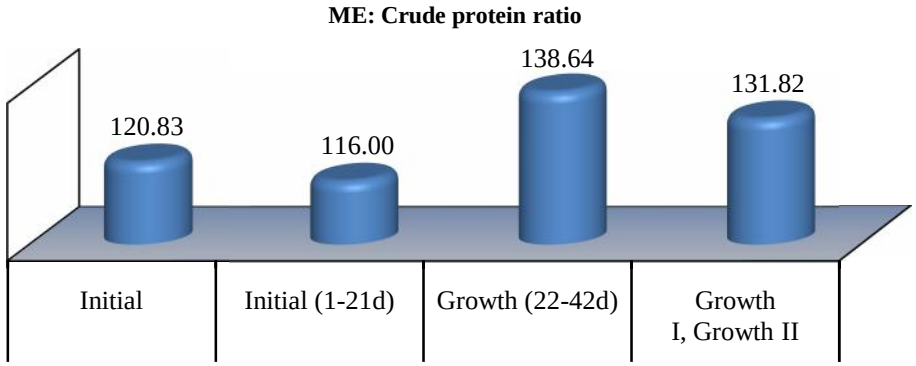

Figure1. Metabolizable energy: Crude protein ratio in the initial phases of Japanese quails according to the suggested recommendations by NRC (1994), Silva & Costa (2009) and Rostagno et al. (2011).

The variation shown in Figure 1 represents a great variation that can surely influence the performance of Japanese quails in the initial stages directly and possibly affect the performance in the later stage, ie laying phase. The appropriate metabolizable energy: crude protein ratio is vital for the perfect performance of Japanese quail and the metabolizable energy: amino acids ratio seems to expose in more details the best diet for Japanese quail today, especially, because it is not worth a diet with high crude protein if this is not of good quality, ie, there is disbalance of their fundamental units, the amino acids.

Also based on Figure 1, we see that the metabolizable energy: crude protein ratio recommended by the NRC (1994), is similar to that recommended for the same age, 1-21 days, by Silva & Costa (2009). However, Rostagno *et al*. (2011), by joining the phases of Growth I and Growth II in just a recommendation, can, in one of two phases of growth, be overestimating this ratio or not, because if we compare the ratio recommended by

Silva & Costa (2009), such ratio is lower, so there is more crude protein in relation to the metabolizable energy level, which could, into what we highlighted in the previously text, change the metabolism of Japanese quail and influence on the protein and fat tissue deposition of the quail.

Knowing the importance of lysine in the in the ideal protein concept, is perceived that there is significant variation between the recommendations presented here. This variation on the metabolizable energy: lysine ratio is more significant in the laying phase of the Japanese quail. We can see in Figure 2 that there is a variance of at least 400 points between the ratio recommended by the NRC (1994) and Rostagno *et al*. (2011).

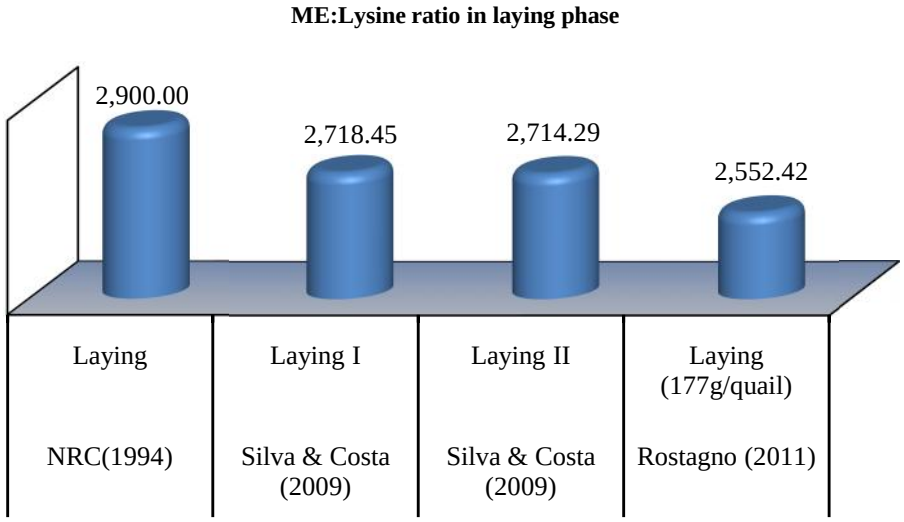

Figure 2. ME: Lysine ratio in laying phase of Japanese quail according to the recommendations suggested by NRC (1994). Silva and Costa (2009) and Rostagno et al. (2011).

Comparing the recommendations in a chronologically way, the reduction in the ME: Lysine ratio, does not indicate an elevation of the metabolizable energy recommendations, but actually a higher growth of the daily requirement of lysine in the Japanese quail diets, since this level increased from 1.0 to 1.12% from 1994 to 2011. Bearing in mind that the data recommended by the NRC (1994) are based on total amino acids, while the recommendations made by Silva & Costa (2009) and Rostagno *et al*. (2011) are based in digestible amino acids, what shows even more growth.

For the comparative presented here, is perceived that there is a discrepancy between the recommendations in several aspects and that the formulation of a diet should, with no doubt, have these dots well connected. Especially in energetic level, lysine level and levels of the remaining amino acids, for all may suffer important variations, and any erroneous manipulation in mentioned items above can directly compromise the performance and efficiency of the Japanese quail production system.

Comparative of a practical formulation

Based on what was mentioned in this material about metabolizable energy: nutrient ratio and nutritional recommendations of Japanese quail suggested by NRC (1994), Silva & Costa (2009) and Rostagno *et al*. (2011), we will simulate practical diets formulations for these Japanese quails. The intention is to correlate in a clear and practical form the recommendations and, if possible, comment about the levels suggested and practiced, especially, on the economic effects caused by the choice.

Thus, in order to maintain the homogeneity of the data to be used in the diets formulations and economic evaluation, feed intake and daily egg production will be set at 25.5 g and 87.5%, respectively. The diets formulations presented in Table 3 are for Japanese quails in 40-240 days of age, and to generate data more

convincing and real as the quail economic production, we will repeat this evaluations in the same time, ie simulate the production of four lots of quail under the same conditions.

Table 3. Diets according to the recommendations suggested by NRC (1994), Silva & Costa (2009) and Rostagno *et al.* (2011)

Item		NRC (1994)	Silva & Costa (2009)	Rostagno *et al.* (2011)
Corn		54.227	57.219	61.215
Soybean meal		35.367	31.362	29.011
Limestone		5.237	7.272	6.675
Soybean oil		3.026	2.191	0.954
Phosphate dicalcium		1.583	1.048	1.080
Salt		0.333	0.535	0.322
Choline		0.07	0.07	0.07
DL- Methionine		0.056	0.135	0.356
Vitaminic Mix		0.05	0.05	0.05
Mineral Mix		0.05	0.05	0.05
L-Lysine			0.017	0.159
L-Tryptophan				0.013
L-Isoleucine			0.052	
L-Arginine				0.044
Total		**100.00**	**100.00**	**100.00**
Cost/kg	U$	0.863	0.830	0.827
Crude Protein	%	20.00	20.00	18.80
Calcium	%	2.50	3.15	2.922
Available phosphorus	%	0.40	0.30	0.304
Metabolizable Energy	kcal/kg	2900	2800	2800
Total Arginine	%	1.26		
Digestible Arginine	%		1.26	1.273
Total Isoleucine	%	0.90		
Digestible Isoleucine	%		0.87	0.713
Total Lysine	%	1.00		
Digestible. Lysine	%		1.03	1.097
Total Methionine + Cystine	%	0.70		
Digestible Methionine + Cystine	%		0.70	0.90
Total Threonine	%	0.74		
Digestible Threonine	%		0.67	0.658
Total Tryptophan	%	0.19		
Digestible Tryptophan	%		0.18	0.23
Total Valine	%	0.92		
Digestible Valine	%		0.87	0.823
Sodium	%	0.15	0.23	0.146
Chlorine	%	0.14	0.24	0.240*
Potassium	%	0.40	0.46	0.460*

*Recommendations of Silva & Costa (2009)

Observing the diets, is perceived that industrial amino acid supplementation was, as expected, higher in the diets formulated based on the current recommendations, which can be explained due to the higher recommended levels. This greater amino acid supplementation has made it possible the easily attendance of the crude protein, which in the case of the recommendations suggested by Rostagno *et al*. (2011) is lower than the others. Furthermore, the amino acid supplementation provided a reduction in the Soybean meal and Soybean oil as well as an increase of the level of Corn, such as industrial amino acids have, in addition of amino acids at issue, metabolizable energy and crude protein, and with their use in feed, allows an enrichment of these items, reducing the importance of these sources of crude protein and metabolizable energy in feed currently practiced in the production of Japanese quail. Based on data, provided by the diets formulated, presented in Table 3, was calculated the cost per pound of diet, which made possible the economic evaluation, shown in Table 4.

Soon the production cost of a dozen eggs is influenced and, logically, the profir gross margin and, when we compare the data with the diet at levels recommended by the NRC (1994) we realized that, although diets have

subsequent industrial amino acid supplementation, what appears to be a factor of higher costs, the relative gross margin was higher than 1.65 and 1.80% in the diets formulations that attended the recommendations of Costa & Silva (2009) and Rostagno *et al*. (2011), respectively.

Table 4. Economic evaluation of diets formulated according to the recommendations suggested by NRC (1994) Silva & Costa (2009) and Rostagno *et al*. (2011)

Variables	Used Recommendations		
	NRC	Silva & Costa	Rostagno
Feed Intake in Period of 200 days, kg/quail	5.10	5.10	5.10
Cost of Feed in the Period, U$/quail	4.40	4.23	4.22
Egg Dozen, dozen/quail	14.58	14.58	14.58
Cost of Feed for Egg Dozen, U$/ dozen	0.30	0.29	0.29
Gross Income, U$ (U$ 1,00/dozen)	14.58	14.58	14.58
Gross Margin U$	10.18	10.35	10.37
Relative Gross, %	100.00	101.65	101.80

Although it seems a small difference in a gross margin, let remember that the calculations were made based on taking only one Japanese quail of 40-240 days old. If we consider that a commercial production shed of Japanese quail houses an average of 10 thousands quails, the gross margin of diets based in the recommendations suggested by Silva & Costa (2009) and Rostagno *et al*. (2011) would have an increase of U$ 1,683.00 and U$ 1,836.00, respectively. If we consider the repetition of these diets formulations on time, as mentioned previously, in a hypothetical situation, we would have a savings of U$ 6,732.00 and U$ 7,344.00, respectively.

So even though the changes in nutritional recommendations for these birds, we realized that the metabolizable energy enforces great deciding factor in the diets formulations, because as we have seen it is not very discrepant in the recommendations presented, but the other nutrients are, in particular the amino acids, and this causes clear changes in production performance, so far as to even keeping the same zootechnical parameters, yet there is economic loss. Hence, the maintenance of the metabolizable energy levels of diets is interesting, but for any modifications of it, is necessary to balance the diets based on the other nutrients and to verify the influence of any changes on the economic impact due to the importance of animal production activity, ie, it must be able to promote greater financial return, the profit, so that it can maintain and develop efficient and fulfill, in quantity and quality, the prerequisites of any food production, as previously mentioned at the beginning of this material .

Final Considerations

Despite of its great economic significance, few researches has been done concerning nutritional requirement of Japanese quails, being the diet formulation for this birds usually made based on charts, like the National Research Council - NRC (1994) and Institute National de la Recherch Agronomique – INRA, or by extrapolation of the requirements for some laying hens or broiler chickens. Nevertheless, Silva & Costa (2009) published the Japanese and European Quails Tables, where it's possible to obtain several nutritional recommendations, and yet Rostagno *et al*. (2011) besides that had already contemplate nutritional recommendations of laying hens, broiler chicken and breeders on poultry's recommendation, included in this last edition, the Japanese quails. Although, the data presented by Silva & Costa. (2009) are more complete and the information amount is bigger than the cited recommendations, ensuring a greater reliability for the quail farmer.

The use of inappropriate levels of amino acids may lead to a lower performance of Japanese quails, since the deficiency results in the limitation of the protein synthesis, with consequent decrease of weight gain and egg production in the same way that the excess can result in deviation of the energy for the process of excretion, reducing, thus, the energy to be used for the egg production, besides environmental cost of increased nitrogen excretion. This effect is more real when there is a constant search for reduction of the contents of crude protein of the feed, since this reduction is not totally efficient, since it can lead to a situation in which other amino acids such as threonine and tryptophan, for example, become bounds to the best performance of Japanese quails. In

this way, to achieve the increased performance of Japanese quails submitted to these feed diets with higher crude protein reductions; one should take care to establish more precisely the nutritional requirements of amino acids.

Another aspect based on the information that the energy regulates feed intake of the birds is that it is not enough to recommend the level of a nutrient, such as lysine, but recommend the daily intake of it, since the influence of the energy can, due to factors previously commented, alter feed intake and logically intake of amino acids. If there is a higher energy density, then the diet should be more concentrated in certain nutrients, so that when the Japanese quail satisfy the hunger it can be possible to meet their nutritional needs in the most complete and as satisfying form as possible. This way the nutritional recommendations can be made based on the energy content of the diets, in other words, the amino acid mg/kcal per kg of diet.

REFERENCE

Baião, N.C., Lara, L.J.C. 2005. Oil and fat in broiler nutrition. R. Bras. Ciência Avícola, 7, n.3, 129-141.

Barreto, Sergio Luiz de Toledo. 2006. Níveis de energia metabolizável para codornas japonesas na fase inicial de postura. *R. Bras. de Zootec.* vol.36, n.1, pág. 79-85.

Bertechini, A.G. 2006. Nutrição de monogástricos. Lavras: Editora UFLA, 301p.

Costa, F.G.P., Rodrigues, V.P., Goulart, C.C. 2008. Exigências de lisina digestível para codornas japonesas na fase de postura. *R. Bras. de Zootec*, 37,12,2136-2140.

Costa, F.G.P.; Souza, H.C.; Gomes, C.A.V. *2004*. Níveis de proteína bruta e energia metabolizável na produção e qualidade dos ovos de poedeiras da linhagem Lohmann Brown. C. Agrot., 28, 6, 1421-1427.

Deponti, B.J., Faria, D.E., Faria Filho, D.E. *2007*. Exigências de triptofano e padrão de recuperação do desempenho de poedeiras comerciais após alimentação com rações deficientes em triptofano. *R. Bras. de Zootec*, 36, 5, 1324-1330.

Freitas, A.C., Fuentes, M.F.F., Freitas, E.R. 2005. Efeito de níveis de proteína bruta e de energia metabolizável sobre o desempenho de codornas de postura. *R. Bras. de Zootec*, 34, 3, 838-846.

Garcia, E.A., Mendes, A.A., Pizzolante, C.C. 2005. Protein, Methionine+Cystine and Lysine Levels for Japanese Quails During the Production Phase. Braz. J.l of Poul. Sci., 7, 1, 11-18.

Harms, R.H. & Russel, G.B. 2000. Evaluation of tryptophan requirement of the commercial layer by using a corn-soybean meal basal diet. Poul. Sci., 79, 740-742.

Leeson, S., & Summers, J.D. 2005. Commercial poultry production. 3rd ed. University Books, Guelph, Ontario, Canada.

Lima, M.R., Perazzo Costa, F.G.P., Guerra, R.R. et al. 2013. Threonine:lysine ratio for Japanese quail hen diets. J. Appl. Poult. Res. 22, 260-268.

Lima, M. R., Costa, F.G.P. 2012 . Digestible tryptophan:lysine ratio for laying hens. R. Bras. de Zootec, 41, 10, 2203-2210.

Moura, G.S., Barreto, S.L.T., Donzele, J.L. 2008. Dietas de diferentes densidades energéticas mantendo constante a relação energia metabolizável: nutrientes para codornas japonesas em postura. R. Bras. de Zootec, 37, 9, 1628-1633.

Moura, G.S., Barreto, S.L.T., Lanna, E.A.T. 2010. Efeito da redução da densidade energética de dietas sobre as características do ovo de codorna japonesa. *R. Bras. de Zootec*, 39, 6, 1266-1271 .

Novak, C.H,. Yakou.,S. 2004. The combined effect of dietary lysine and total sulfur amino acid level on egg production parameters and egg components in dekalb delta laying hens. Poul. Sci., 83,977–984.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. National Academics Press, Washington, DC.

Page, J.W., Andrews, J.W. 1973. Interaction of dietary levels of protein and energy on Channel catfish (*Ictalurus punctatus*). Jour. of Nutri., 103, 1339-1346.

Pinheiro, S.R., Barreto, S.L.T., Albino, L.F.T. 2008. Efeito dos níveis de triptofano digestível em dietas para codornas japonesas em postura. *R. Bras. de Zootec*, 37, 6, 1012-1016.

Pinto, R., Ferreira, A.S., Donzele, J.L. 2003. Exigência de lisina para codornas japonesas

em postura. *R. Bras. de Zootec*, 32, 5, 1182-1189.

Ribeiro, M.L.G., Silva, J.H.V., Dantas, M.O. 2003. Exigências nutricionais de lisina para codornas durante a fase de postura, em função do nível de proteína da ração. *R. Bras. de Zootec*, 32, 1, 156-161.

Rizzo, P.V., Guandolini, G.C., Amoroso, L. 2008. Triptofano na alimentação de codornas japonesas nas fases de recria e postura. *R. Bras. de Zootec*, 37, 6, 1017-1022.

Rostagno, H.S., Albino, L.F.T., Donzele, J.L. 2011. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. Viçosa, MG: Universidade Federal de Viçosa.

Shim, K.F., P. Vohra. 1984. A Review of the Nutrition of Japanese Quail. W. Poul. Sci., 40, 261-271.

Shim, K.F., Lee, T.K. 1993. Effect of dietary essential amino acids on egg production of laying Japanese quail. Sing. Jour. of Prim. Indust., 21,.2, 72-75.

Silva, J.H.V. & Costa, F.G.P. 2009. Tabelas para codornas Japonesas e Européias. 2ª Ed. 107p.

Silva, J.H.V., Costa, F.G.P., Silva, E.L. 2007. Exigências nutricionais de codornas. In: 3º CONGRESSO INTERNACIONAL DE COTURNICULTURA. 42-62., 2007. Palestra... Lavras: UFLA – MG.

Silva, J.H.V., Silva, M.B., Jordão Filho, J. *2004.* Exigências de mantença e de ganho em proteína e energia em codornas japonesas (Coturnix coturnix japonica) na fase de 15 a 32 dias. *R. Bras. de Zootec.*, 33, 5, 1220-1230.

Umigi, R.T., Barreto, S.L.T., Donzele, J.L. 2007. Níveis de treonina digestível em dietas para codornas japonesas em postura. R. Bras. Zootec., 36,6, 1868-1874.

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Vaccination with a Streptomycin-Resistant Strain of *Salmonella enteric serovar Enteritidis* lacking *pefA* and *spvC* Genes Reduces Cecal Colonization and Organ Invasion in *SPF* Chicks

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ABSTRACT

Salmonellosis is one of the most important food-borne diseases and remains an important pathogen of poultry. In this study was evaluated the protection of a vaccine containing a streptomycin-resistant strain of *Salmonella entericas erovar Enteritidis* lacking *pefA* and *spvC* genes with respect to cecal colonization, organ invasion and excretion in SPF chicks and the potential use as a vaccine candidate was tested. Streptomycin-mutant strain was obtained by exposure to high concentration of streptomycin. Parent and resistant strain were evaluated phenotypically by measuring biochemical properties, growth rate and antibiotic resistance, and genetically for expression of twenty-three genes. Mutant strain was tested in SPF chickens by testing excretion, and by challenge using a wild-type isolate three weeks after immunization evaluating cecal colonization and organ invasion. Streptomycin-resistant strain showed lack of expression of *pefA* and s*pvC* when compared to the parent strain. DNA sequencing of a PCR-amplified *gyrA* fragment detected one point of mutation Ser15→Phe. Biochemical properties did not change. Growth rate differences were observed between parent and mutant strains, showing a generation time was increased five-fold in the mutant strain. Excretion of the vaccine strain was reduced 50% at the second week compared to the positive control group and no excretion of the vaccine strain was detected at the third week. Cecal colonization and organ invasion were significantly reduced in the vaccinated group, 80% and 70%, respectively. The vaccine strain was not detected in cecal and organ samples at the end of the trial. Attenuated strains produced by selecting for resistance to streptomycin have been described in mice. This study showed that streptomycin-resistant strain may be an important factor in the attenuation, suggesting that after exposure to streptomycin the parent isolate lost the expression of *pefA* and *spvC* genes and it could be a vaccine candidate to protect chicks against a *Salmonella* Enteritidis challenge.
Key words: Salmonella Enteritidis, streptomycin-resistant strain, potential vaccine strain.

INTRODUCTION

An alternative approach to the development of bacterial vaccines is the development of attenuated strains for use as live vaccine candidates by exploiting antimicrobial resistance. Bacteria exhibiting resistance to rifampicin typically have reduced virulence (Bhatagar et al., 1994). A direct approach has been tested in mice, demonstrating that *Salmonella* Typhimurium mutants that are resistant to streptomycin, rifampicin, and nalidixic acid are avirulent in this species (Bjorkman et al., 1998). The purpose of the present study was to assess the protective effect a vaccine containing a streptomycin-resistant strain of *Salmonella enterica*serovarEnteritidis against a virulent challenge. We evaluated expression of virulence genes, cecal colonization and organ invasion by this potential vaccine candidate in specific-pathogen-free chicks.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions for DNA Extraction

Field and antibiotic-mutant resistant strains of *Salmonella*Enteritidis were evaluated for temperature sensitivity, generation time, and minimum inhibitory antibiotic concentrations. Polymerase chain reactions used Salmonella Typhimuirum ATCC 14028 as a reference strain. A volume of 200 µL of each pure strain was used for DNA extraction.

PCR Analysis and Sequencing

Twenty-three sets of primer pairs were used to evaluate the expression profiles of wild and antibiotic-mutant resistant *Salmonella*Enteritidis strains. Target genes, oligonucleotide sequences (Invitrogen, Carlsbad, CA, USA), amplification region, PCR conditions and references or accession numbers are described in Table 1. DNA extractions were performed in parent and antibiotic-mutant, as described by Boom et al. (1990). All purifications and PCR reactions used ATCC 14028 and E. coli K12 as positive and negative controls, respectively. PCR was performed with 0.75 µL of DNA sample, except for the *agfA* target gene where 1.5 µL of DNA sample was used, 50 nM $MgCl_2$, 200 nMTris pH 8.4, 500 mMKCl, 1.25 nMdNTP mix, 1U *Taq* DNA polymerase (Invitrogen, Carlsbard, CA, USA), and 10 pmol of each primer for a final volume of 25 µL. Primers for *fliC*, *sifA*, *sopB*, *gyrA*, *rpoB*, and*rpsL* were designed based on the GenBank sequence database. Electrophoresis of PCR products was performed on 1.5% agarose gel with a 100-bp ladder (Invitrogen Carlsbad, CA, USA) as the molecular weight marker. Blue Green Loading Dye (LGC Biotecnologia, Cotia, SP, Brazil) was used to mix the samples and ladder 1:10. DNA sequencing of an amplified PCR gyrA, rpoB and rpsL fragments were evaluated.

Antimicrobial Susceptibility Tests

Antimicrobial susceptibility tests were performed using the parent and antibiotic-mutant resistant strains. The disk diffusion method used 15 antibiotics. Two different tests were performed. AST by microdilution and disk diffusion tests were performed in antibiotic-mutant strains obtained after exposure to high concentrations of antibiotics. The first method was the antimicrobial susceptibility test using a microdilution commercial test AviPro® Plate (Lohmann Animal Health GmβH, Cuxhaven, Germany). This microdilution test quantitatively measures the in vitro activity of an antimicrobial agent against a given bacterial culture isolated from poultry. The round-bottom wells were pre-coated with various concentrations of antibiotics. Each plate contained the following drugs: amoxicillin, ceftiofur, colistin, enrofloxacin, erythromycin, gentamicin, lincomycin, neomycin, oxacilin, penicillin, rifampicyn, spectinomycin, streptomycin, tiamulin, tetracycline, lincomycin/spectinomycin, and trimethoprim/sulfamethoxazole. The test was performed as recommended by the manufacturer and interpreted according the manufacturer guideline. The second test was the disk diffusion method on Mueller-Hinton agar (Difco, Sparks, MD, USA), using fifteen antibiotics (CefarDiagnostica, São Paulo, SP, Brazil) following standard protocols and interpretative guidelines from CLSI (2008). *E. coli* ATCC 25922 was used as reference strain. The following antibiotic disks were tested: ampicillin (AMP) 10 µg; ciprofloxacin (CIP) 5 µg; chloramphenicol (CLO) 30 µg; colistin (COL) 10 µg; doxycicline (DOX) 30 µg; enrofloxacin (ENRO) 5 µg;

erythromycin (ERY) 15 µg; florfenicol (FFN) 30 µg; gentamicin (GEN) 10 µg; kanamycin (KAN) 30 µg; nalidixic acid (NAL) 30 µg; rifampicyn (RIF) 30 µg; streptomycin (STR) 10 µg; trimethoprim/sulfametoxasole (TMP/SMX) 1.25/23.75 µg; and tetracycline (TET) 30 µg. Overnight cultures grown on LB broth were spread on Mueller-Hinton plates and incubated at 37^{o}C for 24 hours.

Evaluation of the Parent and Antibiotic-Mutant Strains in SPF Chicks

A total of 180 one-day-old SPF Ross chicks of the same hatch were used. The birds were housed in floor pen facilities with pine-shaving litter and were provided *ad libitum* with water and a balanced non-medicated diet. The housing environment and paper pads were tested according to ISO 6579:2002/Amd 1:2007. Additionally, house environment and paper pads samples were pre-enriched in tetrathionate broth (Difco, Sparks, MD, USA) and cultured on xylose-lysine-deoxycolate (XLD) agar (Difco, Sparks, MD, USA) and xylose-lysine-tergitol-4 (XLT_4) agar (Difco, Sparks, MD, USA).Groups 1 and 2 contained 60 birds each, and these chicks were vaccinated at one day of age by oral gavage with at least 1×10^8cfu/mL of the parent strain or the antibiotic-mutant resistant strain, respectively. These chicks were challenged three weeks later. Groups 3 and 4 contained 20 birds each, and these chicks were challenged at three weeks of age. Group 5 was maintained as a negative control. Chicks were observed for 21 days. Clinical signs and mortality rates were recorded. After the observation period, birds were killed by CO2 inhalation. Postmortem examination of cecal contents, livers, and spleens was performed.

RESULT

The antibiotic-mutant resistant strain was sensible to ceftiofur, colistin, rifampicin and tetracycline. The biochemical properties of the bacteria did not change. Growth rate differences were observed between the parent and antibiotic-mutant resistant strains, and the generation time was increased five-fold for the mutant strain. The streptomycin-resistant strain showed no expression of the *pefA*and *spvC* genes, which were expressed by the parent strain. Parent (SEO) and antibiotic-mutant resistant (SEAM) strains were not different with respect to the of *gyrA*, *rpoB* and *rpsL* genes, while DNA sequencing of a PCR-amplified *gyrA* fragment showed differences in the antibiotic-mutant (Genbank accession number HQ237456) resistant strain (Phe15) compared to the parent strain (Genbank accession number HQ237457), as shown in Figure 1.

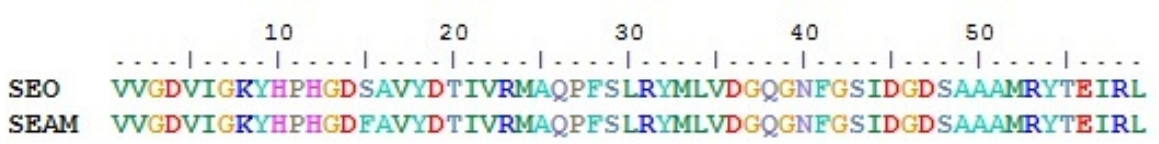

Figure 1. Sequencing of a PCR-amplified *gyrA* fragment

Before inoculation, *Salmonella* was not detected in the following areas: paper pads, pine-shaving litter, house environment, equipment, balanced diet food, feeders, water, or water containers. Cecal colonization showed significant differences ($P \leq 0.05$) in groups vaccinated when compared to unvaccinated group challenged orally. The group challenged subcutaneously did not show any evidence of cecal colonization. Liver and spleen invasion showed a significant ($P \leq 0.05$) reduction in birds vaccinated and challenged orally when compared to the group vaccinated orally and challenged subcutaneously. Positive control groups showed at least 90% of organ invasion. No clinical signs or mortality was recorded during the trial in the groups inoculated with the parent and antibiotic-mutant SE strain.

As shown in Figure 2, evaluation of the cecal colonization revealed a significant ($P<0.05$) reduction in recovery of the challenge strain in the vaccinated groups compared to the positive control group that was challenged orally. Liver and spleen invasion was significantly ($P<0.05$) reduced in the vaccinated groups as compared to the groups challenged orally and subcutaneously.

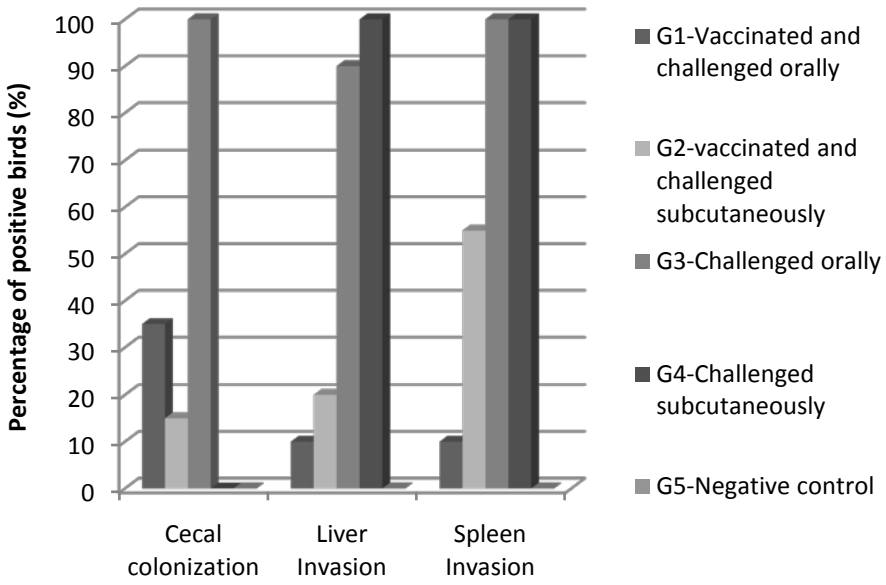

Figure 2. Recovery of challenge strain

DISCUSSION

Bjorkman et al. (1998) demonstrated that restrictive mutations and resistance to streptomycin are associated with a loss of virulence in the mouse model system. Streptomycin resistance is often attributable to aminoglycoside modifying enzymes (Vakulenko and Mobashery, 2003). The stability of antibiotic mutation should be guaranteed by no less than 2 markers, which can provide vaccine strains with an optimal attenuation for a particular animal species, conferring full protection after a single vaccination (Linde et al., 1990). p*efA*and *spvC*mutations were observed after exposure of a field isolate of SE to high levels of streptomycin. Nontyphoidserovars that lack the *spv*genes are less able to proliferate beyond the superficial epithelial layer (Fluit 1995), because *spv* genes are required for the systemic phase of the disease. This fact could explain the reduced organ invasion obtained in groups vaccinated because mutation in this gene causes various defects in *Salmonella* virulence (Rotger e Cadesús, 1999). Our results suggest that streptomycin may have a negative effect on the expression of the *pefA*and *spvC*genes in SE field strains, inducing a mutation that has a positive effect in vivo and thereby reducing cecal colonization and organ invasion by the resistant strain. Further research can be done to determine if differences in sequence of the *gyrA* fragment are related to reduction in virulence.

CONCLUSION

- Vaccination with a streptomycin-resistant strain of *Salmonella entericaserovar Enteritidis* that lacks *pefA* and *spvC* genes protected SPF chicks against a challenge with a virulent strain of wild-type *Salmonella*.
- DNA sequencing of a PCR-amplified *gyrA* fragment showed differences in the antibiotic-mutant resistant strain compared to the parent strain.
- The antibiotic-mutant resistant SE had diminished cecal colonization and organ invasion abilities in SPF chicks, and this strain could be a potential vaccine candidate.

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REFERENCE

Bhatnagar N, Getachew E, Straley S, Williams J, Meltzer M, Fortier A. Reduced virulence of rifampicin-resistant mutants of *Francisellatularensis. J Infect Dis* 1994; **170**:841–7

Bjorkman, J., Samuelsson, P., Andersson, D.I., and D. Hughes. 1998. Virulence of antibiotic-resistant *Salmonella* Typhimurium. Proc. Natl. Acad. Sci. USA95:3949–53.

Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M., and J. Van der Noordaa. 1990. Rapid and simple method for purification of nucleic acids. J.Clin.Microbiol.28:495-503

Fluit, A.C. 2005.Towards more virulent and antibiotic-resistant *Salmonella*?FEMS Immunol. Med. Microbiol.43:1–11.

Linde, K., Beer, J., andV. Bondarenko. 1990. Stable *Salmonella* live vaccine strains with two or more attenuating mutations and any desired level of attenuation. Vaccine8:278–82.

Rotger, R., and J. Cadesús. 1999. The virulence plasmids of *Salmonella*. Int. Microbiol. 2:177-184.

Vakulenko, S.B., and S. Mobashery. 2003. Versatility of aminoglycosides and prospects for their future. Clin.Microbiol.Rev.16:430–450.

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Efficacy of Antibiotic, Probiotic, Prebiotic and Synbiotic on growth performance, organ weights, intestinal histomorphology and immune response in broiler chickens

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ABSTRACT

A feeding trial was conducted to investigate the effects of dietary supplementations of antibiotic, probiotic, prebiotic and synbiotic on broiler performance, histomorphologic measurements of small intestine and immune response. A total number of 432, day-old broiler chicks (Ross308) were obtained and randomly assigned to 1 of 9 dietary treatments for 6 weeks. The dietary treatments were: 1) basal diet ; 2,3) basal diet plus (400, 600) g of phosphomycin product/ton of starter and grower feeds, respectively, 4,5) basal diet plus (150,200) g of probiotic product/ton of the starter feed and (100,150) g/ton of the grower feed, respectively, 6,7) basal diet plus (500, 1000) g of a prebiotic product /ton of starter and grower feeds, respectively, 8 and9) basal diet plus (1000,1250) g of synbiotic product /ton of the starter feed and (500,750) g/ton of the grower feed, respectively. Birds supplemented with the synbiotic had a greater ($P < 0.01$) feed intake and body weight gain compared with those of others treatments. Feed conversion rate was lower in birds supplemented with all additives than in control birds ($P < 0.01$). The carcass weight was significantly increased in feed additives compared with that of control treatment group ($P < 0.05$). The villus height was significantly increased in feed additives compared with that of control group ($P < 0.01$). Synbiotic treated animals showed increase ($p<0.05$) in antibody titers against NDV compared to those of the control groups at 28, 35 and 42 days of age. The result of the present study revealed that these products had promising effects as alternatives for antibiotics in parallel to demand for elimination of growth-promotant antibiotics.

Key words: broiler, feed additives, performance, histomorphology, immune response

INTRODUCTION

Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics in broiler industry. Therefore, it is highly essential to improve feed efficiency in poultry to produce meat economically and, food safety is also more seriously considered than before. On the other hand, economy of food production is also a factor that cannot be ignored. A huge amount of antibiotics have been used to control diseases and improve performances in livestock. The mechanisms for the observed improvement in productive parameters (body weight gain and feed conversion) have not been completely elucidated. However, it is suspected that an overall reduction in bacterial load within the intestine is responsible for increased availability of nutrients to the animal. Theoretically, a decrease in pathogenic bacteria and their metabolites could contribute to reduce subclinical lesions on the intestinal mucosa. Since the healing process involves the use of resources to repair the damaged cells, less epithelial damage can be indeed an efficient way to save energy. However, the use of dietary antibiotics has resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1999), the presence of antibiotic residues in poultry meat and eggs that may have deleterious effects on human consumers, imbalance of normal microflora (Andremont, 2000), and the ban on subtherapeutic antibiotic usage in many countries. There is increasing interest in finding alternatives to antibiotics for poultry production.

Because of the general problem of increased resistance of bacteria and the decreasing acceptance of the consumers for Antibacterial Growth Promoters (AGPs), different substances, referred as Natural Growth Promoters (NGPs), have been identified as effective and safe alternatives to AGPs. At present, there is a large number of NGPs available in the market, including probiotics, prebiotics and synbiotics.

Substitution of conventional and prohibited AGPs with probiotics has received much attention in the recent years. One of the major reasons for increased interest in the use of probiotics is because they are natural alternatives to antibiotics for growth promotion in poultry. Recently, it was shown that addition of probiotic containing Enterococcus faecium microorganisms to broiler diets has increased the jejunal villus height (Chichowski et al., 2007) and ileal villus height (Samli et al., 2007). Probiotics act through six different means (Menten, 2002): (a) adherence to the binding sites of the intestinal epithelium (competition with pathogenic bacteria); (b) direct antagonism through the production of bactericidal substances; (c) stimulus to the immune system; (d) facilitating the digestion and absorption of nutrients; (e) suppression of ammonia production, which might be toxic to intestinal cells; and (f) neutralization of enterotoxins. The health promoting effect of probiotic in the gastrointestinal tract has been mainly associated with their capacity to stimulate the immune response and to inhibit the growth of pathogenic bacteria (Barnes et al., 1972).

Prebiotics are substances that act as microbial modulators and are defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve host health” (Gibson and Roberfroid, 1995). This definition was revised in 2004 and prebiotics are now defined as “selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health” (Gibson et al., 2004). Intake of prebiotics can either significantly modulate the colonic microbiota by increasing the number of specific beneficial bacteria such as lactobacilli and bifidobacteria (Rycroft et al., 2001) or reducing undesired intestinal colonization of pathogenic bacteria by mimicking their attachment sites on the intestinal mucosa (Iji and Tivey, 1998). Moreover, increased intestinal villi height was reported after addition of Bacillus subtilis in association with prebiotics (Pluske et al., 1996). Several studies have shown that administration of prebiotics can improve weight gain, feed intake and feed convention rate in broiler (Rodrigues et al., 2005). However, some reports indicated that prebiotic supplementation did not affect body weight gain, feed intake or feed convention (Stanczuk et al., 2005).

Synbiotics is defined as a mixture of probiotics and prebiotics that beneficially affects the host by activating the metabolism of one or a limited number of health promoting bacteria and/or by stimulating their growth selectively, improving the host’s welfare (Gibson and Roberfroid, 1995). Synbiotic products contain viable

bacterial cultures that establish easily in the gut while the prebiotic present in the synbiotic serve as a source of nutrient for the probiotics in addition to dietary sources. Recent research and development of synbiotic products have been increasingly focused on functional benefits including resistance to gastrointestinal bacterial infection, antibacterial activity, and improved immune status in broiler chicks. In addition, Mohnl et al., (2007) found that the synbiotic had a comparable potential to improve broiler performance as avilamycin (an antibiotic growth promoter). It seems that synergistic effects of prebiotics and probiotics can be useful in stimulating beneficial bacteria and improving the health of the gut. To the best of the authors knowledge there is scarce information available to date on synbiotics and the possible mechanisms in broiler chickens. Little information is available regarding the effect of adding synbiotic product to broiler diets on the immune status of broiler chickens. The aim of the present study was to assess the effects of antibiotic, probiotic, prebiotic and synbiotic on the performance, intestinal histomorphology and immune response of broiler chickens.

MATERIALS AND METHODS

Birds and Housing

A total number of 432, day-old broiler chicks (Ross308) were obtained from a commercial hatchery. A completely randomized experimental design was used and chicks were divided into nine treatment groups, with four replicates per treatment, each group with the equal numbers of male and female included and 12 chicks per replicate. Chicks were raised in floor pens with *ad libitum* access to feed and water and controlled ventilation. Temperature was maintained at 32 °C for the first 4 days and then gradually reduced. According to normal management practices a temperature of 22 °C was achieved at day 28. The lightning regimen was 23 hours of light and 1 hour of dark.

The dietary treatments

The dietary treatments were: 1) basal diet (control) ; 2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively, 4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively, 6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively, 8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750) g/ton of the grower feed, respectively. All diets were formulated to provide 3000 kcal of ME/kg and to meet the amino acid ratios and all other nutrients as suggested by the NRC, 1994 for broilers from 0 to 6 week of age (Table 1).

Growth Performance Traits

All birds were weighed individually after their arrival from the hatchery to the experimental farm (initial weight) and chicks of a uniform body weight (BW) were placed in individual pens and average initial body weight was 48 g. Weekly weight gain for each dietary treatment was calculated. Feed consumption was recorded weekly and in the course of the whole experiment for each treatment, and subsequently the feed conversion rates were calculated.

Organ Weights and Carcass Weights

At the end of experiment, after weighing, 8 birds per treatment were randomly selected and euthanized by cervical dislocation. The gizzard, heart, liver, pancreas, proventriculus, spleen, bursa of Fabricius, small intestine, (duodenum, jejunum, and ileum) and cecum were excised and weighed. The gastrointestinal tract was weighed after removal of the content. Afterward, the birds were scalded, defeathered, and carcasses were eviscerated. The head, neck, and feet were removed, and the carcass subsequently was ready to cook (RTC). The RTC carcass weight was then determined.

Histomorphological Samples

The tissue samples for histology were taken from the ileum. 10 cm proximal to the ileocecal junction (from Meckel's diverticulum to the ileocecal colonic junction) was referred as the ileum.

Light Microscopy

The samples were fixed in 4% buffered formalin for 48 h. The processing consisted of serial dehydration, clearing, and impregnation with wax. Semithin sections, 5 μm thick (3 cross-sections from each sample), were cut by a microtome and were mounted on slides. A routine staining procedure was carried out using hematoxylin and eosin. The slides were examined under an Olympus AX70 microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera (Sony DXC-930P, Sony Corporation, Tokyo, Japan). The images were analyzed using stereological image software, Cast Image System (Version 2.3.1.3, Visiopharm Albertslund, Hørsholm, Denmark).

Histomorphological Measurements

The intact well-oriented crypt-villus units were selected in triplicate for each intestinal cross- section for each sample. The criterion for villus selection was based on the presence of intact lamina propria. Villus height was measured from the tip of the villus to the villus-crypt junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi.

Vaccination and serology

At 9th day chicks were vaccinated with Hitchner B1 NDV (Newcastle Disease Virus) vaccine via eye I/O route and bivalent killed vaccine (Newpasol 102, Inactivated W/O Emulsion ND + AI (H9N2) Vaccine, Pasouk Biological Co) by I/M route. Blood samples were collected every week from the wing veins of broiler chickens and their sera were separated and inactivated at 56° C for 30 min and kept at −20° C until analysis of NDV antibody level. Serum Antibody titer was measured using hemagglutination-inhibition test as described by Alexander et al.,1983 (Alexander et al., 1983) on d7, 14, 21, 28, 35, and 42.

Table 1. Composition of experimental diets[1]

Ingredients %	Starter (0-21 d)	Grower (22-42)
Corn grain	52.89	64.09
Soybean meal	38.87	30.23
Soybean oil	4.04	2
Oyster shell	1.63	1.69
Ca phosphate	1.52	1.09
Salt	0.38	0.28
Mineral premix[2]	0.25	0.25
Vitamin premix[3]	0.25	0.25
DL-methionine	0.17	0.03
	Nutrient composition	
ME, kcal/kg	3000	3000
Crude protein %	21.6	18.75
Lys%	1.3	0.9375
Met %	0.4874	0.3659
Ca %	0.937	0.843
P (Total) %	0.42	0.33
Antibiotic[4]		
Probiotic[5]		
Prebiotic[6]		
Synbiotic[7]		

[1]Calculated from NRC (1994).
[2]provides per kilogram of diet: Cu (CuSO4-5 H2O), 4.0 mg; I (potassium iodate), 1.0 mg; Fe (ferrous sulfate-7 H2O), 60 mg; Mn (manganese sulfate-H2O), 60 mg; Se (sodium selenite), 0.1mg; Zn (zinc sulfate-7H2O), 44 mg; and Ca (calcium carbonate), 723 mg.
[3]For experiment, provides per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D3, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 2.4 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; pyridoxine (pyridoxine_HCl), 6.4 mg;riboflavin, 15 mg; and thiamin (thiamin_HCl), 15.2 mg.
[4]phosphomycin -Bedson co S.A.,La Lonja,Argentina.
[5]Probiotic- each kilogram contained: *Lactobacillus plantarum*, $1.89 \cdot 10^{10}$ cfu; *Lactobacillus delbrueckii* subsp. *bulgaricus*, $3.09 \cdot 10^{10}$ cfu; *Lactobacillus acidophilus*, $3.09 \cdot 10^{10}$ cfu; *Lactobacillus rhamnosus*, $3.09 \cdot 10^{10}$ cfu; *Bifidobacterium* 10^{10} cfu; *Aspergillus oryza*, $7.98 \cdot 10^{9}$ cfu; *Candida pintolopesii*, $7.98 \cdot 10^{9}$ cfu. Protexin Compounder, Novaritis Inc., Istanbul, Turkey.
[6]Prebiotic- Techno Mos (25% mannan-oligosaccharides, Alltech, Nicholasville, KY).
[7]Synbiotic- each kilogram contained 5×10^{8} cfu/kg- Biomin IMBO, Etouk Farda Feed Additives Co,Tehran,Iran.

Statistical analysis

When the chicks reached 42 d of age, the feeding trial was terminated. Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software. The treatment means with significant differences were compared using Duncan's new multiple range tests. All statements of differences were based on significance level set at $P \leq 0.05$.

RESULT

Mortality was low (<1%) and not treatment associated.

Feed Intake

The effects of treatments on feed intake (FI) are presented in Table 2. Birds supplemented with the synbiotic had a greater ($P < 0.05$) FI compared with that of control and other treatments. Moreover, prebiotic supplemented birds had a greater ($P < 0.05$) FI than that of probiotic and phosphomycin supplemented birds. No significant differences on feed intake were observed between the probiotic and phosphomycin-treated birds in the entire experimental period ($P > 0.05$).

Body Weight Gain

The initial body weight (BW) of chicks did not differ significantly ($P > 0.05$) between the dietary treatments (48 g). Responses to dietary treatments were significant ($P < 0.01$) for BWG (Body weight gain) in starter and grower periods (Table 3). At the end of the experiment (d 42), birds supplemented with the synbiotic had a greater ($P < 0.01$) BWG compared with that of control and other treatments. Moreover, prebiotic supplemented birds had a greater ($P < 0.01$) BWG than that of probiotic and phosphomycin supplemented birds. However, birds supplemented with the probiotic had a greater BWG than phosphomycin supplemented birds but difference was not significant ($P > 0.05$).

Feed Conversion Rate

Feed conversion rate (FCR) was lower for birds supplemented with synbiotic, prebiotic, probiotic and phosphomysin than that of control birds ($P < 0.01$). In addition, no significant differences on FCR were found among treatments (Table 4).

Carcass Weight and Live Weight

The means of carcass weight and live weight are shown in Table 5. The carcass weight was significantly higher in synbiotic treated group compared with control and phosphomycin treated groups ($P < 0.05$), and it was significantly increased for prebiotic and probiotic compared with that of control treatment ($P < 0.05$). No significant differences on carcass weight were found between synbiotic, prebiotic and probiotic treatments with each other ($P > 0.05$). Birds supplemented with the synbiotic had a greater ($P < 0.01$) live weight compared with

that of control and other treatments. Moreover, prebiotic supplemented birds had a greater ($P < 0.01$) live weight than probiotic and phosphomycin supplemented birds. However, birds supplemented with the probiotic had a greater live weight than that of phosphomycin supplemented birds but the difference was not significant ($P > 0.05$). Both probiotic and phosphomycin increased live weight ($P < 0.01$) compared with that of the control group.

Weights of Organs

The means of the absolute weights of organs for dietary treatments are presented in Table 6a and b. The weight of liver, pancreas and small intestine were decreased ($P < 0.05$) for the synbiotic-supplemented group compared with that of the control group and other dietary supplemented groups. Moreover, the synbiotic- supplemented group showed a decrease ($P < 0.01$) in heart weight compared with that of either the control group or other treatments groups. The weight of small intestine was significantly greater ($P < 0.05$) in the probiotic-supplemented group than that in the control group and other treatment groups. The weight of heart was increased ($P < 0.01$) in the prebiotic-supplemented group compared with that of the control group and other treatment groups. In addition, the absolute weights of gizzard, proventriculus, spleen, cecum and bursa did not show any significant differences among the dietary treatments.

The means of weight of organs relative to the BW are shown in Table7a and b. The weight of heart, liver, small intestine, pancreas relative to the BW tended to be lower ($P < 0.01$) for synbiotic-fed birds than those of control group and other product-fed birds. The relative weight of heart, liver and small intestine were significantly greater ($P < 0.01$) for probiotic compared with synbiotic-fed birds. In addition, the relative weights of proventriculus, cecum, spleen, and bursa remained unaffected by dietary supplementations.

Histomorphological Measurements

Ileum. The means of ileal villus height, crypt depth, and villus height: Crypt depth ratios for dietary treatments are shown in Table 8. The villus height was significantly increased for synbiotic compared with that of control and other treatment groups ($P < 0.01$), and it was significantly increased for prebiotic compared with that of control, probiotic and phposphomycin- treatments ($P < 0.01$). The villus height were significantly increased ($P < 0.01$) for both probiotic and phosphomycin compared with that of control but no significant differences were found between these treatments with each other. Moreover, synbiotic supplementation increased the villus height: crypt depth ratio compared with that of control and other treatment groups ($P <0.01$). The villus height: crypt depth ratio was significantly increased ($P < 0.01$) for prebiotic compared with that of control, probiotic and phposphomycin- treatments. In addition, the crypt depth remained unaffected by dietary supplementations ($P > 0.05$).

Immunological Measurements

Antibody titers

The effect of treatments on antibody production against NDV in broilers from 7 d to 42d are presented in Table 9. On the day 7th , no differences among antibody titers of experimental groups were observed. Animals of synbiotic treatment showed increase ($p<0.05$) in antibody titers against NDV as compared to those of the control at 28, 35 and 42 days of age. Other supplementation of the diet showed increase in antibody titers against NDV compared to those of the control but were not significant ($P > 0.05$). No significant differences on antibody titers were found among the prebiotic, probiotic and phosphomycin-treated birds in the entire experimental period ($P > 0.05$).

Table 2. Effect of feed supplementations on feed intake of broiler chickens (g)

Dietary treatment[1] (n=12)[3]	FI **(0-3)week**	FI **(3-6)week**	FI **(0-6)week**
T1	1057.79 ± 74.36^{b}	3222.80 ± 91.82^{c}	4280.64 ± 136.12^{c}
T2	1131.03 ± 21.63^{ab}	3263.49 ± 18.80^{bc}	4394.52 ± 16.23^{ab}
T3	1101.85 ± 29.55^{ab}	3223.54 ± 18.28^{c}	4325.40 ± 26.29^{bc}
T4	1062.13 ± 12.93^{ab}	3263.85 ± 46.73^{bc}	4325.98 ± 43.72^{bc}
T5	1108.63 ± 55.59^{ab}	3257.83 ± 66.39^{bc}	4341.45 ± 62.53^{bc}
T6	1085.00 ± 39.40^{ab}	3233.23 ± 65.73^{ab}	4447.23 ± 99.66^{ab}
T7	1136.63 ± 21.82^{a}	3343.48 ± 152.67^{abc}	4477.85 ± 154.62^{ab}
T8	1125.93 ± 28.18^{ab}	3338.50 ± 73.14^{abc}	4440.70 ± 107.17^{ab}
T9	1111.40 ± 77.09^{ab}	3397.08 ± 55.95^{a}	4508.52 ± 88.68^{a}
P-value	*[2]	*	*

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-d Means within a column with differing superscripts are significantly different at $P < 0.05$.
3 n=the number of birds/pen

Table 3. Effect of feed supplementations on body weight gain of broiler chickens (g)

Dietary treatment[1] (n=12)[3]	Initial BW	BWG (0-3)week	BWG (3-6)week	BWG (0-6)week
T1	48	619.77 ± 14.55^{c}	1570.29 ± 2760^{e}	2190.06 ± 18.29^{f}
T2	48	686.08 ± 42.94^{ab}	1666.84 ± 49.00^{d}	2353.00 ± 32.12^{e}
T3	48	709.49 ± 54.02^{ab}	1712.95 ± 6593^{cd}	2224.45 ± 97.00^{de}
T4	48	703.50 ± 7.33^{ab}	1718.63 ± 37.38^{cd}	2422.13 ± 33.62^{de}
T5	48	703.50 ± 71.1^{ab}	1734.75 ± 32.11^{cd}	2438.25 ± 31.19^{de}
T6	48	715.25 ± 11.09^{ab}	1755.50 ± 97.58^{bc}	2470.75 ± 101.10^{dc}
T7	48	713.50 ± 9.47^{ab}	1813.75 ± 32.85^{ab}	2527.25 ± 40.26^{bc}
T8	48	753.74 ± 33.25^{b}	1850.77 ± 18.28^{a}	2593.25 ± 54.29^{ab}
T9	48	755.12 ± 19.35^{b}	1874.11 ± 32.53^{a}	2629.21 ± 41.57^{a}
P-value	ns[2]	**[2]	**	**

The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-f Means within a column with differing superscripts are significantly different at $P < 0.01$.
3 n=The number of birds/pen

Table 4. Effect of feed supplementations on feed conversion rate of broiler chickens

Dietary treatment[1] (n=12)[3]	FCR(0-3)week	FCR(3-6)week	FCR(0-6)week
T1	1.70 ± 0.08^{a}	2.05 ± 0.06^{a}	1.95 ± 0.06^{a}
T2	1.65 ± 0.09^{ab}	1.96 ± 0.06^{ab}	1.86 ± 0.02^{b}
T3	1.53 ± 0.16^{bc}	1.88 ± 0.08^{bcd}	1.78 ± 0.06^{c}
T4	1.50 ± 0.02^{c}	1.90 ± 0.02^{bcd}	1.79 ± 0.01^{c}
T5	1.54 ± 0.02^{bc}	1.89 ± 0.03^{bcd}	1.79 ± 0.04^{c}
T6	1.53 ± 0.13^{bc}	1.92 ± 0.12^{bc}	1.77 ± 0.06^{c}
T7	1.59 ± 0.06^{abc}	1.82 ± 0.10^{cd}	1.73 ± 0.07^{c}
T8	1.46 ± 0.08^{c}	1.80 ± 0.03^{d}	1.71 ± 0.05^{c}
T9	1.46 ± 0.07^{c}	1.81 ± 0.05^{cd}	1.71 ± 0.04^{c}
P-value	**[2]	**	**

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2] a-d Means within a column with differing superscripts are significantly different at $P < 0.01$.

3 n=The number of birds/pen

Table 5. Effects of dietary treatments on live weight and carcass weight (g) of broiler chickens (42 day)

Dietary treatment[1] (n=12)[4]	Carcass weight	Live weight
T1	1303.60 ± 118.27^{d}	2243.09 ± 18.34^{f}
T2	1387.18 ± 48.00^{cd}	2405.98 ± 32.14^{e}
T3	1403.25 ± 102.10^{bcd}	2475.33 ± 97.02^{de}
T4	1408.68 ± 43.18^{abcd}	2475.13 ± 33.62^{de}
T5	1429.70 ± 27.75^{abc}	2491.25 ± 31.19^{de}
T6	1425.50 ± 63.33^{abc}	2523.75 ± 101.10^{dc}
T7	1453.63 ± 38.20^{abc}	2580.25 ± 40.26^{bc}
T8	1515.53 ± 108.37^{ab}	2646.23 ± 54.32^{ab}
T9	1529.50 ± 70.47^{a}	2682.20 ± 41.56^{a}
P-value	*[2]	**[3]

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.

[2] a-d Means within a column with differing superscripts are significantly different at $P < 0.05$.

[3] a-f Means within a column with differing superscripts are significantly different at $P < 0.01$.

4 n=The number of birds/pen

Table 6a. Effect of dietary treatments on absolute organ weights of broiler chickens (g)

Dietary treatment[1] (n=12)[5]	Heart	Liver	Cecum	Small intestine	Proventriculus
T1	46.20 ± 5.04^{abc}	39.68 ± 3.48^{ab}	11.33 ± 3.02	74.38 ± 5.40^{ab}	7.43 ± 0.59
T2	48.40 ± 4.58^{ab}	39.73 ± 2.90^{ab}	10.53 ± 0.72	64.35 ± 11.08^{abc}	6.90 ± 0.65
T3	40.80 ± 7.59^{bc}	37.18 ± 0.59^{b}	11.10 ± 2.10	62.73 ± 7.78^{bc}	6.45 ± 0.24
T4	49.63 ± 5.45^{a}	40.43 ± 3.11^{ab}	11.25 ± 1.01	77.53 ± 1.36^{a}	7.10 ± 1.28
T5	52.00 ± 5.05^{a}	43.68 ± 1.14^{a}	12.28 ± 1.86	77.25 ± 3.99^{a}	7.40 ± 0.35
T6	47.00 ± 4.34^{abc}	43.93 ± 3.12^{a}	10.88 ± 3.30	71.85 ± 10.83^{ab}	8.28 ± 0.61
T7	54.43 ± 3.81^{a}	40.30 ± 5.06^{ab}	11.38 ± 0.98	68.48 ± 6.67^{abc}	7.00 ± 0.37
T8	45.70 ± 4.10^{abc}	37.38 ± 2.35^{b}	11.50 ± 2.15	62.48 ± 10.35^{bc}	6.35 ± 1.31
T9	39.30 ± 7.54^{c}	36.98 ± 3.23^{b}	11.55 ± 1.39	55.88 ± 12.52^{bc}	6.83 ± 0.97
P-value	[2]**	[3]*	ns[4]	*	ns

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-c Means within a column with differing superscripts are significantly different at $P < 0.01$.
[3] a-c Means within a column with differing superscripts are significantly different at $P < 0.05$.
[4] $P \geq 0.05$.
5 n=The number of birds/pen

Table 6b. Effect of dietary treatments on absolute organ weights of broiler chickens (g)

Dietary treatment[1] (n=12)[4]	Gizzard	Pancreas	Bursa	Spleen
T1	42.00 ± 7.16	5.35 ± 0.17^{a}	٣.٦٨ ± ٠.٤٢	2.50 ± 0.58
T2	40.38 ± 7.28	4.00 ± 0.67^{b}	4.00 ± 1.23	2.35 ± 0.72
T3	38.48 ± 3.13	4.35 ± 1.26^{ab}	3.28 ± 1.33	2.15 ± 0.53
T4	39.65 ± 6.63	4.78 ± 0.66^{ab}	3.23 ± 0.26	2.48 ± 0.35
T5	42.55 ± 5.91	4.70 ± 0.54^{ab}	3.30 ± 0.27	2.23 ± 0.32
T6	37.58 ± 6.03	4.48 ± 0.33^{ab}	3.28 ± 0.13	2.35 ± 0.35
T7	36.33 ± 3.17	5.05 ± 0.64^{ab}	3.18 ± 0.48	2.33 ± 0.58
T8	37.58 ± 6.35	4.00 ± 0.47^{b}	3.28 ± 0.38	2.65 ± 0.13
T9	36.18 ± 5.67	4.23 ± 0.86^{ab}	0.93 ± 1.44	2.23 ± 0.39
P-value	ns[2]	*[3]	ns	ns

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] $P \geq 0.05$.
[3] a-b Means within a column with differing superscripts are significantly different at $P < 0.05$.
4 n=The number of birds/pen

Table 7a. Effect of dietary treatment on organ weights relative to BW of broiler chickens (g/100g)

Dietary treatment[1] (n=12)[4]	Heart	Liver	Cecum	Small intestine	Proventriculus
T1	2.05 ± 0.21[a]	1.76 ± 0.16[a]	0.50 ± 0.13	3.31 ± 0.26[a]	0.33 ± 0.03
T2	2.01 ± 0.21[a]	1.65 ± 012[abc]	0.43 ± 0.03	2.67 ± 0.47[bc]	0.28 ± 0.03
T3	1.65 ± 0.36[bc]	1.50 ± 0.08[dc]	0.44 ± 0.09	2.53 ± 0.25[dc]	0.28 ± 0.05
T4	2.00 ± 0.24[a]	1.63 ± 0.15[abc]	0.45 ± 0.04	3.13 ± 0.08[ab]	0.28 ± 0.05
T5	2.09 ± 0.22[a]	1.75 ± 0.05[ab]	0.49 ± 0.08	3.10 ± 0.18[ab]	0.29 ± 0.02
T6	1.86 ± 0.06[ab]	1.74 ± 0.12[ab]	0.43 ± 0.15	2.85 ± 0.51[abc]	0.29 ± 0.04
T7	2.11 ± 0.17[a]	1.55 ± 0.23[bcd]	0.44 ± 0.04	2.65 ± 0.29[bc]	0.27 ± 0.02
T8	1.63 ± 0.17[bc]	1.41 ± 0.08[d]	0.43 ± 0.08	2.35 ± 0.35[dc]	0.41 ± 0.33
T9	1.46 ± 0.26[c]	1.37 ± 0.13[d]	0.42 ± 0.05	2.07 ± 0.44[d]	0.25 ± 0.04
P-value	[2]**	**	ns[3]	**	ns

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-d Means within a column with differing superscripts are significantly different at $P < 0.01$.
[3] $P \geq 0.05$
4 n=The number of birds/pen

Table 7b. Effect of dietary treatment on organ weights relative to BW of broiler chickens (g/100g)

Dietary treatment[1] (n=12)[5]	Gizzard	Pancreas	Bursa	Spleen
T1	1.87 ± 0.32[a]	0.24 ± 0.01[a]	0.16 ± 0.02	0.11 ± 0.03
T2	1.67 ± 0.31[ab]	0.16 ± 0.03[b]	0.16 ± 0.05	0.10 ± 0.03
T3	1.55 ± 0.14[ab]	0.17 ± 1.05[b]	0.13 ± 0.05	0.08 ± 0.03
T4	1.28 ± 0.79[ab]	0.19 ± 0.03[b]	0.13 ± 0.01	0.10 ± 0.01
T5	1.71 ± 0.25[ab]	0.18 ± 0.02[b]	0.13 ± 0.01	0.09 ± 0.01
T6	1.49 ± 0.27[ab]	0.17 ± 0.02[b]	0.13 ± 0.01	0.09 ± 0.01
T7	1.08 ± 0.63[a]	0.19 ± 0.03[b]	0.12 ± 0.02	0.09 ± 0.03
T8	1.02 ± 0.57[a]	0.15 ± 0.02[b]	0.12 ± 0.01	0.10 ± 0.01
T9	1.34 ± 0.19[ab]	0.16 ± 0.03[b]	0.14 ± 0.05	0.09 ± 0.02
P-value	*[2]	**[3]	ns[4]	ns

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-b Means within a column with differing superscripts are significantly different at $P < 0.05$.
[3] a-b Means within a column with differing superscripts are significantly different at $P < 0.01$.
[4] $P \geq 0.05$
5 n=The number of birds/pen

Table 8. Effect of feed additive supplementations on histomorphological parameters of the ileum in broilers chickens

Dietary treatment[1] (n=12)[4]	Villus height (µm)	Crypt depth (µm)	Villus height:crypt depth
T1	523.00 ± 27.17[d]	131.00 ± 4.32	۳.۹۹ ± ۰.۱۰[e]
T2	563.00 ± 10.80[c]	136.00 ± 8.12	4.15 ± 0.28[e]
T3	564.25 ± 22.91[c]	137.00 ± 6.27	3.48 ± 18.74[e]
T4	581.00 ± 17.68[c]	134.25 ± 8.54	4.34 ± 0.26[de]
T5	578.75 ± 31.94[c]	133.00 ± 7.12	4.36 ± 0.29[de]
T6	676.25 ± 11.84[b]	141. 50 ± 4.93	4.79 ± 0.23[bc]
T7	656.75 ± 10.90[b]	139.25 ± 5.56	4.72 ± 0.24[dc]
T8	724.00 ± 8.87[a]	134.75 ± 8.10	5.39 ± 0.29[a]
T9	714.75 ± 4.35[a]	139.50 ± 11.12	5.15 ± 0.41[ab]
P-value	[2]**	ns[3]	**

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] a-e Means within a column with differing superscripts are significantly different at $P < 0.01$.
[3] $P \geq 0.05$
4 n=The number of birds/pen

Table 9. Effect of feed additive supplementations on NDV antibody titers in broiler chicenks from 7 to 42 days of age

Dietary treatment[1] (n=12)[5]	Antibody titers					
	7th day	**14th day**	**21st day**	**28th day**	**35th day**	**42nd day**
T1	6.15 ± 0.45	5.03 ± 0.55[ab]	4.68 ± 0.49	5.13 ± 0.43[c]	4.40 ± 0.20[c]	4.48 ± 4.40[c]
T2	6.03 ± 0.36	4.58 ± 0.05[b]	4.93 ± 0.49	5.33 ± 0.13[c]	4.95 ± 1.27[bc]	4.93 ± 4.95[c]
T3	5.73 ± 0.17	5.05 ± 0.52[ab]	4.52 ± 0.04	5.35 ± 0.70[c]	5.15 ± 0.90[abc]	5.30 ± 5.15[bc]
T4	5.75 ± 0.06	5.00 ± 0.58[ab]	4.92 ± 0.45	5.30 ± 0.70[c]	5.35 ± 0.70[abc]	5.00 ± 5.35[c]
T5	5.90 ± 0.80	5.25 ± 0.50[ab]	4.83 ± 0.39	5.95 ± 0.53[abc]	5.20 ± 0.83[abc]	4.53 ± 5.20[c]
T6	5.85 ± 0.44	4.78 ± 0.49[ab]	4.93 ± 0.49	5.73 ± 0.55[bc]	5.98 ± 0.50[ab]	5.03 ± 5.98[c]
T7	5.73 ± 0.15	5.15 ± 0.44[ab]	4.88 ± 0.43	5.98 ± 0.61[abc]	5.60 ± 0.91[abc]	5.10 ± 5.60[bc]
T8	5.73 ± 0.83	5.53 ± 0.78[a]	5.10 ± 0.40	6.28 ± 0.83[ab]	6.28 ± 0.05[a]	5.89 ± 6.28[ab]
T9	5.88 ± 8.96	5.25 ± 0.50[ab]	5.13 ± 0.43	6.80 ± 0.54[a]	6.18 ± 0.47[a]	6.29 ± 6.18[a]
P-value	ns[2]	*[3]	ns	**[4]	*	**

[1] The dietary treatments were: T1) basal diet (control) ; T2,3) basal diet plus (400, 600) g of phosphomycin product(Bedson co.) /ton of starter and grower feeds, respectively; T4,5) basal diet plus (150,200) g of probiotic product (Protexin™) /ton of the starter feed and (100,150)g/ton of the grower feed, respectively; T6,7) basal diet plus (500, 1000)g of a prebiotic product (Techno Mos) /ton of starter and grower feeds, respectively; T8 and9) basal diet plus (1000,1250) g of synbiotic product (Biomin IMBO) /ton of the starter feed and (500,750)g/ton of the grower feed, respectively.
[2] $P \geq 0.05$
[3] a-c Means within a column with differing superscripts are significantly different at $P < 0.05$.
[4] a-c Means within a column with differing superscripts are significantly different at $P < 0.01$.
5 n=The number of birds/pen

DISCUSSION AND CONCLUSION

In the recent decades, deficiencies in feed formulation and management practices have been masked by the routine use of antibiotic growth promoters (AGP). However, the ban of AGP in Europe has driven the implementation of alternative strategies in order to maintain health and performance status and optimizing digestion in poultry production. Several feed additives have been used to manipulate microbial communities in the digestive tract. However, their efficacy has not always been proven and their modes of action require further research. The present study focused on the role and the efficacy of the antibiotic, probiotic, prebiotic and synbiotic products as potential modulators of gut health, immune responses and growth performance in poultry production.

Data of this research showed that probiotic can be good alternative for antibiotic because it had positive influence on growth performance, organ weights, intestinal histomorphology and immune response in broiler chickens compared with those of the control group (but in some parameters it is not significant). Improvement in growth performance and feed efficiency of broiler chickens fed probiotics (Falaki et al., 2011; Naseri et al., 2012; Houshmand et al., 2012) is thought to be induced by the total effects of probiotic action including the maintenance of beneficial microbial population (llerFu, 1989), improving feed intake and digestion (Nahanshon et al., 1993), and altering bacterial metabolism (Jin et al., 1997). The mechanism that explains the action of probiotics is focused on gastro intestinal tract, because, most of these products are not absorbed and are not efficient as growth promoters in germ-free animals (Coates et al., 1963). Therefore, it may be speculated that there is a strong interaction between probiotics and the intestinal micro flora. Hence, this improvement in performance due to the action of probiotics on the micro flora can be interpreted in two ways: the first is related to the reduction in the utilization of nutrients by micro organisms and the second is the decrease of microbial metabolites that interfere with host growth (Anderson et al., 1999). In addition, maintaining the integrity of the intestinal mucosa results in high energy requirements, and the decrease of pathogens and intestinal metabolites can also decrease intestinal cell turnover, resulting in more energy available for production. Finally, the reduction of opportunistic pathogens and subclinical infections can also be associated with the use of probiotics (Dibner and Richards, 2005). In the present study, the beneficial effects of probiotic product on broiler performance parameters, histomorphological parameters and immune responses are in agreement with previous studies (Midilli et al., 2008; Awad et al., 2009; Ashayerizadeh et al., 2011).

Serum antibody titers against Newcastle disease virus based on hemagglutination-inhibition test (HI) in broiler chickens fed commercial diet supplemented with probiotic (group 4and5) was higher than those of chickens in group 1. The positive effect of feeding diet containing probiotic on the immune response indicates the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The direct effect might be related to stimulate the lymphatic tissue (Kabir et al., 2004), whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract. Shoeib et al. (1997) reported that the bursa of probiotic-treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Christensen et al., (2002) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Commensally, bacteria presented in intestinal microbiota are in close contact with cells of the immune system. It has recently been demonstrated that resident dendritic cells (DC) in the intestinal lamina propria have the capacity to directly sample the gut lumen by projecting their dendrites through the tight junctions of epithelial cells (Rescigno et al., 2001). The recognition of commensal bacteria or their structural components by Toll like receptors (TLR) presented on surfaces of DC could lead to the activation and maturation of these cells (Rakoff-Nahoum et al., 2004). Differential activation of DC by commensal bacteria promotes the establishment of T-helper 1 (Th1), Th2, and Th3 responses and the secretion of cytokines, such as interleukin 4 (IL-4), IL-10, and transforming growth factor β, that are important for antibody production and isotype switching (Christensen et al., 2002; Di Giacinto et al., 2005).

In this study we found that, broilers fed prebiotic were more efficient than probiotic and antibiotic on broiler performance parameters, immune responses and histomorphological parameters, however, in some parameters

this differences was not significant. Prebiotics can serve as substrate for beneficial bacteria mainly located in the hind gut. We think they can enhance the digestibility and performance parameters by creating the favorable conditions for beneficial bacteria. However, they are exclusively fermented by beneficial bacteria such as Lactobacillus, Bifidobacteria and Bacteroides, thereby having the potential to modulate the composition of microbial communities in the gut (Chen et al., 2005). According to our data, prebiotic supplementation of the diet showed increase in antibody titers against NDV compared to that of the control but were not significant ($P > 0.05$). Much of the nature of mechanism accountable for immunomodulation associated with the prebiotic remains to be delineated. One hypothesis is that defense cells in the gut-associated lymphoid tissue (GALT) detect the presence of microbes by recognizing molecules unique to microorganisms that are not associated with host cells. We think it is also possible that prebiotic may enhance the secretion of plasma serum IgG and intestinal mucosa IgA, increasing the number of lymphocytes and/or leukocytes in the GALT and in peripheral blood (Kaufhold et al., 2000). In the intestine, secretory IgA binds to pathogenic organisms and provides protection by preventing their attachment to mucosal cells (Abbas et al., 2000). It is the most prominent antibody present at mucosal surfaces, and provides passive immunoprotection against invading pathogens in the gastrointestinal tract. Gao et al. (2008) reported that birds fed prebiotic -supplemented diets had greater sIgA content in the duodenum so with increasing concentration of dietary prebiotic, IgA content increased linearly. We speculated that it may stimulate the humoral immune system to produce more antibodies therefore increased antibodies cover the surface of intestinal mucosa and can protect villi from damage (Toloei et al., 2010; Ghahri et al., 2010). However, in a commercial poultry setting, the impact of nutrition on the immune competence that underpins the production traits is difficult to quantify. As such, laboratory research is essential for a better understanding of the immunomodulatory properties of feed additives, particularly in light of the need for alternatives to in-feed antibiotics.

Conclusions obtained by this study represent that synbiotics had a positive effect on growth performance, immune responses and histomorphological parameters, that is in agreement with previous studies (Awad et al., 2009; Naseri et al., 2012) It might be combination of probiotics and prebiotics, also referred as synbiotics, improve the survival rate of probiotics during their passage through the digestive tract, thus contributing to enhancement of the probiotic effects. A synbiotic relationship between a prebiotic substance and a probiotic organism suggests synergism. There are many discussions about synbiotic but their mechanism of action is not completely known and there are a few articles about the effectiveness of this product in the world. Our data indicated that synbiotic supplementation of the diet increased antibody titers against NDV compared to that of the control on 28, 35 and 42 days ($P < 0.05$). These results are in agreement with that of Haghighi et al. (Haghighi et al., 2006) who found that probiotics enhance the systemic antibody response to some antigens in chickens and Talebi et al. (2008) who found that administration of a multi-strain probiotic improve the antibody responses to ND. It is possible that, binding of structural components of commencal bacteria to Toll-like receptors expressed on the surface of macrophage and dendritic cells in the lamina propria may lead to their activation and differentiation. Upon its activation, they promote the activation and differentiation of different subsets of other immune system cells, leading to the production of cytokines such as IL4, IL10 and transforming growth factor ß, that are important for antibody production and isotype switching (Di Giacinto et al., 2005; Mohamadzadeh et al., 2005).

In the present study, supplementation of broilers with probiotic, prebiotic and synbiotic increased the villus height and villus height: crypt depth ratio in ileum significantly ($P < 0.01$), suggesting an increased epithelial cell turnover due to feeding of direct-fed microbials. The histomorphological changes in the intestine of broiler chickens reported in the present study provide useful information regarding the potential for using probiotic, prebiotic and synbiotic in broiler feed. Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). The villus crypt is considered as the villus factory and deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demands for tissue. The intestinal epithelial cells originating in the crypt migrate along the villus surface upward to the villus tip and are extruded into the intestinal lumen within 48 to 96 h (Potten, 1998). We believe shortening of the villi and deeper crypts may lead

to poor nutrient absorption, increased secretion in the gastrointestinal tract, and lower performance (Xu et al., 2003). In contrast, increase in the villus height and villus height: crypt depth ratio are directly correlated with increased epithelial cell turnover and longer villi are associated with activated cell mitosis (Dunham et al., 1993). Longer villi were found in the ileum of chicks and turkeys treated with Lactobacillus reuteri (Dunham et al., 1993) and in the ileum of adult male layers with slight improvement in feed efficiency after dietary addition of Bacillus subtilis var. natto (Samanya and Yamauchi, 2002). Feeding of probiotics has been shown to induce gut epithelial cell proliferation in rats (Ichikawa et al., 1999). In addition, longer villi were induced by dietary amylase (Ritz et al., 1995). The concentrations of amylase in broiler intestine were increased after supplementation of diet with either a single strain of Lactobacillus acidophilus or a mixture of Lactobacillus strains (Jin et al., 2000). However, amylase concentrations were not estimated in the present study, and further experiments are needed to verify this effect. It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes, and nutrient transport systems (Pluske et al., 1996). It is understood that greater villus height is an indicator that the function of intestinal villi is activated (Shamoto and Yamauchi, 2000). This fact suggests that the villus function is activated after feeding of dietary probiotic, prebiotic and synbiotic.

In conclusion, the present study indicated that the synbiotic had the best effect on performance, immune responses, histomorphological parameters in comparison with probiotic and prebiotic products. Also prebiotic had better effect than probiotic and, both of them had better effect than phosphomycin. Therefore, these products might be promising alternatives for antibiotic growth promoters as pressure to eliminate antibiotic growth promoters in animal feed increases. The synbiotic offers a good alternative to improve poultry production.

REFERENCE

Abbas, A.K., A.H. Lichtman, and J.S. Pober. 2000 . Cellular and molecular immunology, 4th edn. Page 553: W.B. Saunders, Philadelphia .

Alexander, D.J., W.H. Allan, and P.M. Biggs. 1983.A standard technique for hemagglutination inhibition tests for antibodies to avian infectious bronchitis virus. Vet Rec. 16; 113(3):64.

Anderson, D.B., V.J. McCracken, R.J. Aminov, J.M. Simpson, R.J. Mackie, M.W.A. Vestegem, and H.R. Gaskins . 1999 .Gut microbiology and growth promoting antibiotics in swine. Pig News and Information. 20:115 - 122.

Andremont, A. 2000.Consequences of antibiotic therapy to the intestinal ecosystem. Ann. Fr. Anesth Reanim .19:395–402.

Ashayerizadeh, A., N. Dabiri, K.H. Mirzadeh, and M.R. Ghorbani. 2011 . Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. J Cell Anim Biol . 5(8): 152-156.

Awad, W.A., K. Ghareeb, S. Abdel-Raheem, and J. Bohm . 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult Sci. 88: 49-56.

Barnes, E. M., G. C. Mead, D. A. Barnum, and E .G. Harry. 1972 . The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br Poult Sci. 13:311–326.

Burgat, V. 1999. Residues of drugs of veterinary use in food. Rev Prat.41:985–990.

Caspary, W. F. 1992. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr .55:299S–308S.

Chen, Y.C., C Nakthong, and T.C. Chen. 2005. Improvement of laying hen performance by dietary prebiotic chicory oligofructose and inulin. Int J Poult Sci. 4: 170-178.

Chichowski, M., W.J. Croom, F.W. Edens, B.W. MacBride, R. Qiu, C.C. Chiang, L.R. Daniel, G.B. Havenstein ,and M.D. Koci. 2007 . Microarchitecture and spatial relationship between bacteria and ileal, cecal and colonic epithelium in chicks fed a direct- fed microbial, PrimaLac, and Salinomycin. Poult Sci. 86: 1121-1132.

Christensen, H. R., H. Frokiaer,and J. J. Pestka . 2002. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol.186:171-178.

Coates, M.E., R. Fuller, G.F. Harrison, M. Lev,and S.F. Suffolk. 1963. Compand in a conventional environment, with and without dietary supplementations of penicillin. Br J Nut.17:141-151.

Di Giacinto, C., M. Marinaro, M. Sanchez, W. Strober, M. Boirivant . 2005. Probiotics ameliorate recurrent Th1-

mediated murine colitis by inducing IL10 and IL 10 dependant TGF-beta-bearing regalatory cells. J. Immunol.174: 3237-3246.

Dibner, J.J.,and J.D. Richards . 2005. Antibiotic growth promoters in agriculture: History and mode action. Poult Sci. 84:634-643.

Dunham, H.J., C. Williams, F.W. Edens, I.A. Casas ,and W.J. Dobrogosz. 1993 . Lactobacillus reuteri immunomodulation of stressor-associated diseases in newly hatched chickens and turkeys. Poult Sci.72(Suppl. 1):103. (Abstr.)

Falaki, M., M. Shams Shargh, B. Dastar,and S. Zerehdaran. 2011. Effect of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens.J Anim Vet Adv. 10 (3):378-384.

Fuller, R. 1989 .Probiotic in man and animal. J Appl Bacteriol.66:365–378.

Gao, J., H.J. Zhang, S.H. Yu, S.G. Wu, I. Yoon, J. Quigley, Y.P. Gao, and G.H. Qi. 2008 . Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poult Sci. 87:1377–1384.

Ghahri, H., T. Toloei,and M. Habibi. 2010 . Efficacy of esterified glucomannan, sodium bentonite and humic acid to counteract experimental aflatoxicosis on antibody titers against Newcastle disease in broilers. Afr J Biotechnol.9(26): 4127-4131.

Gibson, G.R., H.M. Probert, J.V. Loo, R.A. Rastall, M.B. Roberfroid . 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev. 17: 259-275.

Gibson, G.R., and M.B. Roberfroid. 1995. Dietary modulation of human colonic microbiota: Introducing the concept of prebiotic. J Nutr. 125:1401–1412.

Haghighi, H.R., J. Gong, C.L. Gyles, M.A. Hayes, H. Zohu, B. Sanei, J.R. Chambers,and S Sharif . 2006. Probiotics stimulate production of natural antibodies in chickens. clin and Vaccine Immunol.13(9): 975-980.

Houshmand, M., K. Azhar, I. Zulkifli, M.H. Bejo,and A. Kamyab. 2012 . Effects of non-antibiotic feed additives on performance, immunity and intestinalmorphology of broilers fed different levels of protein. S Afr J Anim Sci .42:22-32.

Ichikawa, H., T. Kuroiwa, A. Inagaki. 1999 . Probiotic bacteria stimulate epithelial cell proliferation in rat. Dig Dis Sci.44:2119–2123.

Iji, P.A.,and D.R. Tivey. 1998. Natural and synthetic oligosaccharides in broiler chicken diets. Worlds. Poult Sci .54: 129-143.

Jin, L. Z., Y.W. Ho, N. Abdullah ,and S. Jalaludin. 1997 . Probiotics poultry: Modes of action. World's Poult Sci J.53:352–368.

Jin, L.Z., H.W. Ho , N. Abdullah,and S. Jalaludin. 2000. Digestive and bacteria enzyme activities in broilers fed diets supplemented with Lactobacillus cultures. Poult Sci.79:886–891.

Kabir, S.M.L., M.M. Rahman, M.B. Rahman,and S.U. Ahmed . 2004. The dynamics of probiotics on growth performance and immune response in broilers. International Journal of Poult Sci. 3: 361-365.

Kaufhold, J., H.M. Hammon,and J.W. Blum Fructo. 2000 .Oligosaccharide supplementation: effects on metabolic, endocrine and hematological traits in veal caves. J Vet Med A. 47:17–29.

Menten, J.F.M. 2002 . Probióticos, prebióticos e aditivos fitogênicos na nutrição de aves. In: 2º Simpósio sobre Ingredientes na Alimentação Animal. Uberlândia Minas Gerais. Brasil. p. 251-276.

Midilli, M., M. Alp, N. Kocabagli, O.H. Muglali, N. Turan, H. Yilmaz,and S. Cakur. 2008 . Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. S Afr J Anim Sci.38: 21-27.

Mohamadzadeh, M., S. Olson, W. Kalina, G. Ruthel, G.L. Demmin, K.L. Warfield, S. Bavari,and T.R. Klaenhammer. 2005 .Lactobacilli activate human dendritic cells that shew T cell toward T helper 1 polarization. Proc. Natl. Acad. Sci. USA. 102: 2880-2885.

Mohnl, M., Y. Acosta Aragon, A. Acosta Ojeda, B. Rodriguez Sanchez,and S. Pasteiner . 2007.Effect of synbiotic feed additive in comparison to antibiotic growth promoter on performance and health status of broilers. Poult Sci.86(suppl. 1), 217.

Nahanshon, S.N., H.S. Nakaue,and L.W. Mirosh. 1993 . Effects of direct fed microbials on nutrient retention and parameters of Single White Leghorn pullets. Poult Sci.72(Suppl .2):87. (Abstr.)

Naseri Alavi, S.A., A. Zakeri, B. Kamrani,and Y. Pourakbari. 2012 . Effect of Prebiotics, Probiotics, Acidfire, Growth Promoter Antibiotics and Synbiotic on Humural Immunity of Broiler Chickens. Glob Vet. 8 (6): 612-617.

Pluske, J.R., M.J. Tompson, C.S. Atwood , P.H. Bird , I.H. Williams ,and P.E. Hartmann . 1996.Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. Br J Nutr.76:409–422.

Potten, C.S . 1998. Stem cells in the gastrointestinal epithelium: Numbers, characteristics and death. Philos. Trans. R. Soc. Lond. B Biol Sci.353:821–830.

Rakoff-Nahoum, S., J. Paglino, F. Eslami-Varzaneh, S. Edberg,and R. Medzhitov. 2004.Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. Cell.118:229–241.

Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl,and P. Ricciardi Castagnoli. 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol.2:361–367.

Ritz, C. W., R.M. Hulet, B.B. Self ,and D.M. Denbow. 1995 . Growth and intestinal morphology of male turkeys as influenced by dietary supplementation of amylase and xylanase. Poult Sci.74:1329–1334.

Rodrigues, T.A., Sartor C, Higgins SE, Wolfenden AD, Bielke LR, Pixley CM ,Sutton L, Tellez G , Hargis M. 2005 . Effect of Aspergillus meal prebiotic (fermacto) on performance of broiler chickens in the starter phase and fed low protein diets. J Appl Poult Res. 14: 665-669.

Rycroft, C.E., M.R. Jones, G.R. Gibson,and R.A. Rastall . 2001. A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. J Appl Bacteriol. 91: 878-887.

Samanya, M.,and K. Yamauchi. 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. Comp. Biochem. Physiol. 133:95–104.

Samli, HE., N. Senkoylu, F. Koc, M. Kanter,and A. Agma. 2007. Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and microbiota. Arch Anim Nutr. 61:42–49.

Sell, J.L.1994. National Research Council. Nutrient Require ments of Poultry. 9th ed. Washington, DC, USA: Natl Acad Press:27.

Shamoto, K.,and K. Yamauchi. 2000. Recovery responses of chick intestinal villus morphology to different refeeding procedures. Poult Sci. 79:718–723.

Shoeib, H.K., A.N. Sayed, S.A. Sotohy,and S.K. Abdel Ghaffar. 1997. Response of broiler chicks to probiotic (pronifer) supplementation. Assiut Vet Med J.36: 103-116.

Sorum, H.,and M. Sunde. 2001. Resistence to antibiotics in the normal flora of animals. Vet Res.32:227–241.

Stanczuk, J., Z. Zdunczyk, J. Juskiewicz,and J. Jankowski. 2005 . Indices of response of young turkeys to diets contaning mannanoligosuccharide or inulin. Vet Zootech. 31: 98-101.

Talebi, A., B. Amirzadeh, B. Mokhtari,and H. Gahri. 2008 . Effects of a multi-strain probiotic (PrimaLac) onperformance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. Avian Pathol. 37(5): 509-512.

Toloei, T., H. Ghahri,and A. Talebi. 2010. Efficacy of mannanoligosaccharides and humateon immune response to Avian Influenza (H9) diseasevaccination in broiler chickens. Vet Res Commun. 34:709–717.

Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan, M.Q. Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci.82:1030–1036.

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Effect of Treated Barley Grain with Sodium Hydroxide, Urea and Formaldehyde on Degradability of Crude Protein Using In situ

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ABSTRACT

The present study was carried out to determine the influence of treated barley grain with sodium hydroxide, urea and formaldehyde on degradability of crud protein and dry matter using in situ technique in Gizel sheep. Two fistulated sheep with average BW 45±2.5 kg were used in a complete randomized design. The ruminal dry matter (DM) and crud protein (CP) disappearance was measured at 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. The experimental treatments were A: barley grain treated with 3.5% sodium hydroxide and 1.5% urea, B: barley grain treated with 3.5% sodium hydroxide and 0.4% formaldehyde, C: barley grain treated with 3.5% urea and 0.4% formaldehyde. Parameters of crud protein (CP) for soluble fractions were (a) 41.063, 9.58 and 45.9% and fermentable fractions were (b) 51.16, 85.13 and 51.77% for treatments A, B and C, respectively. According to the survey results, it is clear that grain treated with chemical digestion has high feed potential and if further investigation in ruminant diets can be used as an alternative feed.

Key words: Barley grain, Formaldehyde, In-situ, Sodium hydroxide, Urea.

INTRODUCTION

Barley is a cheap cereal which is used in many countries, especially in European countries, to reduce production costs (Sharifi et al, 2005). Barley grain is a widely used source of energy and easily digestible carbohydrates in ruminants particularly cattle diets (Yang et al., 2000). The average crude protein content of barley grain is about 12% dry matter which is of low quality and lacks the lysine (Soufisiavash and Janmohammadi, 2004). Despite the low percentage of protein, due to the large amount of this substance in the diet, it provides a substantial portion in diet. Due to the high rate of solubility and the protein composition of barley grain, its degradability is so high that increases the rate and extent of rumen fermentation of

carbohydrates, bloating, acidosis, lameness, and hepatic disorders (Yang et al., 2000). The process of grain can be effective in reducing the rate and extent of ruminal degradation of dry matter and protein, and decreasing metabolic disorders (Dehghan Banadaky et al., 2007b). Various techniques have been developed for processing grains and feed ingredients to increase the efficiency of high cereal diets to improve palatability. Therefore, to improve animal performance, processed cereal grains can affect digestibility, rate, and site of digestion (McNiven et al., 1995). Several processing methods are known to increase the efficiency of digestion and nutritional value of grains for animals (Rowe et al., 1999). However, due to the high cost of physical and mechanical processing, more attention is needed to review the use of chemical methods in improving the nutritional quality of cereals in animal food (Dehghan Banadaky et al., 2007b). Chemical treatments including hydroxides are used to reduce the resistance of the seed coat and formaldehyde is used to reduce the microbial digestion of protein. Formaldehyde is a chemical treatment of barley that reduced ruminal degradability of crude protein and starch grains (Ahmadi, 2011). To obtain the best performance of the seeds, their digestibility should be determined (Rowe et al., 1999). Fermentation pattern and digestion sites can have a significant effect on the nature of available nutrients in animals (Dehghan Banadaky et al., 2007a). Also, due to the limited sources of energy and fuel, high cost of physical and mechanical processing cereal grain, it is necessary to review the chemical methods to improve the nutritional quality of cereals in ruminants (Dehghan Banadaky et al., 2007a). Therefore, the purpose of this study was to investigate the effect of processing grain with urea, sodium hydroxide, and formaldehyde on degradability of protein and optimum use of processing chemicals using nylon bags technique.

MATERIALS AND METHODS

Barley grain collection

Barley grain was obtained from *Ajhdar* local variety of department of agriculture of Malekan, Iran. The experimental treatments were A: barley grain treated with 3.5% sodium hydroxide and 1.5% urea, B: barley grain treated with 3.5% sodium hydroxide and 0.4% formaldehyde, C: barley grain treated with 3.5% urea and 0.4% formaldehyde that were prepared in the laboratory. Besides, 3 parts of solution and 1 part of barley were mixed in plastic containers and were kept in room temperature and away from sunlight for 60 days; samples were taken out of the containers and dried in the sunlight and milled in a 2 mm size to be used in other phases of the experiment. Animals used in this experiment were fed at maintenance level. The animals were fed with a mixture of 60% forage and 40% concentrate diet (Ørskov and McDonald, 1979).

Chemical composition

Feedstuffs dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30), and crude protein (CP, method ID 984.13) were determined by procedures of AOAC (1999). The neutral detergent insoluble fiber (NDF) and acid detergent fiber (ADF) concentrations were determined using the methods of Van Soest et al. (1991), without sodium sulphite. Neutral detergent insoluble fiber was analyzed without amylase with ash included.

Measured in situ

To estimate the degradability of the nylon bag technique, the food samples were milled with a special mill and 2-mm sieve (Moghaddam et al., 2012). 5 grams of each nutrient were poured into bags made of synthetic polyester fiber as 6 × 12 cm and pore diameter of 50 mm. Two fistulated sheep with average BW 45±2.5 kg were used in a complete randomized design To determine the degradation at time zero, sample bags were washed under tap water for 15 minutes. Incubation times were 0, 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. After each incubation time, the bags were removed and rinsed with cold water until the water is completely cleared out. After washing, bags were incubated for 24 h at a temperature of 65 ° C to evaporate and for 24 h at 105 °C in oven (Moghaddam et al., 2012). Degradation parameters (soluble, insoluble, and fixed rate of degradation) were calculated with Naway. For matched degradation data used from $P=a+b(1-e^{-ct})$ that a= The degradation of soluble fraction (%), b= The degradation rate of insoluble fraction (%), c= The constant degradation rate (%/h), t=The incubation time (h), e= The constant factor (2.718) and P= The degradation rate at the time t. Effective degradability was calculated at $ED=a+(b\times c)/(c+k)$ that k is passage rate which were considered in this study 0.02.

Statistical analysis

The obtained data from in situ study was analyzed according to a completely randomized design with 4 replicates by the GLM procedure (SAS, 2002). The treatment means were compared by the Duncan test.

RESULT AND DISCUSSION

The chemical composition of treatments is presented in Table 1. The data show that treatment A had the most (95.5%) and treatment B had the least (94.5%) amount of dry matter which were in agreement with the findings of Parand and Taghizadeh (2009), for barley grain (93.8%) and Nikkhah et al., (2007), for Barley grain processed with 3.5% hydroxide sodium (88.6%) ($P<0.05$). Regarding the percentage of crude protein treatment C (16.31%) and treatment B (9.66%) had the highest and the lowest amount of crude protein. The findings of this study in this regard are in line with the findings of Nikkhah et al., (2007), for barley treated with 3.5% urea (15.01%) and barley treated with 3.5% hydroxide sodium (10.51%). However, the findings of the present study differed from the data reported by Taghizadeh and Nemati (2008), (11.56%), Taghizadeh et al., (2003), (10.5%) and Yang et al., (2000), (13.5%) for barley grain. This difference can be attributed to processing barley with urea, NaOH, and formaldehyde in this study. Significant differences were observed among treatments of crude protein in this study ($P<0.05$). These differences are pertained to the used urea for per 1.5% used urea solution about 3% is added to the crude protein in grain. According to table 1, there were significant differences in dry matter, crude protein, acid detergent fiber, and neutral detergent fiber in tested food ($P<0.05$). There were also differences between the amounts of acid detergent fiber, neutral detergent fiber, crude protein, and neutral detergent fiber obtained in this study and the NRC (2001). These differences can be attributed to the effects of the treatments.

Table 1. The chemical composition of feeds (% DM)*

Treatments	DM	CP	NDF	ADF	ADIN
A	95.5^{a}	12.9^{b}	36.6^{a}	8.1^{b}	1^{b}
B	94.5^{c}	9.66^{c}	35.5^{b}	10.1^{a}	1.2^{a}
C	95.1^{b}	16.31^{a}	35.9^{b}	6^{c}	0.75^{c}
SEM	0.1105	0.04055	0.1972	0.1914	0.03785

*DM=dry matter, CP=crude protein, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADIN=acid detergent insoluble nitrogen.

a,b,c Within a column, means without a common superscript letter differ ($P< 0.05$).

**Standard error means of the difference amount three treatments means.

According to the results reported in tables 2 and 3 at different times of incubation, treatments A and C are the highest and lowest DM disappearance values, respectively. Also, according to the results obtained at 0 h of incubation, treatment C (15.41%) had the lowest and treatment A (23.52%) had the highest rate of dry matter disappearance that there were significant differences in all treatments ($P<0.05$). Taghizadeh et al., (2001), reported (10.6%) of barley grain ruminal DM disappearance at 0 h. Taghizadeh and Nemati (2008), reported the rate of DM disappearance in unprocessed barley (18.145%). Also, showed significant differences in the incubation time 0 h among unprocessed barley grains, processed by autoclaving at 120 °C for 5 min and 20 min, and treated at 100 °C for 5 min and 20 min for the disappearance of DM, respectively. The differences between the results of the experiments can be attributed to the plant species, climatic conditions of the region, conditions of grain growth, and other environmental conditions. Since the DM is a mixture of crude protein, fat, carbohydrates, and vitamins and the tested foods treated with formaldehyde were different with regard to these nutrients, therefore the reduced ruminal degradation of grain starch and crude protein is not due to the toxicity effects of formaldehyde on microorganisms in the rumen, but rather it is due to the methylene crosslinking proteins in the barley field that reduced the sensitivity of microbial degradation of barley protein and microorganisms access to starch. Consequently, it increases the delay phase in rumen degradation of protein and starch (Dehghan Benadaki et al., 2007a). The findings of the present study indicated that treatment A had the highest DM degradation in all hours of incubation which can be caused by urea and sodium hydroxide effects. In addition, a large amount of DM degradation can be as a result of high levels of NDF and ADF of this grain.

Treatments a (20.94%) and B (10.54%) had the highest and lowest a coefficient value for DM, respectively, that due to the high solubility of urea, these results are predictable and justifiable. Taghizadeh and Nemati (2008), reported DM solution at zero time (coefficient a) value of (19.5%) for unprocessed barley grain that regardless of the treatment effect is consistent with the results of the present experiments. Also, Taghizadeh et al., (2001), reported a coefficient value for barley (14%). Treatments B (89.28%) and C (73.23%), had the highest and lowest fermentable material (coefficient b), respectively. Taghizadeh and Nemati (2008), reported coefficient b value for barley (78%) that are similar to the values obtained for treatment A in this study. The results reported in this study revealed that the coefficients a and b indicated significant differences among treatments which were due to the treatment effects (P<0.05). Barley treated with NaOH improves fiber digestibility and reduces fluctuations in rumen pH and rumen degradability of starch and nitrogen (McNiven et al., 1995).

Table 2. Means of dry matter degradation of feeds by incubation at different times in the in situ method (% DM)

Treatment	Incubation times (h)								
	0	2	4	6	8	16	24	36	48
A	23.52^{a}	32.62^{a}	45.79^{a}	54.66^{a}	69.93^{a}	83.97^{a}	94.55^{a}	95.89^{a}	96.64^{a}
B	20.24^{b}	32.23^{a}	36.96^{b}	47.2^{a}	69.54^{a}	83.94^{a}	86.66^{b}	95.56^{a}	95.65^{a}
C	15.41^{c}	24.35^{b}	36.6^{b}	41.93^{a}	69.01^{a}	80.64^{b}	83.33^{c}	88.8^{b}	90^{b}
SEM**	0.5458	0.6283	0.5777	0.4138	1.0255	1.0177	0.6059	0.2186	0.2421

a,b,cWithin a column, means without a common superscript letter differ (P< 0.05).
**Standard error means of the difference amount three treatments means.

Table 3. The parameters estimated from the dry matter degradability coefficients of feeds

Treatments	Degradation coefficients			ED	RSD
	a	b	c		
A	20.94^{a}	77.62^{b}	0.099^{a}	84.13^{a}	4.99^{b}
B	10.54^{c}	89.28^{a}	0.098^{a}	84.67^{a}	6.99^{a}
C	17.97^{b}	73.23^{c}	0.102^{a}	79.23^{b}	6.55^{a}
SEM**	0.6924	0.7944	0.0213	0.179	0.1926

a=Dry matter solution at zero time (%), b=Fermentable material (%), c=Constant degradability coefficients at time t (%/h), ED=Effective degradation (The passage of time r=0.02), RSD= Residual standard deviation.
a,b,cWithin a column, means without a common superscript letter differ (P< 0.05).
**Standard error means of the difference amount three treatments means.

Means of the data presented in tables 4 and 5 show that in zero-hour of incubation, treatments A (49.98%) and C (10.01%) had the highest and lowest rumen CP disappearance (P<0.05). This difference can be due to processing barley with urea that is highly soluble and hydroxide sodium which decreases ruminal pH fluctuations, and therefore in increases proteolytic activity of the rumen microorganisems that it has caused higher CP degradation. Considering the high solubility of urea and the effect of formaldehyde fixation properties, these findings are reasonable.Taghizadeh et al., (2001), reported untreated barley grain CP degradation of (5.85%) in 0 h incubation. Apart from the effects of animals, plant species, and environmental conditions, and because of processing the grain with the chemicals, these differences in CP disappearing are justifiable. The data obtained from this study showed that treatments A (96.2%) highest and C (97.87%) lowest CP disappearance in 48 h incubation. Taghizade et al., (1380), and Taghizadeh and Nemati (2007), found unprocessed barley grain CP degradation in 48 h incubation about (68%) and (59.59%), respectively. These differences can be attributed to planet species, animal effect, conditions for plant growth, environmental conditions and treatments effects. Since the proportion of rumen ammonia nitrogen and urea nitrogen transfer rates correlate negatively with the rumen, therefore the change in the proportion of dietary nitrogen digested in the rumen can alter the rumen and urea nitrogen recycle and this effect can be extensively increased by processing barley. It can also increase ruminal starch digestion and subsequent nitrogen recycle and urea nitrogen microbial degradation to rumen (Kiran and Mutsvangwa, 2007). In cereal starch granules are protected

by a combination of protein which can combine with aldehydes, form covalent bonds through the amino group of amino acids and this protective protein keeps starch granules from being degraded by microorganisms. Therefore, treating barley with formaldehyde can reduce rumen degradation of starch. As a result of this, reproduction and growth rate of microorganisms is delayed and consequently proteolytic activity gets limited and the rate of protein degradation does not increase (Row et al., 1999).

Crud protein degradability coefficients of the treatments presented in table 5 show that coefficient a had the highest and lowest values for treatments C (45.9%) and B (9.58%), respectively (P<0.05). These results were predictable due to the high solubility of urea. Treatments B (85.13%) and A (51.16%) had the highest and lowest coefficient b that were not significantly different, but the high difference between treatment B and other treatments can be due to the hydroxide OH factor that causes the destruction of protective starch protein graniles and the penetration of microorganisms into the feed particles and facilitates digestion (Row et al., 1999). With urea, microorganisms can readily get the needed nitrogen from urea and the feed protein is preserved, but due to increased late stage of protein and starch breakdown in the rumen with the presence of formaldehyde, causes the protein fermentation during over time in the rumen (Navidshad and Jafari Sayadi, 2000). It has been reported that the low nutritional value of this product is due to the presence of trypsin, beta-glucan and phytase as anti-nutritional factors.. These data are less than results of the study done by Taghizadeh and Nemati (2008), for the coefficient b (65%) for unprocessed grain. Chemical treatments included hydroxides to reduce the resistance of the seed coat and formaldehyde consumption to reduce microbial digestion of proteins. Also formaldehyde can be bonded with a protein and Inhibit invasion microorganisms to protein degradation. Endosperm contains starch granules which is surrounded with a combination of protein and Non-starch polysaccharides, using the solubility properties of proteins can be attached to the endosperm in such glutelins which are soluble in alkanes and alcohols, with the addition of chemical compounds eliminated the protective protein (Row et al., 1999).

Table 4. Means of crude protein degradation of feeds by incubation at different times in the in situ method (% DM)

Treatment	Incubation times (h)								
	0	2	4	6	8	16	24	36	48
A	49.98^{a}	52.94^{a}	65.21^{a}	71.84^{a}	79.67^{a}	83.19^{a}	90.15^{a}	94.10^{a}	96.2^{a}
B	40.93^{b}	51.36^{a}	54.55^{b}	55.13^{b}	77.05^{ab}	82.85^{a}	86.97^{b}	92.95^{b}	95.2^{b}
C	10.01^{c}	27.79^{b}	41.91^{c}	44.66^{c}	74.53^{b}	77.35^{b}	86.10^{b}	92.77^{b}	94.87^{b}
SEM**	0.4948	0.7451	0.3757	4.7580	0.7856	0.6135	0.7427	0.3255	0.3289

a,b,c Within a column, means without a common superscript letter differ (P< 0.05).
**Standard error means of the difference amount three treatments means.

Table 5. The parameters estimated from the crude protein degradability coefficients of feeds

Treatments	Degradation coefficients			ED	RSD
	a	b	c		
A	41.063^{b}	51.16^{b}	0.1497^{a}	86.167^{a}	3.12^{a}
B	9.58^{c}	85.13^{a}	0.12^{b}	82.633^{b}	6.56^{b}
C	45.9^{a}	51.77^{b}	0.07^{c}	86.167^{a}	5.77^{b}
SEM**	0.795	1.153	0.00415	0.4	0.3436

a=Crude protein solution at zero time (%), b=Fermentable material (%), c=Constant degradability coefficients at time t (%/h), ED=Effective degradation (The passage of time r=0.02), RSD= Residual standard deviation.
a,b,c Within a column, means without a common superscript letter differ (P< 0.05).
**Standard error means of the difference amount three treatments means.

Urea entered into the rumen by bacterial urease is rapidly hydrolyzed to ammonia and so, ruminal ammonia density can rise and the efficient conversion of ammonia into microbial protein requires fulfilling two conditions. First, the ammonia density must be lower than the desired conditions and second, the microorganisms must have a readily available source of energy for protein synthesis. Feeding operations to achieve these requirements is the

urea mixing with other feeds (For a long period of use and the amino). Such feeds should have a small amount of rumen degradable protein and greater amount of easily fermentable carbohydrates (Navidshad and Jafari Sayadi, 2000). In some studies, barley treated with urea has been a significant increase in milk production and reduced the rate of DM degradation (Robinson and Kennelly, 1988). Reduced protein and starch degradation in the rumen without negative effects on digestibility of rumen has been observed due to the effects of ammonia on barley grain. Increased ruminal propionate and decreased acetate and reduction in rumen pH with consumption ammonia grain in lactating cows, can be marked degradation and fiber fermentable and reduction in starch degradation (Campling, 1991). Many studies have been done on the processing of barley grain treated with sodium hydroxide. In some of these studies, increase in milk production, and food consumption and changes in milk composition have been reported (Bull, 1995). McNiven et al., (1995), reported processed barley with NaOH improve fiber digestibility and reduces fluctuations in rumen pH and starch and nitrogen rumen degradability. Also, increasing acetate and reduced ruminal is a sign of improvement in fiber digestion in the rumen due to better pH stability and decrease in its acidity (Dehghan Benadaki et al., 2007a).

To overcome the problems of easily digestible protein system, metabolizablable protein system was offered (Taghizadeh and Farhomand, 2007). To this end, the protein degraded by rumen microorganisms including the protein with rapid degradability of and the protein with slow degradability and is provided in the form of effective degradable protein and was computed that it will enter the intestine in the form of microbial protein. Also, the digestible undegradable which is part of food protein, that is not undegraded in rumen but is digested in small intestine. The data in Table 6, among the treatments in this experiment, treatments C (95.16%) and B (61.64%) had the highest and lowest metabolizable protein that have a significant difference ($P<0.05$).

Table 5. The parameters estimated from the metabolizable protein of feeds

Treatments	ERDP	DUP	MP
A	108.542^{b}	9.212^{a}	78.677^{b}
B	88.295^{c}	5.135^{b}	61.64^{c}
C	141.991^{a}	4.287^{b}	95.16^{a}
SEM**	0.64	0.7	0.4496

ERDP=Effective ruminal degradable protein, DUP=Digestible undegradable protein, MP=Metabolizable protein.

The differences in the amounts of metabolizable protein in treatments A, B and C could be related to the differences in crude protein and degradation properties of these materials that has led to a decrease in MP in treatment B. The existing difference in the MP of the studied samples can be due to differences in chemical composition, climate variability, variety of different protein, especially insoluble protein in buffer and insoluble protein in acid, and also is related to differences in the cell wall and especially to the protein trapped in the wall (ADIN).

REFERENCE

Ahmadi, A. 2011. Effect of treated barley grain with sodium hydroxide, urea and formaldehyde on degradability of crude protion and dry matter and gas production in Gizel sheep. M.Sc Thesis, Department of Animal Science, Faculty of Agriculture, Islamic Azad University, Maragheh, Iran.

AOAC. 1990 .Official Methods of Analysis. Vol. II. 15th ed. Association of Official Analytical Chemists ,Arlington, VA.

Besharati, M., and A. Taghizadeh. 2009. Evaluation of dried grape by-product as a tanniniferous tropical feedstuff. J. Anim. Feed Sci. Technol. 152:198-203.

Besharati, M., A. Taghizadeh, H. Janmohamadi, and GH. Moghaddam. 2007. The Determaination of degradability of grape by-product using in situ and gas production techniques. Iranian J. Agri. Sci. 18(3):173-185.

Bull, R. C. 1995. A nutritional guide to feeding pacific northwest barley to ruminants. University of Idaho. Moscow.

Campling, R. C. 1991. Processing cereal grain for cattle. a review. Livest. prod. Sci. 28:223-234.

Cone, J. W. 1998. Influence of protein fermentation on gas production profiles. Proc. Soc. Nutr. Physiol. 7:36-43.

Dehghan Banadaky, M., A. Nikkhah, H. Amanlou, M. Danesh Mesgaran, and H. Mansori. 2007a. Effects of chemical treatment of barley with sodium hydroxide, ammonia or formaldehyde on blood metabolites and productive performance of lactating Holstein dairy cow. Pajouhesh & Sazandegi. 74:189-194.

Dehghan Banadaky, M., A. Nikkhah, H. Amanlo, M. Danesh Mesgaran, and H. Mansori. 2007b. Rumen and post abomasal disappearance of amino acids and some nutrients of barley grain treated with sodium hydroxide, formaldehyde or urea in lactating cows. j. Pak. biological sci.10(9):1525-1531.

Fedorak, P. M., and D. E.Hurdy. 1983. A simple apparatus for measuring gas production by methanogenic cultures in serum bottles. Environ. Technol. Leu. 4:425-432.

Kiran, D., and T. Mutsvangwa. 2007. Effect of barley garin processing and dietary ruminally degradable protein on urea nitrogen recycling and nitrogen metabolism in growing lambs. J. Anim. Sci. 85:3391-3399.

McDougall, E. I. 1948. The composition and output of sheep in saliva. J. Bio Chem. 43:99-109.

McNiven, M. A., M. R. Weisbjerg, and T. Hvelpluno. 1995. In fluence of roasting or sodium hydroxid Treatment of barley on digestion in lactaing cows. J. Dairy Sci. 78:1106-1115.

Moghaddam, M., A. Taghizaeh, A. Nobakht, and A. Ahmadi. 2012. Nutritiv value of grape pomace and raisin vitis leaves using nylon bags and gas production techniques. Iranian j. Anim. Sci. Res. 3(4):435-443.

National Research Council (NRC). 2001. Nutrient Requrments of Sheep sthed. Natl, Acad. Sci., Washington, DC.

Navidshad, B., and A. Jafari Sayadi. 2000. Animal nutrition (1996 ed). Farhang jame publication, Tehran, Iran.

Ørskov, E. R., and P. McDonald. 1979. The estimation of protein digestibility in the rumen from incubation measurements weighed according to rate of passage, Cambridge. J. Agric. Sci. 92:499-503.

Parand, E., and A. Taghizadeh. 2009. Examination of Digestibility of Processed Barley Grain with Different Methods, Using Gas Production Technique with two Sources of Inocola. . Iranian J. Res. Anim. Sci. 20.4(2):1-13.

Robinson, P. H., and J. J. Kennelly. 1988. Infuence of ammonatiation of high moisture barley on its in situ rumen degradability and in fluence on rumen fermentation in dairy cows. can. J. Anim. Sci. 68:839-851.

Rowe, B., M. choct, and D. W. pethick. 1999. processing cereal grains for animal feeding. Aust. J. Agric. Res. 50:21-736.

SAS Inc. 2002. SAS User's Guide: Statistics. Statistical Analysis Systems Institute Inc., Cary, NC.

Sharifi, S. D., F. Shariatmadari, A. Yaghobfar, S. A. Mirhadi, and S. M. Nayeb Aghayee. 2005. Determination of the effects of the enzyme and Hull-less barley on Broiler Performance. J. Agric. Sci. Naur. Resour. 12(3):83-92.

Soufisiavash, R., and H. Janmohammadi. 2004. Animal nutrition. 5th ed. Amidi publication, Tehran, Iran.

Taghizadeh, A., and P. Farhoomand. 2007. Scientific feeding of dairy cow. jihad Daneshgahi of West Azerbaijan unit publication, Orumiyeh, Iran.

Taghizadeh, A., and Z. Nemati. 2008. Degradability characteristics of treated and untreated barley grain using in situ technique. American j. Anim. veter. Sci. 3(2):53-56.

Taghizadeh, A., M. Danesh Mesgaran, R. Valizadeh, and F. Eftekhar shahrodi. 2003. Evaluation model of rumen digestion of feedstuffs dry matter and crude protein using mobile nylon bags method. Iranian J. Agri. Sci. 13(1):101-113.

Taghizadeh, A. J. Shoja, H. Janmohamadi, and P. Yasan. 2001. The determination of degradability of some roughage and concentrate using in situ method in sheep. . Iranian J. Agri. Sci. 11(3):93-100.

Yang, W. Z., K. A. Beauchemin, and L.M. Rode. 2000. Effect of barley grain processing on extent of digestion and milk production of lactating cows. J. Dairy Sci. 83:554-568.

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Effect of Month of Production on Reproductive Traits of Barred Plymouth Rock Parent Stock in the Humid Tropics

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ABSTRACT

Seasonal effects on reproductive performance of birds had been overblown in previous study with little or no mentioning of influence of individual month that comprised the season. It is imperative to understand the impact of each month in a season or year on the reproductive performance of birds for effective planning and productive management decisions. The main objective of this study, therefore, was to determine the effect of individual month of production on reproductive traits of Barred Plymouth Rock layer breeder hens. Data used for this study were collected from farm records kept between 2002 and 2005. Analyzed data showed that month of production significantly affected all the reproductive traits, and that coldest months, that is, June-September favored high egg production, fertility and hatchability rates, while hottest months, that is, October-May impacted negatively on birds' performance. In addition, mortality rate was low during coldest months but highest in the hottest periods. The month of July recorded the highest (peak) production levels for all the traits, while the lowest was observed in November. This study revealed that prevailing environmental temperature in individual month determines the productivity level of breeder hens, and hence the profit margin of the stockholders. Therefore, concerted efforts should be made towards massive production of fertile eggs during the aforementioned cool and favorable months in order to meet the increasing demand for day-old chicks by poultry farmers. Possible intervention to increase broiler chickens production is that producers should plan their re-stocking in such a way that the birds will commence egg laying at the onset of cold months in order to maximize the positive influence of these months on performance of breeder birds.

Key words: Breeder, egg, fertility, hatchability, month, mortality

INTRODUCTION

The fluctuating environmental temperature typical of tropical weather is a source of concern to poultry farmers especially breeder farmers in this country because of its negative effect on birds' reproductive performance (Olawumi, 2011). The differences in genetic make-up coupled with the birds' inherent abilities to adjust and adapt to fluctuating weather conditions are the major factors enhancing the reproductive performance of any breed of chickens reared in any production environment (Aganga et al., 2003). The two most important

weather variables that have direct bearing on birds' activity are temperature and relative humidity. Research evidence had shown that throughout the whole range of practical environmental temperature, laying hens have physiological responses that affect their productive performance (Keener et al., 2006). Irshad et al. (2012) posited that it is not the degree of heat alone that causes distress to animals in the tropics but its combination with humidity and the duration of these conditions.

The relationship between ambient temperature and reproductive traits had been much studied in poultry. Olawumi and Ogunlade (2010) documented significant negative correlation between egg production and high temperature in layer breeders. In ostrich, Rozenboim et al. (2007) reported that egg production and weight decreased from naturally and experimentally high temperature. Previous researchers had documented that year, season and month of production have direct bearing on production and reproductive performance of animals (Chowdhury et al., 2004). The authors observed that individual month and season influenced the hatchability of duck eggs. In addition, significant effect of month on egg production (Ipek and Sahan, 2004; Wohr and Erhard, 2005; Elsayed, 2009), fertility and hatchability (Elsayed, 2009) had been reported in Ostrich. In chickens, Malau-Aduli et al. (2003) reported positive effects of age, year and season on egg production and mortality. In general, reproductive efficiency of birds depends on both genetic and non-genetic factors (Olawumi, 2011). Egg production, fertility and hatchability are important reproductive traits that determine the success of any chick production industry (Islam et al., 2002). These reproductive traits are lowly heritable, and are affected largely by environment such as nutrition, management practices, health status of the birds and farm hygiene (Olawumi, 2011).

Seasonal influence on egg production (Bawa et al. 2001; Olawumi, 2011), fertility and hatchability (Olawumi, 2007) and mortality (Bawa et al., 2001; Olawumi, 2007) had been documented in literature. Some of the negative and indirect effects of high temperature on birds' performance are reduced feed intake (Njoya and Piccard, 1994) and declining immune response (Dauda et al., 2006) of the birds to invading pathogens. Yolk formation in birds according to Elsayed (2009) is essentially a continuous process, while oviposition, ovulation and the formation of other egg components are discreet events within the cycle. The author posited that this later events are more susceptible to prevailing environmental temperature. Aside from the reasons already advanced, age of hens, egg size, shell quality, incubation temperature and relative humidity contribute to, and determine fertility and hatchability rates of a certain flock of breeder hens (Robinson et al., 1991; Olawumi, 2007).

The humid zone of Nigeria is characterized by high temperature and relative humidity. Information is limited in literature regarding the effect of individual month of production on egg production, fertility, hatchability and mortality rates of breeder hens in Nigeria. Most previous studies had focused mainly on effects of season which is broad and could be misleading. With this idea in view, present investigation therefore, was carried out to determine the effect of individual month of production on reproductive traits of Barred Plymouth Rock breeder birds reared for the production of commercial day-old chicks in Nigeria.

MATERIALS AND METHODS

Site of Study

Data on egg production, fertility, hatchability and mortality were collected from farm records of Ajanla Farms (CHI Ltd.), Ibadan covering a period from 2002 to 2005. Ibadan is situated at an elevation of 200m above sea level and lies about $7^{0}28^{1}$ and $3^{0}54^{1}$. The city enjoys two distinct seasonal periods namely, rain (May-October) and dry season (November-April). The minimum and maximum temperatures on average during the year are 20^{0}C and 30^{0}C, respectively.

Parent stock birds and their management

The exotic parent stocks studied were Barred Plymouth Rock (BPR) hens, and they were managed on the floor throughout the production period for natural mating at ratio 1male to 10females. The cocks were declawed to prevent injury during copulation, and were separated from the females during growing (rearing) period until about two weeks to the laying time. This method adopted was to prevent pre-cocious mating, and it afforded the cocks an opportunity to reach the prescribed weight and maturity. Management practices on the farm during the observed period were uniform. Cleanliness, bio-safety and bio-security measures were strictly adhered to, while vaccinations against viral diseases were administered as and when due. Fertility percent was determined on the candling (18^{th}) day, while hatchability percent was taken on the hatching (21^{st}) day.

Hatchery management

Temperatures and relative humidity during incubation were as follows:

a. Setting temperature- 99.75^0F (37.64^0C, 1-18days)

b. Setting humidity- 83%RH (1-18days)

c. Hatching temperature- 99^0F (37.22^0C, 19-21days)

d. Hatching humidity- 85%RH (19-21days)

Statistical Analysis

Data collected were subjected to analysis of variance (one way) using the General Linear Model (SAS, 2001), and the significant differences between means of months were determined by Duncan New Multiple Range Test of the computer package

The appropriate statistical model used for egg production, fertility, hatchability and mortality was:

$Y_{ij} = \mu + S_i + €_{ij}$

Y_{ij}= Observation of the j^{th} population, of the i^{th} month

μ= common mean

S_i= fixed effect of month (i=12)

$€_{ij}$= random errors assumed to be normally and independently distributed with zero mean and common variance.

RESULTS

Temperature and relative humidity readings for the period of study were presented in Table 1. Moderate and not too high temperature was recorded between June and October, while very high temperature was recorded during the remaining months. Relative humidity was low between November and March and higher in other months.

Table 2 demonstrates the effect of month of production on breeder hens' egg production. There was highly significant (P<0.001) effect of month of production on egg production in this flock. The month with peak egg production per hen per week was July (5.39±0.23eggs), while the lowest number of egg production per hen per week was recorded in November (3.76±0.20eggs).

Table 1. Average Temperature and relative humidity readings for the months

Months	Temperature (^{0}C)	Relative humidity (%)
January	26.65	59.05
February	28.91	64.04
March	29.4	67.06
April	27.55	75.56
May	27.32	75.85
June	25.71	79.45
July	24.87	81.05
August	24.48	83.23
September	25	79.83
October	25.86	78.31
November	27	71.35
December	26.73	60.28

Table 2. Least square means showing the effect of month on egg production

Factors	**No. (weeks)**	**LSQ**	**±SE**	**P-value**
January	18	4.60^{bc}	0.18	0.001
February	16	4.35^{c}	0.20	0.001
March	18	4.26^{cd}	0.18	0.001
April	13	5.02^{ab}	0.22	0.001
May	15	5.14^{ab}	0.20	0.001
June	12	5.15^{ab}	0.23	0.001
July	12	5.39^{a}	0.23	0.001
August	15	5.33^{a}	0.20	0.001
September	12	5.06^{ab}	0.23	0.001
October	12	4.86^{abc}	0.23	0.001
November	16	3.76^{d}	0.20	0.001
December	20	4.38^{c}	0.16	0.001

^{abcd} means along columns with different superscripts are significantly different

Similarly, month wise fertility of BPR breeder hens was presented in Table 3. There was highly significant (P<0.001) effect of month of production on fertility rate of breeder layers. The highest fertility rate was recorded in July, August and September, while the lowest was in December, January, February, March and April.

In this study (Table 4), there was highly significant (P<0.001) effect of month of production on hatchability of BPR breeder layers. Highest hatchability rate was recorded in July, August and September, while the lowest was recorded for December, February and March.

In the current study (Table 5), month of production has significant (P<0.001) effect on mortality rate of BPR breeder birds. Highest mortality rate was found in January and February, while the lowest rate was recorded in June, July, August and September.

Table 3. Least square means showing the effect of month on fertility

Factors	No. (weeks)	LSQ	±SE	P-value
January	16	78.74^{d}	1.22	0.001
February	14	75.27^{d}	1.34	0.001
March	18	74.92^{d}	1.22	0.001
April	13	79.12^{d}	1.40	0.001
May	15	83.85^{c}	1.34	0.001
June	12	87.53^{abc}	1.46	0.001
July	12	91.06^{a}	1.46	0.001
August	15	89.99^{a}	1.34	0.001
September	12	90.03^{a}	1.46	0.001
October	12	89.12^{ab}	1.46	0.001
November	16	$85.67b^{c}$	1.46	0.001
December	20	76.01^{d}	1.60	0.001

^{abcd} means along columns with different superscripts are significantly different

Table 4. Least square means showing the effect of month on hatchability

Factor	No. (weeks)	LSQ	±SE	P-value
January	14	66.23^{e}	1.63	0.001
February	14	60.83^{f}	1.63	0.001
March	16	60.9^{f}	1.43	0.001
April	13	71.46^{d}	1.69	0.001
May	14	75.44^{cd}	1.63	0.001
June	12	79.75^{abc}	1.76	0.001
July	12	83.43^{a}	1.76	0.001
August	14	80.81^{ab}	1.63	0.001
September	12	80.51^{ab}	1.76	0.001
October	12	76.93^{bc}	1.76	0.001
November	12	71.61^{d}	1.76	0.001
December	10	60.86^{f}	1.93	0.001

^{abcdef} means along columns with different superscripts are significantly different

DISCUSSION

In general, many factors influence the number of eggs produced in each month by breeder hens in this country. These include breed, weather, nutrition, obesity, health and physiological factors (Aganga *et al.*, 2003; Olawumi *et al*., 2008). The obtained results on egg production were similar to those reported in previous studies in ducks (Ipek and Saha, 2004; Wohr and Erhard, 2005; Elsayed, 2009) and in commercial layers (Malau-Aduli *et al*., 2003). The month (July) with the highest egg numbers happened to be the month with lowest temperature range (24.48^{0}C), while the month (November) with the lowest egg production coincided with the month with highest temperature range (27^{0}C). This result confirmed previous findings that high temperature impacted

negatively on the laying performance of hens (Olawumi and Ogunlade, 2010). The result also corroborates what was reported by Rozenboim *et al.* (2007) in Ostrich. The decrease in egg production in this study during hottest months was probably due to decrease in feed consumption by the hens as a result of heat load, thereby reducing the amount of nutrients available for production. The small amount of feed consumed was used for body maintenance. In this study, the most favourable months for egg production were April, May, June, July, August and September, while the unfavourable months, that is, months with lowest egg production were October, November, December, January, February and March which incidentally happened to be the hottest periods in this country (Olawumi, 2007).

Table 5. Least square means showing the effect of month on mortality

Factor	No. (weeks)	LSQ	±SE	P-value
January	16	20^{ab}	2.07	0.001
February	14	23.43^{a}	2.24	0.001
March	16	14.81^{bc}	2.07	0.001
April	13	8.77^{cd}	2.32	0.001
May	14	9.21^{cd}	2.24	0.001
June	12	5.75^{d}	2.41	0.001
July	12	6.25^{d}	2.41	0.001
August	14	5.71^{d}	2.23	0.001
September	12	7.58^{d}	2.42	0.001
October	12	9.0^{cd}	2.42	0.001
November	11	6.73^{d}	2.16	0.001
December	18	10.78^{cd}	1.86	0.001

^{abcd} means along columns with different superscripts are significantly different

In a recent study which was comparable with this findings, Melesse *et al.* (2013) reported that hen-housed egg production decreased significantly in all heat-stressed genotypes compared with those at thermo-neutral environment. It is important therefore, that concerted efforts be made towards massive production of fertile eggs during the aforementioned favourable months in order to meet the increasing demand for day-old chicks by poultry farmers. However, our data showed that egg number per hen per month significantly ($P<0.05$) increased with the advancement of laying month, that is, each hen laid more eggs as the month progressed from commencement of laying to the end of production cycle.

It was observed that the months with highest fertility rate fell within the period with lowest temperature. Also, the months with lowest fertility rate coincided with hottest periods of the year in this country. The result of month's effect on fertility indicates that changes in weather conditions within a year significantly affected this trait, and that those months with lowest temperature range supported high fertility rate than hottest months. In agreement with this result, Jayarayan (1992) and Das and Ali (1999) observed lower fertility rate in summer compared to winter period. It is believed that fertility rate of breeder hens is a very important measure of their reproductive efficiency. According to Gowe *et al.* (1993), egg fertility is generally considered a trait of both parents, and their ability to interact and produce a viable zygote. The grave consequence of high temperature on fertility rate could be traced to reduced feed intake (quality and quantity), reduced mating activity and sperm production (quality and quantity). During hot weather conditions, birds eat less and are usually found clustering around drinkers than feeders in order to cool down their body temperature. In addition, males are also found to be heat-stressed, and unable to mate or sexually arouse the females. The combined effects of these factors result to poor or unimpressive fertility percentage, and this accounts for poor productivity of laying birds between months, within a year (Olawumi, 2007).

The hatchability result followed the pattern reported for fertility rate. The two traits are related, and are affected by both genetic and environmental factors. Months with highest hatchability rate happened to be the cold periods of the year, while the months with lowest production were the hottest periods. And this confirmed previous observations that variations in weather conditions cause observed differences in hatchability rate within a year (Farooq *et al*., 2003; Chowdhurry *et al*., 2004; Olawumi, 2007). According to Sastry *et al*. (1996), temperature was the most critical factor for incubation, and the temperature affected both quantity and quality of hatch. The researchers posited that high incubation temperature results in embryonic mortality, particularly when there was high temperature during the last part of incubation period. The result of this study shows that the best time for hatching in this country falls within July-September, while appreciable and reasonable production could also be obtained in June and October.

With regard to mortality rate, months with highest deaths were the hottest part of the year in this country, while the coldest months (June-September) had the lowest mortality rate. The obtained result confirmed previous findings which found negative effect of high temperature on mortality rate (Bawa *et al*., 2001; Malau-Aduli *et al*., 2003). High mortality rate reported during the hottest periods of the year could be as a result of heat stress which lessened the immune response of the birds to weather fluctuations, thereby predisposing them to heat stroke or pathogenic organisms and eventual death.

CONCLUSIONS

The obtained results showed that month of production significantly affected the overall performance of BPR layer breeders in this country. The most productive months with greater production are June–September when the temperature appeared very low and conducive for increased reproductive activity of breeder hens. For increased production of day-old chicks, replacement pullets should be purchased in December or January so that the birds will commence egg laying in June or July when the weather appears favorable and conducive for good production.

REFERENCE

Aganga, A.A., A.O. Aganga, and U.J. Omphile. 2003. Ostrich feeding and nutrition. Asian Network for Scientific Information. *Pakistan J. Nutr*. 2(2): 60−67.

Bawa, G.S., M.K. Joel, and A.E.O. Malau-Aduli. 2001. Effects of age, season and year on egg production and layer bird mortality. In: Proceedings of the Nigerian Society for Animal Production: Strategies for poverty alleviation. Ahmadu Bello University, Zaria, Nigeria. pp: 36-39. ISBN 0331-2064

Chowdhury, M.M.I., A. Ashraf, S.P. Mondal, N.M.A. Mondol, and M.M. Hasan. 2004. Effect of season on the hatchability of duck eggs. *Int. J. Poul. Sci*. 3(6): 419−421.

Das, G.B., and M.L. Ali. 1999. Raising layer chicken over fish ponds in integrated poultry-fish farming system in rural areas. *Bangladesh J. Anim. Sci*. 28: 121−128.

Dauda, T.O, A.O Adetayo, and A.K. Tiamiyu. 2006. Effects of weather on egg characteristic of Isa Brown layers in Ibadan. *Nigerian Anim. Sci. J.* 77: 117−121.

Elsayed, M.A. 2009. Effect of month of production on external and internal ostrich egg quality, fertility and hatchability. *Egypt Poul. Sci.* 29 (11): 547−564.

Farooq, M., K. Javed, F.R Durrani, K. Irfanullah, and N. Chand. 2003. Hatching performance of backyard hens in Peshawar, *Pakistan. Liv. Res. Rural Dev*. 15.

Gowe, R.S., R.W. Fairful, I. Mcmillan, and G.S. Schmidt. 1993. A strategy for maintaining high fertility and hatchability in a multiple-trait egg stock selection program. *Poult. Sci*. 72: 1433−1448.

Ipek, A., and U. Sahan. 2004. Effect of breeder age and breeding season on egg production and incubation in farmed ostriches. *Br. Poult. Sci*. 45(5): 643−647.

Irshad, A., G. Kandeepan, S. Kumar, K.A. Ashish, M.R. Vishnuraj, and V. Shukla. 2012. Factors influencing carcass composition of livestock: A review. *J. Anim. Prod. Adv*. 3(5): 177−186.

Islam, M.S., M.A.R. Howlider, F. Kabir and J. Alam. 2002. Comparative assessment of fertility and hatchability of Barred Plymouth Rock, white leghorn, Rhode Island Red and White Rock hen. *Inter. J. Poult. Sci*. 1(4): 85-90.

Jayarajan, S. 1992. Seasonal variation in fertility and hatchability of chicken eggs. *Indian J. Poult. Sci*. 27: 36−39.

Keener, K.M., McAvoy, K.C., Foegeding, J.B., P.A. Curtid, Anderson, J.A. Osborne, and D.J. Bush, 2006. Effect of testing temperature on internal egg quality measurements. *Poult. Sci*. 85: 550−555.

Malau-Aduli, A.E.D., G.S. Bawa, and K. Joel. 2003. Factors affecting egg production and layer bird mortality in private poultry farms in the sub-humid zone of Nigeria. *Anim. Sci. J*. 74 (3): 239−242.

Melesse, A., S. Maak, H. Pingel, and G.V. Lengerchen. 2013. Assessing the thermo-tolerance potentials of five commercial layer chicken genotypes under long-term heat stress environment as measured by their performance traits. *J. Anim. Prod. Adv*. 3(8): 254−264.

Njoya, J., and M. Piccard, 1994. Climatic adaptation of laying hens. Tropical Anim. Health and Prod. 26: 180−186.

Olawumi, S.O. 2007. Genotype x season interaction effects on reproductive performance of two breeds of layer breeders in humid zone in Nigeria. *Appl. Tropical Agric.* 12(2): 78−82.

Olawumi, S.O., S.O. Oseni, and J.O. Akinokun. 2008. Comparative assessment of fertility, hatchability and survivability of Bovan Nera and Isa Brown Breeder cocks under Humid Tropical conditions of Nigeria. *Ife. J. Agric*. 23(1): 134−146.

Olawumi, S.O., and J.T. Ogunlade. 2010. Phenotypic Correlations between Reproductive Traits in Layer Breeders and Weather Variables in Humid Zone of Nigeria. *Int. J. Anim. Sci*. 2(1): 113−120.

Olawumi, S.O. 2011. Influence of Breed and Season on Reproductive Traits of Three Strains of Commercial Layers in the Derived Savannah zone of Nigeria. *Int. J. Agric. and Food Sci*. 2: 97−104.

Rozenboim, I., E.Tako, O. Gol-Garber, J.A. Proudman, and Z. Uni. 2007. The effect of heat stress on ovarian function of laying hens. *Poult. Sci*. 86 (8): 1760−1765.

Sastry, N.S.R., C.K. Thomas, and R.A. Singh. 1996. Livestock Production Management, 3rd edition, Kalyani Publishers, New Delhi-Ludhiana. p: 559.

Statistical Analysis System (SAS, 2001). SAS Users Guide. Statistics, 8th edition, SAS Institute Cary, NC, USA.

Wohr, A., and M. Erhard. 2005. Ostrich farming in Germany- an Animal Welfare issue. 3rd International Ratite Science Symposium and X11 World Ostrich Congress. Madrid 14-16 October, 2005. Ed: Carbajio, E., Madrid, Spain. pp: 145−156.

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Effect of Treated Cowpea Seeds on Broiler Chicken

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ABSTRACT

Poultry investment became one of the most important farming activities in Sudan. The aim of this experiment was to investigate the effect of dietary treated cowpea seeds on the performance of broiler chicks. Four rations were formulated that contained 0 cowpea for the control diet (A) and 15% cowpea for the three tested rations. The test diets contained cowpea soaked with no enzyme addition (B), soaked with enzyme addition (C) or roasted (D). One hundred and sixty unsexed chicks were used in a complete randomized design. The results indicated that roasted seeds contained low crude protein, ether extract, crude fiber, ash and metabolizable energy than soaked seeds, while it contained high nitrogen free extract than soaked seeds. Treatment differences had no significant effects on weekly and overall feed intake. There were only significant differences on weight gain in third and fourth weeks. Chicken fed diet C gained the highest weight in week (3267.6) while chicken fed diet C and D gained the highest weight in week 4 (350.6 and 354.1g). Overall weight gain in the four treatments was not significantly different (1598.2 -1737.2 g). Treatments significantly affected feed conversion ratio in week 3 and 4 only diet C and D showed best results in the two weeks than the control and D diet. Over all feed conversion ratio was significantly better for chicks fed cow pea incorporated diets than those fed the control diet (2.40 vs. 2.60 kg feed / kg weight).

Key words: Cowpea Seed, Broiler, Chicken

INTRODUCTION

Many researches were conducted to evaluate the nutritive value of local plant protein sources aiming to reduce the cost of imported concentrates (Algam *et al*., 2012).Cowpea and black common bean are well adapted, cheap legumes that can be used in animal feeds in tropical countries (FAO, 1999).Cowpea is used by human as a nutritious component (Berssani, 1985).Amino acids are balanced in cowpea with the exception of methionine which is deficient

(Carnovale *et al*., 1990) but the amount of lysine is considered high (Akanji, 2002).The limiting aspect in the use of legumes in animal feed is presence of antinutritional factors (Miega, 1987; Wiryawan and Dingle, 1999 and Teguia and Beynen., 2005;).Grain legumes like cow pea contain many anti-nutritional factors as chymotrypsin inhibitors, amylase inhibitors, tannins and phytic acids (Kratzer *et al*., 1968, Singh,1988; Duc, 1996; Amaefuil *et al*., 2005and Teguia and Beynen, 2005).Chicks performance was remarkably reduced when fed raw legumes (Wiryawan and Dinlge, 1999, Bressani, 2002, Teguia *et al*., 2003).Cowpea content of anti-nutritional factors is reduced by roasting (Vaishale *et al*., 1998) and in the rural areas cocking is a conventional method of removing legume toxins (Defang *et al*., 2008)The nutritive value of legumes is increased by cooking and this is due to the decrease in the activity of trypsin inhibitors or the decrease in other toxins (Duke 1981).The purpose of the study is to investigate the effect of cow pea incorporation in broiler feeds.

MATERIALS AND METHODS

Experimental site

This experiment was conducted in the premises of poultry research unit in faculty of Animal Production at Khartoum North. During the experiment the maximum and minimum environmental temperatures were 34.3- 22.7º C and 24.7- 11.3º C while the relative humidity of 20 to 40%.

Housing and Management

The study was carried out in an open side poultry house. The house (5.5×4m) was portioned internally into 15 pens (1×1m) with suitable working place allowance. the house was cleaned and disinfected before the study and saw dust was laid as beddings to each pen. Each pen was provided with manual feeder and drinker. The light was maintained for 24 hours natural and artificially.

Experimental Diets

Vignaunguiculata seeds was purchased from Khartoum state (at a price of 2 SP/Kg 0.50 $) and has been decorticated, then divided into three parts one for roasting, the other for soaking, to one third (soaked) multi enzymes were added (Endo-B-1,4xylanase, Endo-pentosanase, protease, and amylase). Four experimental diets were formulated with 0.00% Vignaunguiculata level in the control and 15 % Vignaunguiculata in the other three diets. These diets were formulated to meet the requirements for broilers as recommended by NRC (1994). Seeds were treated by soaking over night (12 hours) then boiled for 10 minutes or roasted in electric oven at 100º C for 15 minutes.

Experimental birds

One hundred and sixty unsexed, one day old broiler chicks (Ross 508) were obtained from Bageir commercial company after being vaccinated against marek's disease. The chicks were then weighed and allotted randomly into pens of eight chicks as replicate. Each treatment consists of 40 chicks that were replicated 5 times in complete randomized design.

Data collection

Parameters studied were body weight (BW), feed intake (FI), and weight gain (WG) plus feed conversion ratio (FCR) that was calculated for the individual replicates of each dietary treatment. Mortality was recorded when it occurred. The experiment extended for six weeks and at the end of the period 25 chicks were randomly selected from each dietary treatment (5 birds/replicate), leg banded, weighed individually and slaughtered. Hot carcass weight was recorded and dressing out percentage was determined by expressing hot carcass weight to live weight.

Chemical methods

Samples of Vignaunguiculata seeds dry and wet treated were approximately analyzed on dry matter basis for chemical components according to AOAC (1982).

Experimental design and statistical analysis

A complete randomized design was used. The data generated from the experiment were statistically analyzed using SPSS software. Duncan's multiple range tests were used to analyzed the differences between treatment means (Gomez and Gomez, 1984).

Table 1.composition of experimental diets (as feed %)

Ingredient	Diet			
	A	B	C	D
V. unguiculata%	0.00	15.00	15.00	15.00
Sorghum	64.60	54.80	54.80	54.80
Groundnut cake	17.00	15.00	15.00	15.00
Sesame cake	11.00	5.00	5.00	5.00
Super Concentrate*	5.00	5.00	5.00	5.00
Dicalcium phosphate	0.25	1.73	1.73	1.73
Nacl	0.25	0.25	0.25	0.25
Vitamin Premix**	0.25	0.25	0.25	0.25
Lysine	0.25	-	-	-
Vegetable Oil	0.03	3.00	3.00	3.00
Wheat bran	0.10	-	-	-
Total	100	100	100	100

(A): control diet, (B):15% soaking boiling cowpea seeds+ enzymes, (C): 15% soaking boiling cowpea seeds, (D): 15% roasting cowpea seeds

*super concentrate (%) CP 40, lysine 10, methionine 3,methionie+cystine 3.3, ca 10, available phosphate 6.40, CF1.44, C fat 3.99, ME 1750 kcal/kg, crude minerals 39.30

**Vitamin composition per kg of diet: vit A: 200.000 IU,vit D3: 70.000 IU, vit B1:50mg, B2:120mg, B12:180 mg, K3:30mg, niacin:440 mg, zincL: 1.6 mg, copper :450 mg, iodine 550 mg, selenium : 8 mg, cobalt: 9 mg, iron : 580 mg, molyden 20 mg

Table 2.calculated and determined chemical analysis of experimental diets

Parameters	Diets			
	A	B	C	D
Crude protein	22.45	22.54	22.52	22.50
Metabolizable energy (Kcal/ kg)	3100.29	3100.25	3002.20	3003.40
Lysine%	1.11	1.19	1.17	1.16
Methionine%	0.45	0.45	0.43	0.47
Ca%	1.24	1.10	1.14	0.14
Total P%	0.64	0.62	0.63	0.68
Determined analysis				
Dry mater	96.86	96.40	95.30	95.47
Ash	5.72	5.90	5.43	5.14
Ether Extract	5.14	5.03	5.11	4.57
Crude protein	22.45	22.54	22.52	22.50
Crude fiber	3.00	3.20	4.14	4.20

(A); control diet, (B): 15% soaking boiling cowpea seeds+ enzymes, (C): 15% soaking boiling cow pea seeds, (D): 15% roasting cowpea.

RESULTS AND DISCUSSION

The results of chemical composition of treated cowpea were shown in table 3. The results indicated that roasted seeds contained low crude protein, ether extract, crude fiber, ash and ME than soaked seeds, while it contained high nitrogen free extract than soaked seeds. This may be due to heat effects on proteins. On the other hand Azizah and Zainon (1997), and Mahadevamma and Tharanathan (2004) reported that roasting of legumes reduced insoluble dietary fibers and total fibers but increased soluble fibers. This is in agreement with Defang *et al*., (2008).Cowpea seeds in this study contained 96% dry mater, 2.2-2.9 ether extract, 24.2- 21.4 crude protein, 3.4 – 3.0 crude fiber, 4.90 ash, and 14417- 22714 ME (kal/ kg). Eljack *et al*., (2009) reported that cow pea contain 93.3, 20.91, 2.0, 3.4, 4.1, 62.89, 13.4 MJ/kg dry mater, crude protein, ether extract, crude fiber, ash, nitrogen free extract, metabolizable energy respectively.

Table 3.Proximate analysis (%) of treated cowpea (V. unguiculata) seeds

compound	(soaking+ boiling)	(Roasting)
Dry matter	96.40	96.90
Ether extract	2.90	2.20
Crude protein	24.20	21.40
Crude fiber	3.40	3.00
Ash	4.87	4.90
Nitrogen Free extract	61.00	65.40
Metabolizable energy (kcal/ kg)	22714.00	14417.00

Data of table 4 show that treatment differences had no significant (p> 0.05) effects on weekly and overall feed intake. This may be due to the fact estates that treating of cow pea seeds by soaking or roasting lead to enhancement of feed palatability by reducing its content of anti-nutritional factors this idea is in agreement with Gahlawat and Sehgal (1992) who stated that roasting reduces anti nutritional factors in cereals and legumes hence improving their digestibility. As energy content of the four rations formulated was similar, feed intake was expected to be close in the four chick groups (Scott *et al*., 1982). Feed intake in week 1 to week 7 was in the range of 190.1- 216.8, 764.8- 977.4, 407.5- 411.3, 701.0- 742.8, 579.5- 622.5, 1200.1-1263.3 g respectively, these values were lower than those estimated by Musa *et al*., (2012). Total feed intake was 4104.40-4178.70g and this estimate is higher than the range 3278.75- 3325.49 reported by Abdel Atti *et al*., (2011), the range 3144-3660 reported by Eljack *et al* (2009) and the range 3236-3366 reported by Chakam*et al*., (2010).

Table 4. Effect of feeding cowpea (V. unguiculata) (g/bird) on broiler weekly feed intake (g/bird/ week)

Age (weeks)	Diets				
	A	B	C	D	S E
1	208.2	216.8	190.1	192.1	19.0
2	977.40	964.6	930.2	764.8	84.5
3	407.5	409.7	410.7	411.3	19.2
4	742.8	701.0	727.7	731.4	25.7
5	579.4	622.5	622.5	606.7	26.1
6	1263.3	1220.1	1223.7	1200.1	42.2

SE: standard error, (A); control diet, (B): 15% soaking boiling cowpea seeds+ enzymes, (C): 15% soaking boiling cow pea seeds, (D): 15% roasting cowpea
Means with the different superscript are significantly different (p> 0.05)

Results in table 3 shows that treatments had no significant effects (p> 0.05) on weekly feed intake (table 4).

Data in table 5.shows the effects of cowpea on weekly weight gain .There was only significant differences (p< 0.05) on weight gain in third and fourth weeks. Chicks fed diet C gained the highest weight in week (3267.6) while chicks fed diet C and D gained the highest weight in week 4 (350.6 and 354.1g). Overall weight gain in the four treatments was not significantly (p> 0.05) different (1598.2- 1737.2 g); this disagreed with Defang *et al*., (2003). Estimated range was higher than that reported by Chakam*et al*., (2010)and Kana *et al*., (2012) who reported 1287.85- 1536.13-g and 1094.93- 1362.49 g. Eljack *et al*., (2009) estimated a higher range for overall weight gain (1683. 29- 2152.02g).

Table 5. Effect of feeding cowpea (V. unguiculata) (g/bird) on broiler weekly weight gain (g/bird/ week)

Age (weeks)	Diets				
	A	B	C	D	S E
1	69.3 [a]	65.0 [a]	7.00 [a]	60.2 [a]	3.80
2	152.1 [a]	167.2 [a]	166.0 [a]	154.8 [a]	6.90
3	228.0 [a]	256.0[ab]	267.6 [b]	233.4 [a]	8.90
4	292.5 [a]	350.6 [b]	354.1 [b]	303.6 [a]	14.9
5	379.2 [a]	369.3 [a]	395.6 [a]	402.3 [a]	20.8
6	476.8 [a]	529.1 [a]	426.3 [a]	506.3 [a]	34.00

SE: standard error, (A); control diet, (B): 15% soaking boiling cowpea seeds+ enzymes, (C): 15% soaking boiling cow pea seeds, (D): 15% roasting cowpea
Means with the different superscript are significantly different (p> 0.05)

Effects of treatment on feed conversion ratio were shown in table 6. Treatments significantly (p>0.05) affected feed conversion ratio in week 3 and 4 only diet C and D showed best results in the two periods than the control and D diet. Over all feed conversion ratio (Table 7) was significantly (p>0.05) better for chicks fed cow pea incorporated diets than those fed the control diet (2.40 Vs 2.60 kg feed / kg weight). These results were similar to Abdelgani *et al*., (2013) for the control diet and higher for treated cow pea contained diets. Estimated results were lower than those estimated by Kana *et al*., (2012) who found a range of 2.74-3.18but higher than Eljack *et al*., (2010).

Table 6. Effect of feeding V. unguiculata (g/bird) on broiler weekly feed conversion ratio (g/bird/ week)

Age (weeks)	Diets				
	A	B	C	D	SE
1	2.10	3.40 [a]	2.80 [a]	3.20	0.30
2	6.50 [a]	5.80 [a]	5.60 [a]	4.90 [a]	0.60
3	1.80 [a]	1.60[ab]	1.50 [b]	1.80 [a]	0.10
4	2.60 [a]	2.00 [b]	2.10[ab]	2.40 [a]	0.20
5	1.50 [a]	1.70 [a]	1.60 [a]	1.50 [a]	0.10
6	2.70 [a]	2.30 [a]	2.90 [a]	2.50 [a]	0.20

SE: standard error, (A); control diet, (B): 15% soaking boiling cowpea seeds+ enzymes, (C): 15% soaking boiling cow pea seeds, (D): 15% roasting cowpea

Table 7. Effect of treated cowpea (V. unguiculata) seeds on overall performance of broiler chicks (0-45days)

Parameters	Diets				
	A	B	C	D	S E
Total feed intake (g)	4178.70 [a]	4143.10 [a]	4104.90 [a]	4104.40 [a]	84.47
Total weight Gain (g)	1598.20 [a]	1737.20 [a]	1680.10 [a]	1660.70 [a]	39.30
Total Feed conversion ratio	2.60[a]	2.40 [b]	2.40 [b]	2.40 [b]	0.10

SE: standard error, (A); control diet, (B): 15% soaking boiling cowpea seeds+ enzymes, (C): 15% soaking boiling cow pea seeds, (D): 15% roasting cowpea
Means with the different superscript are significantly different (p> 0.05)

Live body weight, carcass weight and dressing percentage were not affected (p> 0.05) by dietary differences this may be related to similar feed intake and diets that were all isocaloricisonitrogrnous. Live weight is higher than that estimated by Abdelgani *et al*., (2013).

Table 8. average live weight, hot carcass weight and dressing percentage of broiler chicks fed diets containing treated cowpea (V. unguiculata) (g/bird)

Parameters	Diets				
	A	B	C	D	S E
Live body weight (g)	1826.70	1859.0	1844.9	1828.2	24.9
Hot carcass weight (g)	1218.20	1233.20	1243.4	1238.3	35.1
Dressing %	66.0	66.0	67.0	67.0	1.0

SE: standard error, (A); control diet, (B): 15% soaking boiling cowpea seeds+ enzymes, (C): 15% soaking boiling cow pea seeds, (D): 15% roasting cowpea
Means with the different superscript are significantly different (p>0.05).

CONCLUSION

Inclusion of treated cowpea seeds in broiler diets resulted in similar performance as in the control diet. The level of anti-nutritional factors in cow pea can be reduced by roasting or soaking.

REFERENCE

A.O.A.C. 1982. Official methods of Analysis. Association of Official Agriculture Chemist, Washington D. C.

Abdelatti, K. A., B. M. Dousa, and A. M. FadelElseed. 2011. Effect of Substitution the imported concentrate by Plant concentrate on The Performance and Blood chemistry of broiler Chicks. *Pakistan Journal of Nutrition*. 10 (11):1000-1003.

Abdelgani, A. A., K. A. Abdelatti, K .M. Elamin, K. Y .Dafalla, H .E. E. Malik, and B. M. Dousa. 2013. Effects of Dietary Cowpea (Vignaunguiculata) seeds on the performance of broiler chicks, *Wayamba Journal of Animal Science*. 5:678-684.

Akanji, A. M. 2002. Enhancement the utilization of some tropical legume seeds in diet of exotic meat type and egg type chicken. Ph.D. thesis, university of Ibadan.

Algam, T. A., K. A. Abdelatti, B. M. Dousa, S. M. Elawad, and A. M. F. Elseed. 2012 .Effect of dietary raw chick pea (Cicerarietinum L.) seeds on broiler Performance and blood constituents. *International journal of Poultry Science*. 11:294-297.

Amaefuil, K. U., and F. M. Osuagwu. 2005. Performance pf pullet chicks fed graded levels of raw bambarra groundnut (Vigna subterranean (L.) Verdc) offal diets as replacement of soybean meal and maize. *Live stock Research for Rural Development*. 17.

Azizah, A. H, and H. Zainon. 1997. Effect of processing on dietary fiber contents of selected legumes and cereals. *Malaysian Journal of Nutrition*. 3 (1): 131-136.

Bressani, R. 2002. Factors Influencing nutritive value in food grain legumes, Mucuna in comparison to other grain legumes. In: Mucuna as a food of feed: current use and the way forward. Edited by M. Flores., M. Eilitta., R. Myhrman., L. Crew and R. Carsky. Workshop held April 26-29, 2000 in Teguiligalapa, Honduras. CIDICCO.

Carnovale, E., L. Mareltta, E. Marconi, and E. Boris .1990. Nutritional and hydration properties in cowpea. Cowpea genetic. Resources: 113. contribution in cowpea exploration, Evolution and Research from Italy and the International Institute of tropical Agriculture.

Chakam, V.P., A. Teguia, and J. Tchoumboue. 2010. Performance of Finisher Broiler Chickens As affected by Different Proportions of Cooked Cowpea (Vignaunguiculata) in the Grower Finisher diet. *African Journal of Food Agriculture Nutrition and Development*. 10(4):2427-2438.

Defang, H. F, T. A. Awash- Ndukum. J. Kenfack, A. Ngoulaand, and F. Metuge. 2008. Performance and carcass characteristicsof broilers fed boiled cowpea (VignaunguiculataL.Walp) and or black common bea (pPhaseolus vulgaris) meal diets. *African Journal of Biotechnology*. 7(9):1351-1356.

Defang, H. F., A.Teguia, J. Awash-Ndukum, A .Kenfack, F. Ngoula, and F. Metuge. 2008.Performance and carcass characteristics of broilers fedboiled cowpea (Vignaunguiculata L Walp) and or blackcommon bean (Phaseolus vulgaris) meal diets. *African Journal of Biotechnology*. 7 (9):1351-1356.

Duc, G. 1996. Valeuralimentaireet usage des graines delégumineuses. INRA. Station de génétiqueet _amelioration de plantsdomaine Epoisses. *Sauve qui peut* ! n°8.

Duke, J. A. 1981. The gen revolution. In: office of technology assessment background papers for innovative biological technologies for lesser development countries. USGPO, *Washington paper* 1:89-150.

Eljack,B. H., I. M. T. Fadlalla,and M. T. Ibrahim. 2009. The effect of feeding cow pea (Vignaungialata) on broiler chicks performance and some carcass quality measurements.*Assiut vet. Med. Journal*. 56(124):173-180.

F.A.O. 1999.Food and Agricultural Organization (FAO) .Production year book.FAO statistics series 156. FAO Rome Italy.

Gahlawat, P., and S. Sehgal. 1992. Phytic acid, saponins and polyphenols in weaning foods prepared from oven heated green gram and cereals. *Cereal Chemistry*. 69(4): 463–464.

Gomez, K. A., and A. A. Gomez. 1984. Statistical procedures for agricultural research. John Wiley and sons, Inc. London, UK (2ed) .13-175.

Kana, J. R., A. Teguia, and A. Fomekong. 2012. Effect of Substituting Soybean Meal with Cowpea (Vignaunguiculata) supplemented with natural Plants charcoals in broiler diet on Growth performance and carcass characteristics. *Iranian Journal of Allied Animal science*. 2(4):377-381.

kratzer, R. S.,S. C. Mitjavila ,G. Lacome, and R. Devache. 1968. Effect of Tanic acid and oxidized tanic acid on the growth of rat and chick. *journal of nutrition*. 106:212-232.

Mahadevamma, S., and R. N. Tharanathan. 2004. Processing of Legumes: Resistant Starch and Dietary Fiber Contents. *Journal of Food Quality*. 27(4): 289-303.

Miega, J. 1987. Les facteurstoxiques des graines de légumineusesUniversité de Geneve, Suisse, p. 34.

Musa , M. M. , K. M. Elamin, K .A. Abdelatti, H. A. Elagib,and A. M. Musa. 2012. Effect of Dietary Levels of Decoticated Cow Pea (Vignaunguiculata) supplemented with Molases on Broiler Chicks Performance and Carcass Traits. *Pakistan Journal of Biological Science*. 15(21):1010-1018.

N.R.C. 1994. Nutrient Requirements of Poultry. Ninth Revised Ed. National Academy Press, Washington, D.C. PP. 19-26.

Scott , M. L., Nesheim, M. C.,and Young, R. S. 1982. Nutrition of the Chicken scatt and associate. Ithaca. New York.

Singh, U. 1988. Anti nutritional Factor of chick pea and pigeon pea and their removal by processing. *Plant Food for Human Nutrition*. 38:251-261.

Teguia, A., A. C. Beynen. 2005. Alternative feedstuffs for broilers in Cameroon.*Livestock Res Rural Dev*. 17(3).http:/www.Cipav.Org.Co/Irrd/Irrd17/3/tegu 173.htm.

Teguia, A., I. B. Japou, and E. C. Kamsu. 2003. Response of broiler chickens to Vignaunguiculata(L) Walp (cowpea), and Phaleolus vulgaris (blackbean) and Voandzeiacubterranean (Bambara groundnut) as feedingredients in replacement of meat meal. *Anim. Feed Sci*. 11: 127-133.

Vaishale, A. j., K. Sadhana, P. Seema, C. Kishore, V. Shashi, S. Agte, S. Joshi, K. Khot, Paknikarand,and S. Chiplonkar.1998. Effect of processing on phytate degradation and mineral solubility impulses. *Journal of Food Science and Technology*. Mysore. 35:330-332.

Wiryawan, K. G.,and Dingle, J. G. 1999.Recent research on improving the quality of grain legumes for chicken growth. *Anim. Feed Sci. Technol*. 76: 185-193.

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Efficacy of Herbal Fly Repellent Product (Keetguard Liquid) to Control Musca Domestica Population in Poultry Egg Layer Farm

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ABSTRACT

The housefly Musca domestica L. is recognized as a public health pest causing a serious threat to human and livestock by vectoring many infectious diseases. Chemical control method commonly used against this pest, though effective, has some major disadvantages, such as development of insect resistance and bioaccumulation. Therefore the efficacy of a herbal fly repellent Keetguard Liquid (supplied by M/S Ayurvet Limited, Baddi, H.P., India) was evaluated in vivo on the layer farm as well as in vitro. For in vivo trial the keetguard was used at the concentrations of 1:20 (Group-I recommended for application on body) in one shed and 1:40 (Group-II, recommended for application in premises) in another shed. Another shed kept as control (Group-III) was sprayed with plain water. The herbal fly repellant product was assessed for fly repellent and larvicidal effect and for its efficacy to minimize the count of larvae. In both the treatment groups, the fly repellency recorded during first 2-6 hrs was satisfactory. However, the fly population observed at the end of the week was encouraging in group-I due to its larvicidal effect. The probit analysis to draw Effective Concentration 50 (EC50) showed 43.66 ml/lit as EC50. The in vitro comparative efficacy study was conducted on third stage larvae of Musca spp. divided equally in 4 groups and exposed to 1:20, 1:40 (two different concentrations) of KEETGUARD LIQUID, a standard pyrethroid insecticide 'Cypermethrin (1%)' and control (plain water) respectively as group-I: 1:20 concentration of Keetguard liquid Group-II: 1:40 concentration of Keetguard liquid, group-III: standard pyrethroids insecticide 'Cypermethrin (1%) and group-IV: control (plain water). In invitro study, higher efficacy (95 %) was recorded in group-I as compared to group-II (66.66%) and the standard insecticide-Cypermethrin-1% (100%) while similar trend of efficacy (90.69% in G-I and only 65.76% in G-II) was observed during in vivo field experimentation. The plant based herbal product Keetguard is efficacious as a fly repellent against house fly Musca spp. in egg layer poultry farm in both 1:20 & 1:40 dilution. In addition, it also has larvicidal potential against 3rd stage of larvae of Musca spp. considering the detrimental effects of chemical fly repellents and insecticides on the environment and human health, Keetguard Liquid was found better option for control of fly population in and around poultry layer farms.

Key words: keetguard liquid, fly repellent, larvicidal, ectoparasiticidal, herbal

INTRODUCTION

The flies are not only nuisance to the health of the birds in the farm but also become annoyance not only to the workers of the farm but also to the people residing in the villages nearby the farms The manure accumulated in commercial caged-layer houses are the major source of attraction for the flies (*Musca* spp.). These flies act as a vector to various disease causing organisms and thus possess a great threat to human and confined poultry health. *Musca* spp. can prosper in wide range of environmental conditions with high reproductive rate and can breed throughout the year (Crespo *et al.,* 1998). Thus control of these potential vectors of disease is a serious concern. The house fly control is largely based upon the use of chemical insecticides such as organochlorines, organophosphates, pyrethroids. However, use of chemical insecticide is not only detrimental to environment and have undesirable effects on non-target organisms, but its long term use also leads to development of resistance among insects (Thomas and Jespersen, 1994). Injudicious use of these insecticides on large scale particularly in proximity to human food may prove to be toxic to man (Bhatia *et al.,* 2006). House flies are notorious for their ability to develop behavioral and metabolic mechanisms to avoid and detoxify chemical insecticides. Resistance to DDT was noticed within a few years of its introduction (Varzandeh *et al*., 1954; Perry, 1958). House flies (*Musca domestica* L.) have resisted human attempts to control them since antiquity, and the global problem of fly resistance to conventional insecticides has resulted in renewed interest in biopesticides as alternative management tools to conventional insecticides (Geden, 2012). In search of environment friendly and effective insecticides, essential oils from plants could be a good approach (Kant and Bhatt, 1994). Presently, bioinsecticides, especially those derived from plant origin, have been increasingly evaluated in controlling insects. Plants contain bioactive organic chemicals in the form of metabolites and plant extracts have been used locally in herbal preparations to cure ailments even before the advent of orthodox medicine in many developing countries (Oyedokun *et al.,* 2011). Flavonoids, Alkaloids, Saponins, Sesquiterpenes, Limonoids, Phenols, Stilbenes and Coumarins of plant origin have been reported to possess toxic, growth regulating and anti-feedant effects against a host of insect pests (Sunita and Lalijee, 2008). The knowledge and use of plants as well as their extracts as protectants against grains and other foodstuffs had been in existence since time immemorial (Dales, 1996; Isman, 2000). Therefore, considering the detrimental effects of chemical fly repellents and insecticides on the environment and human health the present study was undertaken to evaluate the *in vitro* and *in vivo* acaricide efficacy of herbal Fly repellent product (Keetguard liquid).

MATERIAL AND METHOD

Experiment design

The experiment was conducted in a farm near village Yewat, Dist. Pune having huge fly population suitable to undertake the experiment on efficacy of herbal fly repellant Keetguard liquid. The environmental temperature and relative humidity at farm premises was recorded during the period of experiment between 26.6-30.8 ^{o} C and humidity between 45-67 per cent. At this farm, three layer sheds caged with 10 thousand layer birds placed wide apart (100 feet) from each other with sufficient quantity of fresh manure underneath and having approximately identical fly population were selected for the present study. The repellents were applied to the area under (dropping area) and around the sheds by using power spray so that the spray reaches to the side mesh and entrances and tested against *Musca* spp. on the droppings, mesh, grills, grass and other objects and places where these flies were flying freely in the area as shown in figure 1.

In vivo study

Experimental groups

The three selected sheds were marked as three different groups for the study

Group I: Shed treated with Keetguard liquid @ 1:20

Group II: Shed treated with Keetguard liquid @ 1:40

Group III: Control group (Shed treated with plain water)

The product Keetguard liquid comprises of oil of herbs viz. *Eucalyptus globulus*, *Cedrus deodara*, *Pinus longifolia* & many others in a fixed concentration, which has got proven insecticidal, repellant and larvicidal efficacy.

Selection of fly base/bait

A day before starting actual experiment, a pilot trial was conducted to select a best fly base (viz. paper sheets, white gonies, grass sticks etc.) or bait (viz. coloured sugar, egg albumin baits, syrup-soap baits etc), its position (viz. vertical/horizontal/ hanging/ fixed etc.) and location (viz. under/ sides of the shed) around the shed to assess maximum fly population per unit area at three different timings at an interval of 3 hrs throughout a day. Finally, syrup-based baits were preferred and applied on 8 the plain papers / fly sheets. These fly sheets were hanged at the side mesh of each shed so as to get the maximum access to flies in the premises and those escaping – newly emerged imagoes. The method was adopted on the basis of preference by the flies.

Preparation of test solution (Keetguard liquid) for spray

The stock herbal preparation which is sticky, brownish-orange coloured liquid presented in 250 ml plastic bottle was first poured in a plastic jar containing 1 lit of clean water and mixed thoroughly with a clean wooden stick to ensure proper and complete mixing of the drug. Then such stock solution was used to prepare working / spray solution of desired concentration i.e. 1:20 and 1: 40 respectively.

Parameters Estimated

Following parameters were estimated to evaluate the efficacy of the product.

Fly Repellent efficacy against adult Fly population

Total six baited fly sheets (three on either side) were hanged around the shed and kept undisturbed for one hour to settle on the flies as shown in figure 2. The snap shots of all six fly sheets, hanged around each shed, and were taken after 1 hour of its application. Fresh fly sheets were applied and the procedure was repeated 1 hour before every observation. The observations were recorded at 1 hour, 4 hours, 6 hours, 1 day, 3 days, 5 days and 1 week after the treatment. The numbers of flies present on each sheet at the end of an hour were counted on the 15'' screen of LCD monitor (Kirby, 2008).

In Vitro study

The larvicidal activity was assessed by *in vitro* and on field studies.

In vitro larvicidal activity of Keetguard liquid was undertaken to evaluate two parameters viz. EC 50 and comparative efficacy.

I. Probit analyses of EC50

The *in vitro* study was conducted on twenty number of 3rd stage larvae of Musca spp. to evaluate effective concentration of Keetguard liquid by exposing the larvae to a series of 3

concentration of the compound in distilled water (25, 50, 75 ml/L). The larvae were exposed to different concentrations for 60 minutes and percentage mortality was calculated. The probit analyses test was performed to find out the EC 50 value on the basis of log regression and graph method (Finney.D.J and Stevens. W.L, 1948 and Finney. D.J, 1952)

II. In vitro comparative efficacy

The *in vitro* comparative efficacy study was conducted on third stage larvae of *Musca* spp. divided equally in 4 groups and exposed to 1:20, 1:40 (two different concentrations) of KEETGUARD LIQUID, a standard pyrethroid insecticide 'Cypermethrin (1%)' and control (plain water) respectively as

Group-I: 1:20 concentration of Keetguard liquid
Group-II: 1:40 concentration of Keetguard liquid
Group-III: standard pyrethroids insecticide 'Cypermethrin (1%)
Group-IV: control (plain water)

These larvae were later kept for pupation and the number of flies emerged were recorded as shown in Figure 3. Cypermethrin is a synthetic pyrethroid used as standard insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes (Kirby, 2008).

On field larvicidal efficacy

The droppings from middle layer of manure from all the three sheds under study were collected in a plastic jars of the volume of 3"x3"x3" and this volume was considered as unit volume. The samples from all groups under study were collected before an hour and after 24 hrs of application of the test compound. After collection, these droppings were mixed with sufficient quantity of water and filtered to isolate the larvae as shown in figure 4. The larval population of the *Musca* spp. per unit volume of the droppings were counted and compared.

Data recording & analysis

The data of fly counts was properly recorded, organized and analysed statistically by using completely randomized design (CRD-equal) described by Snedecor and Cochran (1989) and software designed by Jangam and Thali (2001) WASP - Web Agri Stat Package (http://www.icargoa.res.in/wasp/index.php) to draw the conclusions and interpret the results.

RESULT AND DISCUSSION

A. Fly Repellent efficacy against adult Fly population

Fly repellent activity against *Musca* spp. after first application

The results for the effect of compound on the population of flies after First application on day 1(1 hr, 4 hrs, 6 hrs and 24 hrs), day 3, day 5 and day 7 are summarized in Table 1

The pre count of fly population in the poultry layer sheds under study were 14.17+0.99, 14.00+2.03 and 13.83+2.21 in the Group-I, Group-II and Group-III respectively which was approximately similar with no statistically significant difference.

The post treatment count of flies on day 1was found to be 1.67, 3.00, 4.50 and 5.83 per unit area after 1 hr, 4 hrs, 6 hrs and 24 hrs and 12.67, 10.33 and 6.33 at day 3, 5 and 7 post applications respectively for Group I. In Group-II and III, the count was 1.83, 3.67, 4.50, 9.00, 13.50, 17.33, and 19.83 per unit area and 13.00, 13.16, 13.50, 15.67, 19.83, 24.00 and 26.00 flies/UA at 1, 4, 6, 24 hrs, 3rd, 5th and 7th day post application respectively.

With earlier concentration, the repellency was high on the day of application particularly during first 4-6 hrs both in case of group I and group II as only the available adult population was exposed to the repellent. The level of aroma of the herbal formulation was gradually decreased after 6 hours of application and the fly population gradually started increasing after 6 hrs both in Group I and II but was still considerably lower as compared to pre-treatment values. There was significant ($P<0.01$) decrease in the flies' population in group I and group II after 24 hrs, but the population increased significantly ($P<0.01$) in Group III.

The fly population started increasing gradually on day second onwards. The increase was more significant in group II as Compared to group I. This may not be due to the return of the flies which already repelled away, but may be due to synchronized effect of two factors viz. continuous emergence of new flies as a major part and decreased level of aroma of formulation.
However, at 5th day and at the week end (7th day) after application, the level of fly population was again decreased in Group I. This may be the result of the larvicidal effect of the formulation which was sprayed on the manure (on droppings of the birds) under the sheds. The larvae came in contact with the formulation might have failed either to moult or pupate. The batch of flies expected to be emerged during this phase failed to develop. Thus it finally resulted in lowering the fly population. However, the population of flies increased significantly ($P<0.01$) in Group II and Group III. The increase in population may be due to decreased aroma in Group II.
Certain plant leaves have fly repellent and feeding deterrent activity against *Musca domestica* in Ethiopia (Wimalaratne *et al.* 1996). It has direct impact of mortality, also several secondary impacts on oviposition, repellence and antifeedancy (Pavela 2008). Similar results were found against mosquitoes & flies (Watanabe *et al.,* 1993), livestock ticks (Lwande *et al.*, 1999), house flies (Singh *et al.*, 1991).

B. Larvicidal efficacy

In vitro larvicidal activity of Keetguard liquid was undertaken to evaluate two parameters viz. EC 50 and comparative efficacy.

I. Probit analyses of EC 50

The *in vitro* study was conducted to evaluate effective concentration Keetguard liquid by exposing twenty number of 3rd stage larvae of *Musca* spp. to a series of 3 concentrations of the compound in distilled water. The highest mortality of 85 % was observed at the 75 ml/L concentration followed by 55 % mortality at 50 ml/L concentration, 15 % mortality at 25 ml/L concentration of the test compound, as observed after 60 minutes. The probit analysis test was applied to find out EC 50 value on the basis of log regression and graph method and given in Table 2. The EC 50 calculated by using probit analysis was found to be 43.66 ml/L.

II. *In vitro* comparative analyses of larvicidal efficacy

The *In-vitro* larvicidal efficacy of Keetguard liquid on larval count of *Musca* spp. and its further development is represented in Table 3. Four larvae from group-I, one from group-II and 5 from group-III were found dead after one hr of treatment while there was no mortality in control group-IV.
The larvicidal efficacy on the basis of fly emergence was assessed which was 95 % in group-I (1:20), 66.66% in group-II (1:40), 100 % in group-III (Cypermethrin 1% as Standard insecticide) while only 5.54 per cent in the control group.

On field larvicidal efficacy (count of larvae in droppings/unit area 3x3x3" under the Shed)

The larvicidal action of different concentrations is presented in Table 4. The larval population of the *Musca* spp. in the droppings was 21.50+1.57 and 24.33+2.16 before application of the test compound which was significantly (P<0.01) reduced to 2.0+0.37 and 8.33+0.92 at 24 hrs after treatment indicating 90.69 per cent and 65.76 per cent reduction in the population of larvae in the group-I and group-II respectively. However, in the control group, there is 15.7 % increase in the larval count after 24 hrs from 21.00+1.65 to 24.30+1.45. These results indicate that keetguard liquid has got larvicidal action at both concentrations but the effect is more pronounced in Group I at the concentration of 1:20. The larvicidal effect of herbal fly repellents were also reported by Khater *et al.*, (2009), Oyedokun *et al.* (2011) and Jesika (2012). In some studies also, it was confirmed that some essential oils, such as that extracted from cedarwood (Adams, 1991; Grace et al., 1994), Litsea cubeba (Lin and Yin 1995), and cinnamo- mum spp. (Lin and Yin 1995), were repellents to termites. (Eisner et al., 1986) also confirmed that the all the known botanical/herb based fly repellants/ feed deterrents occur in varying proportion in wide range of herb extracts volatile or essential oils. (Campbell, 1983) also established the fly repellency or feeding deterrence properties of terpentenoids.

CONCLUSION

From the study conducted it was concluded that the compound Keetguard liquid has got potential fly repellent and larvicidal activity, which is efficacious at both the concentrations of 1:20 and1:40. On the basis of results so obtained in present experiemental trial, the natural or biological ectoparasiticidal & fly repellant product Keetguard Liquid is found to be efficacious as cypermethrin.

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Table 1: Musca spp. population around poultry shed after first application

Groups	Statistics	Pre-treatment count	Post-treatment count						
			1 hr	4 hrs	6 hrs	24 hrs	3rd day	5th day	7th day
G I	Mean	14.17[a]	1.67[d]	3.00[cd]	4.50[bcd]	5.83[bc]	12.67[a]	10.33[a]	6.33[b]
	% Redn.	--	88.21	78.83	68.24	58.86	10.59	27.10	55.33
G II	Mean	14.00[b]	1.83[e]	3.67[e]	4.50[de]	9.00[cd]	13.50[bc]	17.33[ab]	19.83[a]
	% Redn.	--	86.93	73.79	67.86	35.71	3.58	+22.57	+41.64
GIII	Mean	13.83[d]	13.00[d]	13.16[d]	13.50[d]	15.67[cd]	19.83[bc]	24.00[ab]	26.00[a]
	% Redn.	--	6.01	4.84	2.39	13.30	+43.38	+73.53	+87.99

Values in the parenthesis indicate range of observations (n=6). Redn. = Reduction.
Different superscripts indicate that the values differ significantly in a row (P<0.01).

Table 2: Probit values drawn on the basis of log regression of different concentrations of Keetguard liquid.

Concentration	Log 10 Conc.	Total No. of larvae	Dead Larvae	% mortality	Probit value
0	0.00	20	00	00	--
25	1.39	20	3	15	3.96
50	1.69	20	11	55	5.13
75	1.87	20	17	85	6.04

Table 3: *In-vitro* larvicidal efficacy of Keetguard liquid on larval count of Musca spp. and its further development

Conc. of the test compound	No. of larvae of *Musca* spp.		No. of imagoes emerged	Percent reduction in population
	Exposed to treatment	Died after treatment		
Keetguard liquid (1:20)	18	4	1	95
Keetguard liquid (1:40)	18	1	6	66.66
Cypermethrin 1%	18	5	Nil	100
water	18	nil	17	5.5

Table 4: Larval count of *Musca* spp. in the droppings under the egg layer poultry shed after application of Keetguard liquid.

Conc. of compound		Count of *Musca* spp. larvae in droppings/unit area (3x3x3" area) the under the Shed		Percent reduction in population of larvae
		Before treatment	After treatment	
Keetguard liquid (1:20)	Mean±S.E	21.57±1.57	2.0±0.37	90.69
Keetguard liquid (1:40)	Mean±S.E	24.33±2.16	8.33±0.92	65.76
Untreated	Mean±S.E	21.00±1.65	24.30±1.45	+ 15.7

Figure 1.Thick layer of droppings accumulated under the shed which is an ideal breeding place for flies and Spraying of the repellent in area around the shed

Figure 2.Fly sheets to assess the fly population per unit area before and after treatment.

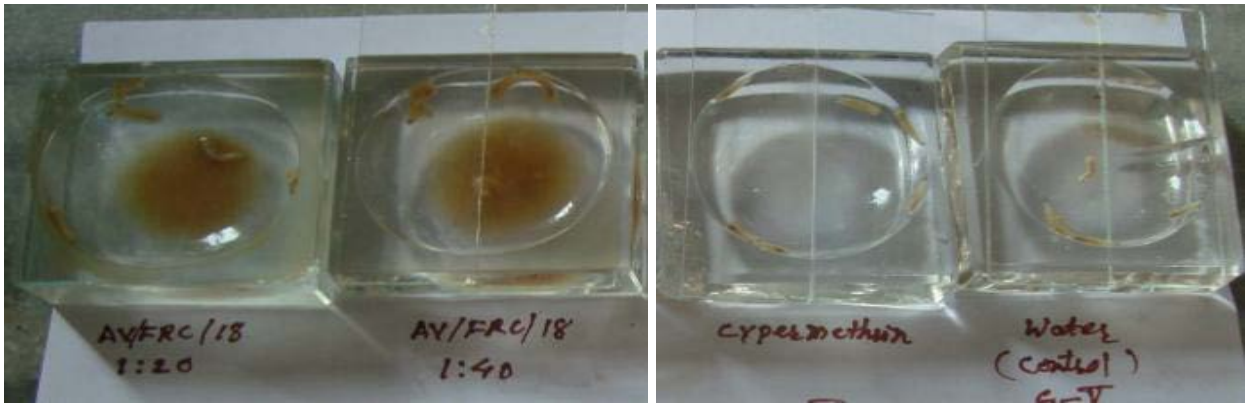

Figure 3. *In vitro* comparative efficacy study

Figure 4. Larvicidal efficacy experiment

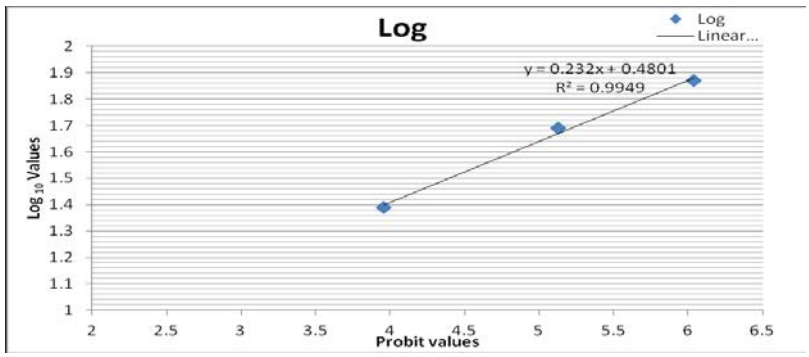

Figure 5. Effective concentration (EC 50) of keetguard liquid was calculated by employing probit analysis on the basis of graph method

REFERENCE

Adams R. P. 1991. Cedar wood oil – analysis and properties. *Modern Methods of Plant Analysis*. 12:159-173.

Bhatia B. B., K. M. L. Pathak, and D. P. Banerjee. 2006. Text book of Veterinary Parasitology. 2nd Edn, Kalyani Publishers. Ludhiana. pp: 248-49.

Crespo D., C. Roberto, E. Lecuona, and A. Jerome. 1998. Biological Control: An Important Component in Integrated Management of *Musca domestica* (Diptera: Muscidae) in Caged-Layer Poultry Houses in Buenos Aires. *Argentina. Biological Control*. 13:16–24

Campbell M. M. 1983. A test of repellency to non-biting flies and a comparison of repellants using *Musca domestica* L. *Pestic Sci*. 14:199-212.

Dales M. J. 1996. A review of plant materials used for controlling insect pests of stored Products. National Resources Institute. UK. 65:1-84.

Eisner T., M. Deyrup, R. Jacob, and J. Meinwald. 1986. Necrodol: anti-insectan terpenes from defensive secretion of carrion beetle (*Necrodes surinamensis*). *J Chem Ecol*. 12:14.

Finney D. J., and W. L. Stevens. 1948. A table for the calculation of working probits and weights in probit analysis. Biometrika. 35(1-2):191-201.

Finney D. J. 1952. Probit Analysis. Cambridge, England, Cambridge University Press.

Geden Christopher J., 2012. Status of biopesticides for control of house flies. *J Biopest*, 5(Supplementary), pp: 1-11.

Grace J. K., and R. T. Yamamoto. 1994. Natural resistance of Alaska-cedar, redwood, an teak to Formosan subterranean termites. *J Fors Prod*, 44(3):41-45.

Isman M. 2000. Plant essential oils for pest and disease management. Crop Protection, 19(8-10):603-608.

Jangam, A. K., and P. Thali. 2001. WASP- Web Agri StatPackage.http://www.icargoa.res.in/wasp/index .php.

Jesika M. 2012. Mixed Flower Waste Extract as Larvicides against *Musca Domestica*. Open Access Scientific Reports, 1(12):1-3.

Kant R., and R. M. Bhatt. 1994. Field evaluation of mosquito repellent action of neem oil. *J Malar*.31:122–125.

Khater Hanem, F. Y. Mohamed, Ramadan, S. Reham, and E. L. Madawy. 2009. The lousicidal, ovicidal, and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. Local publication, Department of Parasitology, Faculty of Veterinary Medicine Benha University, Moshtohor – Toukh, 13736. Egypt.

Kirby, 2008. Fly Management Handbook A Guide to Biology, Dispersal, and Management of the House Fly. The Connecticut Agricultural Experimentation Station. Bulletin 1013.

Lin T. S., and H. W. Yin. 1995. Effects of Litsea cubeba press oils on the control of termite Coptotermes formosanus Shiraki. Taiwan For. *Res. Inst*. New Series. 10:59.

Pavela, R. 2008. Insecticidal Properties of Several Essential Oils on the House Fly (Musca domestica L.). *Phytotherapy Research*, 22:274-278.

Oyedokun A. V., J. C. Anikwe, F. A. Okelana, I. U. Mokwunye, and Azeez O. M. 2011. Pesticidal efficacy of three tropical herbal plants' leaf extracts against *Macrotermes bellicosus*, an emerging pest of cocoa, *Theobroma cacao* L., *J Biopest*. 4(2):131-137.

Perry A. S. 1958. Factors associated with DDT resistance in the house fly *Musca domestica* L. Proceedings, *10th International Congress of Entomology*. 2:157-172.

Snedecor, W G., and C. G. William. 1989. Statistical Methods, Eighth Edition, Iowa State University Press.

Singh D., Singh A.K. 1991. Repellent and insecticidal properties of essential oils against housefly, Musca domestica L. *Insect. Sci. Applic*.12:487-491.

Sunita F., and B. Lalijee. 2008. Study of Various Extracts of *Ayapana triplinervis* for their Potential in Controlling Three Insect Pests of Horticultural Crops.*Tropicultural.* 26(2):119-124.

Thomas G. and J. B. Jespersen. 1994. Non-biting Muscidae and control methods. *Rev. Sci. tech. off int. Epiz.* 13(4):1159-1173.

Varzandeh M, W. N. Bruce, and G. C. Decker. 1954. Resistance to insecticides as a factor influencing the biotic potential of the house fly. *J Eco Ent*. 47:129-134.

Watanabe A.K., Shono Y., Kakimizu A., Matsuo, Saton A., Nishimura H. 1993. New mosquito repellent from Eucalyptus camaldulensis. *J. Agric. Food Chem*. 41:2164-2166.

Wimalaratne P.D.C., K.N. Slessor, J.H. Borden, L.J. Chong and T. Abate. 1996. Isolation and identification of *house fly*, *Musca domestica* L., Repellents from pepper tree, *schinus molle* L., *J. Chemical ecology*. 22(1):49-51.

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Effects of Dietary Ground Ginger (Zingiber Officinale) Root Additive on Broiler performance

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ABSTRACT

This study was conducted to evaluate the effect of ground ginger root (*Zingiber officinale*) addition to the diet of broiler chicks. One hundred and sixty one day old boiler unsexed chicks (cobb strain) were till 42 days of age. Four experimental diets containing 0.25, 0.50, and 0.75% ground ginger root were used. Results showed that dietary ginger incorporation had no significant ($p< 0.05$) effects on feed intake in the first four weeks. *Feed consumption* recorded the lowest estimate by broilers fed 0.50% ginger (841.0 g and 777.0g in the 5th and 6th weeks respectively) where as chicks fed 0.0, 0.25 and 0.755 ginger diets were not significantly($p< 0.05$) different from each other. Weight gain was affect by ginger levels in three weeks. The trait estimated the highest result at level 0.00 in week two, at level 0.75, 0.25 and 0.00 in week four and at level 0.25% in week six. Significantly high results for feed conversion ratio were recorded at level 0.25 0.50, 0.75% in the second week and at level 0.00, 0.50, and 0.75% in the last week. Carcass weight and liver weight were affected by addition of ginger. The traits recorded best results at 0.00, 0.25, and 0.75% ginger level. Broiler chick can tolerate up to 0.75% ground ginger root in the diet without adverse effect.

Key words: carcass, feed, liver, weight gain

INTRODUCTION

There are numerous feed additives of plant origin that are used in broiler feeds as to improve the performance by enhancing growth rate, better feed conversion efficiency and lower morbidity and mortality (Mohamed *et al.*, 2012, Zomrawi *et al.*,2013a).

Recently the use of antibiotics as feed additives is contraindicated due to health concern about their residues in animal tissues and the production of drug resistant bacteria (Zomrawi *et al.*,

2013a). Many researches were conducted to document the benefits of plant feed additives (Kumar, 1991, Babu *et al.*, 1992, Mishra and Singh 2000, Deepak *et al.*, 2002, Jahan *et al.*, 2008) and to evaluate the benefits of using natural phytobiotics as feed additives in poultry diets. Windisch *et al.* (2008) have reported that these natural feed additives have similar effects to antibiotics in improving poultry performance. Ginger is a medicinal herb that have been reported to possess body fat lowering effects (Agarwal 1996, Sharma *et al.*, 1996) it is used for cocking purposes (Zomrawi *et al.*, 2013a) or for its medical effects as it possesses antioxidants, antibacterial, anti-inflammatory, antiseptic, anti-parasitic and immunomodulatory properties (Akhtar *et al.*, 1984, Ali *et al.*, 2008). Incharoen and Yamauchi (2009) reported that ginger stimulate gastric secretion, blood circulation and act as enterokinetic. The objectives of this study were to evaluate the possible improvement in overall performance, reduction in the final cost of feed and to find safe, cheep and efficient natural growth promoter for broiler chicks.

MATERIALS AND METHODS

The experiment was carried out in the premises of Faculty of Animal Production, University of Khartoum, during the period from 21 January to 3 March.

Experimental Diets

Two kilo and half of ginger were purchased from local market beside the other ingredients (sorghum, sesame meal, ground nut meals, dicalcium, salt and super concentrate with multivitamins).Proximate chemical analyses of these ingredients were adopted from Ellis (1981). All nutrient requirements of broiler rations were formulated according to NRC (1984). Chemical composition (%) of experimental diets shown in Table (1), calculated chemical analysis of experimental diets on dry basis in Table (2) and Table (3) shown a proximate analysis of ginger powder. Ginger was added in four experimental diets (0%, 0.25%, 0.5%, and 0.75 %).

Table (1): Chemical composition (%) of experimental diets (As fed)

Ingredient (Kg)	Ground ginger root level %			
	0	0.25	0.5	0.75
Sorghum	62.15	61	62.4	62.54
Ground nut Meal	15	15	15	15
Seasem Meal	15	15	14.25	14
Wheat Bran	0.7	1	0.7	0.7
Super concentrate*	5	5	5	5
Phosphate dicalcium	1	1	1	1.01
Slate	0.2	0.2	0.2	0.2
L-Lysine	0.13	0.2	0.13	0.14
DL- Methionine	0.1	0.1	0.1	0.1
Vegetable oil	0.47	1	0.47	0.31
Premix	0.25	0.25	0.25	0.25
Total	100	100	100	100

*Super-concentrate in%: Crude protein min40.crude fat min 3.9,crude fiber max 1.44,Lysin 10-12,mthionine min 3,mth+cystin min 3.3 ,calcium min10,availablephosphorus min 6.4,energy 1950 kcal/kg, crude mineral 39.30,sodium min 2.77,linoleic acid 0.24,Nacl (salt)6,6-phytase e.c.3.1.3.26.e4a1640 added, mold inhibitor added, vitamin A IU/kg200000,vitamin D_3IU/kg70000,vitamin E mg/kg400,vitamin k_3 mg/kg30,vitamin B_1 mg/kg50,vitaminB_2mg/kg120,vitamin B_6mg/kg50,vitamin B_{12} mg/kg180, D Pantothenic acid mg/kg155,Niacine mg/kg440,Folic acid mg/kg8,Choline Chloride mg/kg5800.Manganese mg/kg1600,Zinc mg/kg1600,Iron mg/kg580,Copper mg/kg450,Iodine mg/kg55,Selenium mg/kg8,Cobalt g/kg9,Molybden mg/kg20

Table 2.Calculated chemical analysis of experimental diet on dry basis

Content	0%	0.25%	0.5%	0.75%
EE%	4.8	4.8	4.7	4.77
CP%	23.1	23.08	22.9	22.86
CF%	4.4	4.53	4.43	4.4
Ash%	5.7	5.82	5.73	5.72
ME(Mj/kg)	2.73	2.75	2.77	2.67

Table 3. Proximate analysis (%) of ginger powder

DM%	EE%	CP%	CF%	ASH%	NFE%	ME/MJ/kg
89.34	2.56	15.5	13.57	8.6	59.78	2.61

DM= dry matter, EE=ether extract, CP= crude protein, NFE= nitrogen free extract, ME= metabolizeable energy
ME was calculated according to Lodhi *et al*. 1970.
ME (P) =1.549+0.0102CP+0.0275oil+0.0148NFE-0.0034CF.

Experimental Birds

One hundred and sixty one-day old Cobb unsexed commercial broiler were bought from commercial company for poultry production and transferred to the poultry production unit at faculty of animal production.

All chicks were assigned to the control diet for the first three days as adaptation period, chicks of approximately equal live weight were randomly allotted into four groups, Each groups contains 40 birds were distributed into four sub-groups as replicate with 10 birds per pen, in a completely randomized design.

Experimental site

The experiment was carried out in an open mesh sided, deep litter poultry house,, the Eastern and Western sides were covered with Jute sacks to prevent conventional heat effects and to control the direct sun rays, the house was subdivided into 16 rooms (m^2) made of wire netting. Enough space for work was left. The house was cleaned, washed and disinfected using formalin and folic acid. Each pen floor was covered with enough wood shavings with allocation of one tubular feed + trough and one round fountain drinker, A 60 watt bulb per pen was used for artificial lighting through evening time.

Management

Daily throughout the experimental period, the house iterance was cleaned early in the morning using folic acid. Feed and water were provided *ad libitum*. Every week live weight was record and feed intake was calculated by difference i.e. offer minus remaining.

Experimental Procedure

At the end of experimental period(sixth weeks), all birds were leg banded, individually weighted and recorded the live weight, then were slaughtered manually, birds were scalded using boiling water, handpicked, washed left to drain. Complete removal of trachea, esophagus, crop, intestinal tract, giblet (heart and gizzard), spleen, bursa of fabricus, kidney, oil glands and reproduction organs. Then the hot carcass and liver were weighted.

Chemical Analysis

Proximate analysis for the chemical components of ginger powder (dry matter, crude protein, ether extract, crude fiber, ME, ash and nitrogen free extract, were determined according to AOAC (1980).

Statistical Analysis

All the data of this experiment were analyzed statistically by using ANOVA. The data generated from experiment were subjected to analysis of variance according to steel and Towrie (1980). Differences among the treatment were tested by the method of Duncan. The analysis was carried out SPSS program (statistical packages for social science

RESULTS AND DISCUSSION

Results in Table 4 showed that treatment had no significant effect ($p<0.05$) on feed intake in the first fourth weeks, although the diets were is caloric and the birds were expected to consume similar feed (Scott et al 1982), however there was a significant increase in feed intake in week five and week six this might be due to enhancement of the appetite of birds by the aroma and flavor of ginger (Kulka, 1967). Results in table 7 showed there was an increase in total feed intake at level 0.25% and 0.75% and decrease in level 0.5%, this iritic increase in feed intake and total feed intake may be due to pungent test or aroma and flavor of ginger. This result agrees with (Purseglove *et al.*, 1981) who reported that the effect of pungent test in feed intake cause by number of components predominated by gingerols followed by shogaols and zingerone. Moreover (Purseglove *et al.*, 1981) mentioned that aroma and flavor of ginger caused by more than 70 constituents present in steam volatile oil obtained from dry ginger. total feed intake in this study ranged from 4150 to 4150g and this is higher than the estimates found by Zomrawi *et al.*, (2013b) but lower than the estimates reported by Fakhim *et al* ., (2013).

Table 4. Effect of dietary ground ginger root on feed intake of broiler chick (g/bird/week)

Weeks	Ground ginger root level %				
	0	0.25	0.5	0.75	SEM
Week 1 **(g)**	258	251	256	235	8
Week 2 **(g)**	476	468	466	453	12
Week 3 **(g)**	768	762	710	680	34
Week 4**(g)**	855	847	832	838	18
Week 5**(g)**	884^{ab}	884^{ab}	841^{b}	928^{a}	24
Week 6**(g)**	835^{ab}	938^{a}	777^{b}	809^{ab}	42

$^{a\ b}$ Values within rows on common superscript differ significantly ($p< 0.05$).; SEM = standard error of the mean.

The significant ($p<0.05$) positive effect of ginger on body weight gain in week 2, 4 and 6 observed in table 5. Similar positive effect of ginger on total weight gain was showed in table 7, at 0.25% ginger level diet there was weight gain was 7.3 % above the control diet fed chicks. Also there was a positive effect in weight gain at 0.75% ginger level, this might be due to *Zingiber officinale* content of volatile oil, fixed fatty oil, proteins, starch and mineral elements or might be due to the fatty oil in ginger which is contained saturated and unsaturated fatty acid, the major component of acids were palmitic, oleic and linoleic (Salzer, 1995).

Table 5.Effect of dietary ground ginger root on weight gain of broiler chick (g/bird/week)

Week	Ground ginger root level %				
	0	0.25	0.5	0.75	SEM
Week 1 **(g)**	170	167	164	161	6
Week 2 **(g)**	316[a]	295[b]	279[b]	281[b]	6
Week 3 **(g)**	383	425	431	389	23
Week 4**(g)**	362[ab]	360[ab]	338[b]	450[a]	33
Week 5**(g)**	398	362	355	417	34
Week 6**(g)**	267[b]	426[a]	269[b]	221[b]	41

[a b] Values within rows on common superscript differ significantly (p< 0.05).; SEM = standard error of the mean.

The significant (p<0.05) positive effect of ginger on body weight gain in week 2, 4 and 6 observed in table 5. Similar positive effect of ginger on total weight gain was showed in table 7, at 0.25% ginger level diet there was weight gain was 7.3 % above the control diet fed chicks. Also there was a positive effect in weight gain at 0.75% ginger level, this might be due to *Zingiber officinale* content of volatile oil, fixed fatty oil, proteins, starch and mineral elements or might be due to the fatty oil in ginger which is contained saturated and unsaturated fatty acid, the major component of acids were palmitic, oleic and linoleic (Salzer, 1995). The overall weight gain in this experiment was 2035- 2035g and this is slightly lower than the estimates reported by Fakhim *et al.* (2013) but higher than the results reported by Zomrawi *et al.* (2013b).

Table 6. Effect of dietary ground ginger root on Feed conversion ratio of broiler chick (g Feed/g weight gain/bird/week)

Week	Ground ginger root level %				
	0	0.25	0.5	0.75	SEM
Week 1 (gF/gW)	1.5	1.5	1.6	1.5	0.022
Week 2(gF/gW)	1.5[b]	1.6[ab]	1.7[a]	1.6[b]	0.019
Week 3(gF/gW)	1.9	1.8	1.7	1.8	0.039
Week 4(gF/gW)	2.4	2.4	2.5	1.9	0.11
Week 5(gF/gW)	2.3	2.5	2.5	2.2	0.129
Week 6(gF/gW)	3.1[ab]	2.3[b]	2.9[ab]	3.7[a]	0.213

[ab] Values within rows on common superscript differ significantly (p< 0.05).; FCR = feed conversion ratio.; SEM = standard error of the mean.

There was significantly positive effect of treatment differences on feed conversion ratio (FCR) as shown in table 7 during the second and the sixth week. This might be due to the effect of supplementation of ginger powder which contains high level of plant proterlytic enzyme (Thompson *et al.*, 1973; Ziauddin *et al.*, 1995). Total feed conversion ratio (2.0-2.2kg F/kg G) is in accordance with the range reported by Herawati and Marjuki 2011, Zomrawi *et al.* 2013[a], Zomrawi *et al.* 2013[b],)

Table 7.Effect of dietary ground ginger root on overall performance of broiler chicks

Parameters	Ground ginger root level %				
	0	0.25	0.5	0.75	SEM
Total feed intake (g)	4074	4150	4150	3943	53
Total weight gain (WTG)	2035[ab]	2035[a]	2035[b]	1918[ab]	28
FCR (gF/gW)	2.2	2.0	2.1	2.1	0.032

[ab] Values within rows on common superscript differ significantly (p< 0.05).; SEM = standard error of the mean.; FCR = Feed conversion ratio.

Results in table 8 showed no significant ($p>0.05$) effects of treatments on dressing percentage, as well as pre- slaughter weight. There was significant effect of treatments on carcass weight which especially at the (0.25%) ginger level where the increase is about 7% higher than in control diet fed chicks. The positive effect in carcass weight might be due to the effect of ginger bioactive compounds on improving protein and fat metabolism (Zhang *et al.*, 2009). Dressings out percentages were in accordance to the results reported by Zomrawi *et al.* (2013[b]). There were significant ($p<0.05$) effects on liver weight, and relative liver weight %, this may be due to photolytic enzyme (Thompson *et al.*, 1973; Ziauddin *et al.*, 1995).

Table 8.Average pre-slaughtered, carcass weight, dressing percentage, liver weight and liver % of broilers fed diet containing ground ginger during 0-6weeks

Parameters	Ground Ginger root level (%)				
	0	0.25	0.5	0.75	SEM
Pre-slaughter weight (g)	2122	2129	1955	2032	57
Carcass (g)	1562[ab]	1591[a]	1450[b]	1541[ab]	38
Dressing %	74.1	75.0	74.3	75.9	1
liver/g	49.8[a]	49.4[a]	40.5[b]	47.5[a]	1.7
Relative liver weigh (%)	2.5[a]	2.3[b]	2.1[b]	2.3[b]	0.1

[ab] Values within rows on common superscript differ significantly ($p< 0.05$).; SEM = standard error of the mean.

Table 9 shows the results of feed cost and profitability of broiler fed diet contain ginger, the highest cost of feed was obtained for birds fed 0.75% ginger then the birds fed 0.5% ginger and the birds fed 0.25% ginger. Birds fed 0.25%ginger showed the highest profitability compared to other bird groups this may be related to the higher weight gain of this group than others, in conclusion the using of ground ginger root at level 0.25% increase carcass yield and return.

Table 9.Feeding benefit of experimental groups

A -Total costs:

Item	Ground Ginger root level (%)			
	0	0.25	0.50	0.75
Chicks purchase (SDG)	108	108	108	108
Feed (SDG)	150.41	154.05	156.20	158.21
Management (SDG)	50	50	50	50
Total costs (SDG)	308.41	312.05	314.2	316.21
Cost/bird/(SDG)	7.7	7.8	7.9	7.9

B-Total returns:

Item	Ground Ginger root level (%)			
	0	0.25	0.50	0.75
Average weight of bird (kg)	1.562	1.591	1.449	1.541
Price kg. of bird (SDG)	11	11	11	11
Total returns (SDG)	17.18	17.50	15.94	16.95
Returns/bird(SDG)	9.48	9.7	8.08	9.0

CONCLUSION

Broiler chick can tolerate up to 0.75% ground ginger root in the diet without adverse effect. Further studies could be done to assess the response of ginger on the physiological and blood biochemical parameters of birds.

REFERENCE

Agarwal, K. C., 1996. Therapeutic actions of garlic constituents. *Med. Res*., 16:111-124.

Akhtar , M. S., H. Afzal, and F. Chaudry. 1984. Preliminary in vitro antibacterial screening of Bakain, and Zarisk against Salmonella. *Medicose*, 9: 6-7.

Ali, B. H., G. Blunden, M. O. Tanira, and A. Nemmar. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. *Food Chem. Toxicol*. 46:409-420.

Babu, M., K. Gajendran, F. R. Sheriff, and G. Srinivasan. 1992. Crown Grow fit supplementation in broilers improved their performance. *Indian Poultry Review*. 23:27-28.

Deebak, G, S. Jogi, A. Kumar, R. Bais, and K. S. Vikas. 2002. Effect of herbal liver stimulants on efficacy of feed utilization in commercial broiler chicken. *Indian Journal of Animal Research.* 36(1):43-45.

Ellis, N. 1981. The nutrient composition of Sudanese Central Animal nutrition Research laboratory KUKU Khartoum North Sudan.

Fakhim, R., Y. Ebrahimnezhad, H. R. Seyedbadi, and T. Vahdatpour. 2013. Effect of different concentrations of aqueous extract of ginger (Zingiber officinale) on performance and carcass characteristics of male broiler chickens in wheat-soybean meal based diets. *Journal of Bioscience and Biotechnology*. 2(2):95-99.

Herawati and Marjuki. 2011. The Effect of Feeding Red Ginger (Zingiber officinale Rosc) as Phytobiotic on broiler Slaughter weight and meat Quality. *International Journal of Poultry Science*.10(12):983-985.

Incharoen, T., and K. Yamauchi. 2009. Production Performance, Egg Quality and Intestinal Histology in Laying Hens Fed Dietary Dried Fermented Ginger. *Poul. Sci*. 8(11):1078-1085.

Jahan, Z. A., U. H. Ahsan, Y. Muhammad, A. Tanveer, and K. Sarzamin. 2008. Evaluation of different medicinal plants as growth promoters for broiler chicks. *Sarhad Journal of Agriculture*. 24(2):323-329.

Kulka, K. 1967. Aspects of functional groups and flavor. *J. Agric Food chem*. 15:48-57.

Kumar, O. M. 1991. Effect of Liv-52® syrup on broiler performance in North Eastern region. *Indian Poultry Review*. April 22:37-38.

Mishra, S. J., and D. S. Singh. 2000. Effect of feeding root powder of Withania somnifera (L.) Dunal (aswagandha) on growth, feed consumption, efficiency of feed conversion and mortality rate in broiler chicks. *Bioved*. (Annual) 11:79-83.

Mohamed, A. B., A. M. Al-Rubaee, and A. Q. Jalil .2012. Effect of Ginger (Zingiber officinale) on Performance and Blood Serum Parameters of Broiler. *International Journal of Poultry Science*.11(2):143-146.

N.R.C. 1984. Nutrient Requirement for Poultry .8th revised edition. Nutritional Academy press .Washington D.C.USA.

Purseglove, J. W., E. G. Brown, C. L. Green, and S. R. J. Robins. 1981. Spices, Vol.2. First Edition, longman Scientific Technical., United State, New York.

Salzer, U. J. 1995. Analytical evaluation of seasoning extracts (oleoresins) and essential oils from seasonings. *Flavours*.11:206-210.

Scott, M. L., M. C. Nesheim and R. J. Young.1982. Nutrition of chicken. Third edition. Scott, M. L ,and Association Publishers, *J. Thaca*.NY.

Sharma, I., D. Gusain and V. P. Dixit. 1996. Hypolipidemic and antiatherosclerotic effects of Zingiber officinale in cholesterol fed rabbits. *Phyto. Res*. 10:517-518.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd.ed. Mc Grow- Hill, New York.

Windisch, W., K. Schedle, C. Plitzner and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. *J. Anim. Sci.* 86(E. Suppl.):E140-E148.

Zomrawi, W. B., K. A. Abdel Atti, B. M. Dousa, A. G. Mahala. 2013a. The effect of dietary Ginger Root (Zingiber officinale) on Broiler Chicks Performance , carcass characteristic and Serum

Constituents . *Journal of Animal science* advances. 3(2):42-47.

Zomrawi, W. B., Atti K. A.Abdel, B. M. Dousa, and A. G. Mahala. 2013b. The effect of dietary Ginger Root powder (Zingiber officinale) on Broiler Chicks Performance, blood and Serum Constituents. *Journal of Animal science advances.* 3(2):42-47.

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