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PERFORMANCE AND IMMUNE RESPONSE OF MALE BROILERS OFFERED GRADED LEVELS OF SAFFLOWER MEAL DURING THE STARTER PERIOD

by
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AN ABSTRACT OF THE THESIS OF

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Title: Performance and Immune Response of Male Broilers Fed Graded Levels of Safflower Meal During the Starter Period.

Two experiments were performed at the Agricultural Research and Education Center of the American University of Beirut to evaluate the performance of broiler chicks in response to a partial or total replacement of soybean meal (SBM) with de-hulled, pressed and extruded safflower meal (SFM) in practical starter rations.
In the first experiment, 252 one week-old Ross 308 males were divided into 36 groups of seven birds each and maintained in Petersime battery brooder pens so that all pens had the same initial average body weight and range. The birds were offered for two weeks, isocaloric (3150 Kcal/Kg as metobolizable energy, ME) and isonitrogenous (23% crude protein, CP) balanced rations containing either SBM (control) or SFM at 20, 40, 60, 80, and 100% substitution rate of SBM. The diets were formulated on the basis of digestible amino acids. There were 6 treatments and 6 replicates per treatment with 7 birds per replicate in a complete randomized design. Feed intake, weight gain, and feed conversion were recorded at 3 weeks of age. In addition, 2 birds per pen representing the average body weight of each pen were slaughtered and their ready to cook carcass (RTC) and internal organs (liver, gizzard, heart and spleen) weight percentages were determined. Data were analyzed using one way ANOVA and means compared using Duncan's multiple range test. SFM 40% gave the greatest weight gain (788 g) which was significantly different ($P<0.05$) only from both SBM and SFM100% (754 and 731 g, respectively). The SFM100% diet resulted in the greatest feed conversion ratio (1.49) in comparison to all treatments whereas the SBM-control diet had the smallest numerical feed conversion value (1.37) that was significantly different only from that of SFM80 (1.42) and SFM100%. RTC carcass yield of birds fed the SFM40 and SFM80 was higher ($P<0.05$) than that of SFM20 and control fed birds. However, weight percentages of internal organs were not affected by any of the dietary treatments.

In the second experiment, the dietary SFM levels varied between 30 and 70% with an incremental increase of 5%. Consequently, a SBM-control diet along with 9 other balanced diets containing 30, 35, 40, 45, 50, 55, 60, 65, and 70% SFM were formulated, on the basis of digestible amino acids, to be isocaloric and isonitrogenous with values similar to those used in the first trial. In this experiment, 350 week-old male broilers were divided into 10 treatments and each fed the different rations in 5 replicates each with 7 birds per replicate. Feed intake, weight gain, and feed conversion were obtained at 3 weeks of age, whereas sera antibody titers of IBD, IB, and NDV were determined and compared among

experimental treatments at specific ages of 2 and 4 weeks. Data were analyzed using one way ANOVA and means were separated by Duncan's multiple range test. No significant differences were detected for the serum antibodies levels among the treatments at 4 weeks of age. SFM40 diet resulted in the highest weight gain (773 g) that was different ($P<0.05$) only from that of SFM30 (720 g). Although feed conversion values were similar among all treatments, both SFM40 and SFM45% had the lowest numerical values. Results of both trials suggested a synergistic effect of SFM and SBM on bird performance when the former is used at a dietary level of 40-45% in starter broiler rations.

Key words: starter broilers, graded-safflower meal, weight gain, feed conversion, anti-body titers.

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ABBREVIATIONS

AREC	Agricultural and research education center
AUB	American University of Beirut
ANOVA	Analysis of Variance
AMEn	Apparent nitrogen corrected metabolizable energy
Apr	April
AOAC	Association of analytical communities
Aug	August
ß	Beta
C	Carbon
cm	Centimeter
CSM	Cold extruded safflower meal
R^2	Correlation coefficient
CP	Crude protein
cyst	Cysteine
Dec	December
°C	Degree Celsius
et al	Et alii (and others)
Feb	February
FAO	Food and agricultural organization
FDA	Food and drug administration
GLM	Generalized linear model
g	Gram
ha	Hectar
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
h	Hour
HCl	Hydrochloric acid
IB	Infectious bronchitis

IBD	Infectious bursal disease virus
IFN	Interferon
IL	Interlukin
IPCC	Intergovernmental Panel on Climate Change
Jan	January
Jul	July
Jun	June
Kcal	Kilo Calorie
Kg	Kilogram
LDL	Low density lipoprotein
Mar	March
ME	Metabolizable energy
m	Meter
Meth	Methionine
µg	Microgram
mg	Milligram
mL	Milliliter
mm	Millimeter
Min	Minute
nm	Nanometer
NRC	National Research Council
NDV	Newcastle disease virus
MEn	Nitrogen corrected metabolizable energy
N	Normality
Nov	November
Oct	October
/	Per
%	Percent
p	Probability
RTC	Ready to cook

SFM	Safflower meal
Sep	September
SBM	Soy bean meal
SAS	Statistical analysis system
TSAA	Total sulfur amino acids
USA	United States of America

To My Family…

CHAPTER 1

INTRODUCTION

Increasing population, climate change, water scarcity, land grabbing, poverty, food security, the economic situation and the food increasing prices are the main worldwide rising issues of present and future times. According to the Food and Agriculture Organization of the United Nation (FAO), world population exceeded seven billion people in 2012, with an estimation to grow to be above nine billion by 2050, which will cause a real problem in the world food security because of the shortage that will rise from the high demand for food faced by an inferior food availability and supply. The Intergovernmental Panel on Climate Change (IPCC, 2007) considers that the average global surface temperature will rise about 1.8 to 4.0 ^{o}C by the end of the twenty first century, which will influence the agricultural and water resources worldwide. All these issues led to the conclusion that the world should start searching for solutions to reduce the effects of the climate change and to secure the increasing demands for food and water.

The newly generated trend of using some crop plants for bio-fuel production has increased the prices of some crops such as corn and soybean which are widely used in the animal production industry as the main components of the diets especially in the poultry and pig sectors. On the other side, scientists from all over the world started searching for alternative feed ingredients replacing the conventional used soybean meal in poultry diets.

In Lebanon, the lack of dams or lakes for water harvesting is reflected in the scarcity of water availability for agricultural purposes especially in the arid and semi-arid regions,

which induce the farmers to rely on rain fed crops. Locally produced alternative feed components have been tested as potential substitution for soybean meal in poultry diets (Daghir, 2008).

Locally produced rain fed barley (*Hordeum vulgare*), vetch (*Vicia sativa*) and safflower (*Carthamus tinctorius L.*) were subjected by Farran *et al*., (2010a) to an economical feasibility study to assess their production cost and yield in addition to their inclusion rates in poultry rations. These researchers concluded that barley could replace corn by 25% when enzymes are supplemented (Farran *et al*., 2010b), vetch is costly because of its manual harvesting and processing but is considered as a promising replacement crop (Farran *et al*., 2001) and finally the de-hulled cold extruded safflower meal could replace SBM up to 75% in Broiler rations when supplemented with lysine and methionine in addition to being a main source for vegetable oil production (Farran *et al*., 2010a).

Recent agronomic research work had strongly recommended the adoption of safflower as a productive crop under semi-arid / rain fed conditions due to its economic, environmental, and agronomic benefits (Yau *et al*., 2008). Accordingly, Farran *et al*. (2008) prepared three safflower meals through the extraction of oil from decorticated seed that has been cultivated under semi-arid conditions. They were able to show that de-hulled extruded, de-hulled hexane extracted, and de-hulled clean extruded safflower meal (SFM) had lower protein efficiency ratio and net protein ratio than soybean meal 44% CP. Moreover, the net protein ratio, crude fat, crude fiber and AMEn of SFM (58.4% CP, 11.7% crude fat, 2.59% fiber, and 2564 kcal AMEn/kg) was higher than the soybean meal 44%.(43% CP, 3.47% crude fat, 6.08% fiber, and 2023 kcal AMEn/kg)

The objectives of this work were to study the effect of feeding graded levels of cold extruded SFM in substitution to SBM on the performance and immune response of male broilers during the starter period and to determine the optimum inclusion rate of SFM in broiler starter diet.

CHAPTER II

LITERATURE REVIEW

A. Conventional Used Chicken Feedstuffs

The poultry industry relies on a few major ingredients for feed formulation. Cereal grains are the principal sources of energy in poultry diets, whereas grain legumes and oilseed cakes are the main sources of protein. Corn, wheat, barley, triticale and sorghum are the key cereal grains and soybean meal, canola meal, fish meal, peas and beans are important protein sources. The industry has always been inclined to use the least expensive ingredients to maximize profit (Iji *et al.*, 2011).

1. Grains

The worldwide mostly used energy concentrates (less than 20% CP) in poultry diets primarily consist of cereals and their byproducts (Pond *et al*., 1995), whilst elsewhere cereal substitutes like roots and tubers, fruits and their by-products were also used (Ravindran and Blair, 1991). Maize or corn is the most common energy feed component fed to poultry worldwide (Leeson and Summers, 1997), although substantial amounts of sorghum, wheat, barley, and rice/rice by-products are also used in poultry diets when price and supply allow for their inclusion.

The feeding value of sorghum is similar to that of maize. But it has higher protein content, quite palatable and maybe used as a replacement of maize. Sorghum-meal is a good source of some amino acids, but costlier than other oilcakes (Acharya, 1997). As for barley, it is not

very palatable because of its high fiber content and can constitute up to 25% of the ration when enzymes were added (Farran *et al.*, 2010b).

Oat is not very palatable because of its high fiber content. It should not constitute more than 20 per cent of the ration. Because of its manganese content, it may help in preventing hock disorders; feather pulling and cannibalism.

The use of higher protein wheat is often economical because of the sparing effect that they exert upon the amount of soybean meal or other protein supplements needed whereas wheat can be used for replacing maize as a source of energy (Scott, 1987). In addition, wheat bran is bulky and quite laxative on account of its high fiber, manganese and phosphorus content.

2. Protein Concentrates

Oilseeds are used for different purposes: food (raw, roasted or boiled and cooking oil), animal feed (pressings, seeds, green material and straw) and industrial raw material and for medicinal purposes. Oilseeds are a reasonable source of dietary mineral especially, potassium, calcium, phosphorus and magnesium; their oil is an excellent source of mono and polyunsaturated fatty acids. They contain about 80% oleic and linoleic acid.
They are good sources of oil, crude fiber, protein, carbohydrate and essential amino acids (Ingale and Shrivastava, 2011).

The optimal use of protein concentrates in poultry feeding programs is essential for at least three reasons: their amino acids content which are critical nutrients for both rapidly growing meat-type birds and high-producing laying hens; in addition, their cost is usually higher than that of energy feedstuffs; finally, the optimal use of dietary amino acids minimizes

the production and excretion of nitrogenous waste products by the birds, thereby reducing the amount of nitrogen released into the environment (Elkin, 2002).

Cottonseed meal has less crude protein, dietary energy, and available lysine and sulphur amino acids content than the other commonly used oilseed meals (Fernandez et al., 1994). The meal is not widely included in poultry diets, unless economic reasons dictate otherwise. Cottonseed meal also contains the anti-nutritional factors of gossypol and cyclopropenoid fatty acids (Cheeke, 1998). Glandless cottonseeds have been developed that almost eliminate gossypol in cottonseed meals.

Another meal, linseed meal, obtained from the flax seeds, a unique among oilseeds because of its high content of alpha-linolenic acid. Although full-fat flax seeds had been traditionally used in ruminant feeds, recently there has been considerable interest in feeding linseed meal to poultry, because of its high content of linolenic acid (Leeson and Summers, 1997). Linseed meal contains 34% CP (NRC, 1994) and 35 to 45% oil, and 45 to 52% of that oil is alpha-linolenic acid (Leeson and Summers, 1997).

Sesame seed meal is a by-product of oil extract. Although it contains 41% CP, sesame meal is also very deficient in lysine, as is the case with safflower meal, is sometimes used to advantage in formulating lysine-deficient diets for experimental purposes (Leeson and Summers, 1997). It is, however, a good source of the sulfur-containing amino acids, including methionine, cystine and tryptophan for both growing chicks and laying hens (Scott *et al*., 1982; Ravindran and Blair, 1992).

Although the soybean is an important legume crop grown for human consumption, particularly in Asia, soybean meal, a by-product of oil extraction, is by far the major plant protein concentrate used in poultry diets (Fernandez et al., 1994; Dale, 1996). Because of the

presence of anti-nutritional factors, whole soybeans must be roasted before they can be included in poultry diets. Nevertheless, soybean meal remains the worldwide standard against which other protein sources are compared (Leeson and Summers, 1997).

Camelina (false flax), contains high levels of omega-3 fatty acids, it is a new by-product meal from oil extraction for biodiesel production. Camelina meal has FDA approval for poultry layer rations up to 10%, broiler feed rations up to 10%, beef cattle rations up to 10% and swine feed rations up to 2% (Kakani *et al*., 2012). The meal contains secondary plant metabolites called glucosinolates that adversely affect broiler performance.

Sunflower meal is a by-product resulting from oil extraction of sunflower seeds. The worldwide production of sunflower meal ranks fourth behind soybean meal, cottonseed meal and canola (Zhang and Parsons, 1994). Seed processing times and temperatures affect the amount of available lysine in the final meal. The fiber level of the meal depends on the extent to which the seed hulls are removed prior to oil extraction (Villamide and San Juan, 1998). Fluctuation in the percentage of hulls remaining after oil extraction is the reason that different sources of sunflower seed meal produce highly variable outcomes in poultry performance. As compared to soybean meal, sunflower meal is relatively richer in sulphur amino acids but markedly lower in lysine and available threonine (Leeson and Summers, 1997)

3. Alternative Feed Ingredients

The increasing trade prices of traditional poultry diet components such as corn and soybean meal are induced by the expansion of production of bio-fuel and the diminished existence of water due to changing environmental conditions. In general, feed represents 60 to 70% of the cost of producing eggs and poultry meat. Due to water scarcity and absence of

environmental conditions conducive to produce cereal grains and beans, developing countries rely almost totally on importing these ingredients which in turn results in increased cost of poultry produce. Thus the issue of using locally produced feedstuff arises.

Searching for substituting the traditional feed ingredients by domestic rain fed crops such as safflower as an oil crop, and barley and legumes such as faba bean and vetch instead of irrigated produce becomes imperative. Cereals and legumes contributed by 5 and 2%, respectively to the aggregate agricultural outcome in Lebanon according to FAO and the ministry of Agriculture of Lebanon(2007).Safflower has been recently planted at AREC and tested for its yield (Yau *et al*., 2004) and the nutritional value of SFM determined by Farran *et al.*,(2010b).

B. Safflower Plant

1. Classification

The safflower plant is selected under:

Kingdom: Plantae
Order: Asterales
Family: Asteraceae or Com positae
Genus: Carthamus
Species: C. tinctorius
Binomial name: *Carthamus tinctorius*

2. History

Safflower is known to have different names across the world as summarized in table 1.

Table 1. Safflower Names around the World (Singh *et al*., 1996)

Country	Common name	Reference	Notes
Afghanistan	Muswar, Maswarah	Knowles 1959	Kabul
	Kajireh	Knowles 1959	Heart
	Kariza	Knowles 1959	Ghazn
Arabia (Iran, Jordan)	Qurtum, Gurtum, Osfur	Knowles 1959	
(Syria, Egypt)	Kurtum, Usfar	Chavan 1961	
Bangladesh	Kusum, Kusumppuli	Chavan 1961	
China	Honghua, Grass safflower, Compositae safflower, Huai safflower, Chuan safflower, Du safflower	Yuan Guobi et al. 1989	
Ethipioa	Suff	Smith 1996	
France	Le carthame		
Germany	Saflor, Färberdistel		
India	Jafran	Chavan 1961	Assamese
	Kusumba	Knowles 1959	Bihar
	Kusumbo	Chavan 1961	Gujarathi
	Kusum karrah	Chavan 1961	Hindi
	Kusuma	Knowles 1959	Hyderabad
	Kusumbe, kusume	Chavan 1961	Kanarese
	Hubulkhurtum, ('seed of safflower')	Knowles 1959	Kashmir
	Kardai, kardi	Chavan 1961	Marathi
	Kasumba	Chavan 1961	Punjabi
	Pavari	Chavan 1961	Sindhi
	Sendurakam	Chavan 1961	Tamil
	Kushumba	Chavan 1961	Telugu
Iran	Golbar aftab	Knowles 1959	Ghom
	Koshe or Kousheeh,	Knowles 1959	Isfahan
	Kajireh, Goplzardu	Knowles 1959	Meshed
	Kajena goli, Khardam	Knowles 1959	Saveh
	Khasdonah, Laba torbak	Knowles 1959	Shiraz
	Zafran-Golu (Turkish)	Knowles 1959	Tabriz
Italy	Cartama		
Japan	Benibana, Benihana	Smith 1996	
Latin America	Cartamó, Azarfrancillo	Smith 1996	
Pakistan	Kusumba	Knowles 1959	
Spain	Alazor, Azafran romí	Knowles 1959	
Turkey	Aspir, Dikken	Knowles 1959	
	Kazhira	Chavan 1961	Persian
	Cnicus, Cnecus, Cnikos	Weiss 1971	Early Greek

Also known as the false Saffron, Safflower is considered as one of the oldest cultivated crops in the world, and it was mainly cultivated in Egypt, Iran, India and China for its carthamin, a red dye found in the flower petals. *Carthamus tinctorius* L. is the latinized name of safflower, originated from the Arabic word *Quartum*, or *Gurtum*, which refers to the dye's color extracted from safflower flowers. (Singh *et al*., 1996)

Safflower was mentioned as *Kusumba* in Indian ancient scriptures and recognized as *hong huain China*. Presently, it is most commonly known as *Kardai* in Marathi and *Kusum* in Hindi. The English name *Safflower* probably evolved from various written forms of *Usfar* , *Affore* , *Asfiore* , and *Saffiore t*o *Safflower* (Singh *et al*., 1996).

According to references from ancient Egypt, safflower was valued as a source of red, yellow and orange dye for coloring cotton and silk, also used to color ceremonial ointments used to smear mummies (Weiss *et al*., 1971). In addition, safflower has been used in the Middle East, India and Africa as purgative and for its alexipharmic (antidote) effects, as well as in a medicated oil, to promote sweating and cure fever (Singh *et al*., 1996).

3. Characteristics

Safflower is an annual, herbaceous extremely branched oilseed plant, innate for arid regions; it can reach a height of 30 to 150 cm and headed with red, yellow, white or orange flowers of globular shape each containing 15 to 20 seeds. Germination is followed by a slow-growing rosette stage, during which numerous leaves are produced near ground level, and strong taproots develop and begin to penetrate deep into the soil, but no long stems form. During this rosette stage, young safflower plants are resistant to cold, even frost, but the crop is very vulnerable to fast-growing weeds (Dajue and Mündel, 1996).

It has an extensive root system with a strong fleshy taproot reaching 2 to 3 meters in depth and thin lateral roots exploring the first 30 centimeters of the soil, which makes it more drought tolerant than small grains (Singh *et al*., 1996). The growth period lengths of the safflower plant are summarized in Table 2.

Table 2. Growth Period Lengths of the Safflower Plant (Smith, 1996)

Crop stage	**Days**
Establishment	4 to 10
Early vegetative (rosette development)	25
Late vegetative (elongation and branching)	60
Flowering	30
Yield formation (seed filling)	25
Ripening	10
Total	**150 to 160**

Safflower is a suitable crop for semi-arid areas receiving winter and spring rainfall, and requires a dry atmosphere during flowering and maturation (Knowles, 1976). In the USA-California, safflower is grown under rain fed conditions, mostly in areas with an annual rainfall of 375–500 mm (Arnon, 1972).

4. World Production

Safflower is a minor crop with a world production of about 591,997 tons of seeds in 2011 (FAO, 2013). Safflower is grown in around 60 countries around the world, with less than 1 million hectares planted, but it plays an important role within the farming systems as indicated in the 7th International Safflower Conference held in Australia in 2008.

Traditionally, safflower has been grown for centuries from China to the Mediterranean region and all along the Nile valley up to Ethiopia (Weiss, 1971). Presently it is grown commercially in India, the U.S., Mexico, Ethiopia, Kazakhstan, Australia, Argentina, Uzbekistan, China, and the Russian Federation. Pakistan, Spain, Turkey, Canada, Iran, and Israel also grow safflower to a limited extent. The international safflower production calendar is summarized in figure 1 and varies according to the geographical country location. Because of its minor status among the agricultural crops, accurate production statistics on safflower are difficult if not impossible to acquire. Suffice to say that India produces approximately half the world's annual production of safflower followed by the USA of which California is the biggest producing State. Safflower acreage and production around the world have witnessed wide fluctuations in the past. Commercial production of safflower in the U.S. was started in the 1950s, and the area rapidly increased to 175,000 ha mainly in the states of California, Nebraska, Arizona, and Montana but later decreased to an area of over 100,000 ha (Esendal, 2001).

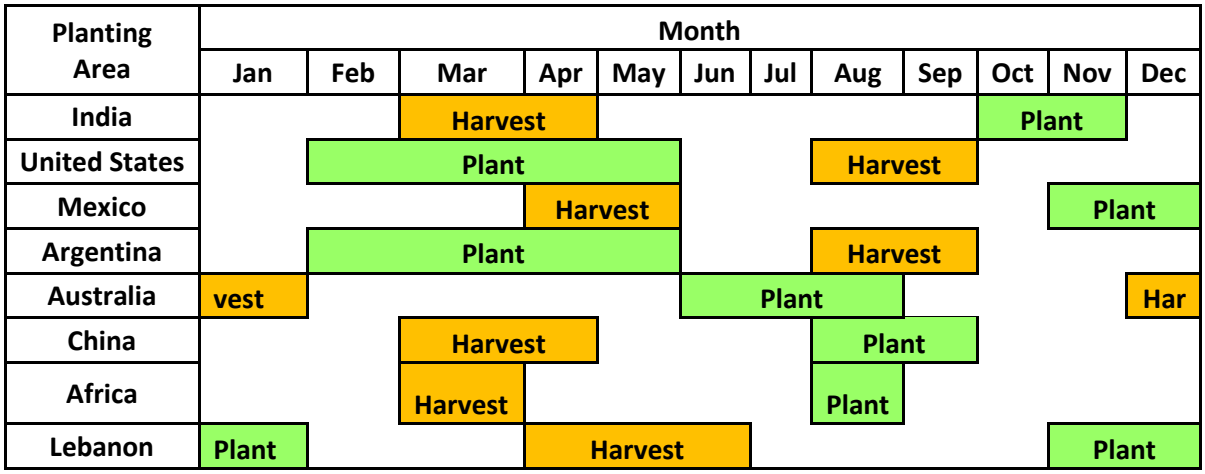

Fig. 1. International Safflower Production Calendar (*7th International Safflower Conference*, *2008*)

5. Uses

a. Whole Plant

All parts of the safflower plant are sold by herbalists in India and Pakistan as 'pansari' to remedy various ailments and as an aphrodisiac (Knowles, 1965). Safflower foliage is used to prepare a tea that can prevent or reduce the incidence of abortion and infertility by women in Afghanistan and India (Weiss, 1983). The whole plant is a promising alternative feedstuff for small ruminants that can be well preserved by ensiling (Ossama, 2002). In India, Pakistan and Burma, immature leaves and thinning are eaten boiled, as a vegetable side dish with curry or rice (Singh *et al*., 1996). Until this century, soot from charred safflower plants was used to make kohl, the Egyptian cosmetic (Weiss, 1983).

b. Flower

Safflower florets are historically used as food and cosmetic coloring agents, replacing the expensive true saffron. The water-soluble yellow dye, carthamidin, and a water-insoluble red

dye, carthamin, which is readily soluble in alkali, can be obtained from safflower florets (Weiss, 1983).

The carthamin dye extracted from safflower florets in China is preferred as a replacement of the other food synthetic coloring agents which can have some health drawbacks (Dajue and Mündel, 1996). Cosmetic rouge can be made from carthamin dye mixed with French chalk, and the Japanese cosmetic (Weiss, 1983) and lipsticks include safflower coloring (Smith, 1996).

The increased production of cheaper synthetic dyes like aniline decreased the use of safflower flowers as a source of edible color gradually during the 20th century (Singh *et al*., 1996). The safflower pollen is valued in China because it is easily collected and contains many nutrients (Dajue and Mündel, 1996).

c. Seeds

Safflower seeds are surrounded by a thick fibrous hull. They are smooth, shiny and angular, about 6-9 mm long, white or brownish and white with grey, brown or black stripes. They generally contain 33-60 % hull and 40-67 % kernel. Thin-hulled varieties have been developed (Dajue and Mündel, 1996).

The majority of the produced bright white safflower seeds are used as bird-seeds for parrots and other domestic birds same as for wild birds and some pets (Peterson, 1996). In the US, Canada, Egypt, Japan and France, the yearly production reached 25 thousand tons in 1995 (Gyulai, 1996) with an estimation to increase in the upcoming years and it reached 591,997 tons in 2011 (FAO, 2013).

In Iran, a paste of seeds is used to hasten cheese curd formation (Knowles, 1965). Ftfit is a well-known drink prepared in Ethiopia, used on fast-days, made of finely pounded safflower kernels mixed with water. Also, roasted seeds, generally mixed with chickpeas, barley or wheat, are eaten as a snack food in Ethiopia and Sudan (Belayneh and Wolde-Mariam, 1991). The Egyptians grind the kernels and mix them with sesame (Knowles, 1965).

d. Oil

Presently, the safflower is being planted for extracting its highly beneficial oil for either cooking or salads and margarine. Safflower oil is stable and its consistency does not change at low temperatures, making it particularly suitable for use in chilled foods. Safflower oil salad dressings have remained stable and satisfactory to –12ºC (Weiss, 1971). In addition, high oleic safflower oils are very stable on heating, and do not give off smoke or smell during frying (Gyulai, 1996).

There are many different cultivars of Safflower where each has its own characteristic in yielding specific oil fatty acids composition. Some cultivars are high in oleic acid, others in linoleic or stearic acid (Table 3).

The increased demand for the Safflower oil, especially in Europe, Canada and Japan was mainly because of its highest poly-unsaturated to saturated fatty acids ratios when compared to other oil types and where it is known that Poly-unsaturated fats are associated with lowering blood cholesterol. Also, mono-unsaturates such as oleic safflower oil tend to lower blood levels of LDL without affecting HDL (Smith, 1996).

Table 3. Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1) and Linoleic (C18:2) Acids Content of Oil of Selected Safflower Lines and Possible Genotypes (Knowles 1989)

	Fatty Acid Content in Safflower Oil (%, range)			
Oil type	C 16:0 Palmitic	C 18:0 Stearic	C 18:1 Oleic	C18:2 Linoleic
Very high linoleic	3-5	1-2	5-7	87-89
High linoleic	6-8	2-3	16-20	71-75
High oleic	5-6	1-2	75-80	14-18
Intermediate oleic	5-6	1-2	41-53	39-52
High stearic	5-6	4-11	13-15	69-72

Safflower oil is considered nutritionally similar to olive oil but with a lower cost (Dajue and Mündel, 1996).

e. Hulls

The hulls may be used in potting mixtures for plant nurseries, to make packing and insulation materials, and as filler for bricks (Oyen *et al*., 2007). As a feedstuff, they are unpalatable, reduce gain, and can constitute only a small part of the roughage requirement (Göhl, 1982). Hulls contain about 60 % crude fiber and 21 % lignin (Hertrampf *et al*., 2000).

f. Meal

After partial or complete hull removal and oil extracting, safflower meal is obtained. The quality of the safflower meal is variable and depends on the amount of hulls and the extent of the oil extraction. Safflower oil can be obtained from the seeds by cold-pressing, expeller

pressing, or solvent extraction (GRDC, 2010). The residual fat varies with the extraction method, from under 2% to 15%. Crude protein also varies: from 20- 25% for un-decorticated meal to more than 50% if hulls are well removed (Dajue and Mündel, 1996). The safflower meal is considered a medium-protein feed suitable for ruminants with 18-21% protein and 34-37% crude fiber (Andrews *et al*., 1961). Safflower meal mixed with barley as feed for dairy cattle (Pittman and Drapter, 1955), decreases the dustiness of the feed and increase its fat and protein content. Also safflower meal is considered a very suitable product for making pellets.

The quality of safflower seed meal for use in poultry diets is considered poor because the meal is deficient in the essential amino acids lysine, methionine, and isoleucine (Darroch et al., 1990). Safflower meal is an excellent source of phosphorus and a good source of zinc and iron. In general, the vitamin content of safflower meal is low, but when compared to soybean meal, safflower meal is a good source of biotin, riboflavin, and niacin (Darroch, 1990).

Although cattle apparently find safflower meal palatable, it has a bitter taste which makes it unacceptable to humans. Protein isolates prepared from de-bittered meal can be used to fortify bread, pasta and nutritional drinks (Dajue and Mündel, 1996).

6. Potential in Mediterranean Region and Lebanon

Safflower adaptation and yield in the low and elevation areas of some Mediterranean Nations (Lebanon, Palestine, Cyprus and Syria) were tested and have provided useful results. The high quality edible oil extracted from safflower seeds, provides a great potential for the crop in order to be widely grown in the Mediterranean region, (Yau *et al*., 1999) suggested this hypothesis based on consideration of crop adaptation, husbandry and economics.

Yau (2004) conducted a three years experiment on safflower, barley, lentil and chickpea in the Bekaa- valley (1000m) where he compared the yield and the economical returns of the four different crops. He concluded that safflower gave a similar seed yield to barley and a higher yield from both chickpea and lentil, but gave a much higher economical return than all the other crops. He advised farmers to adapt safflower in their rotation program to increase the crop diversification and to increase the production of the edible oil.

7. Anti-Nutritional Factors Found in Safflower Seeds

Anti-nutritional factors are defined as naturally occurring substances that interfere with nutrient intake and/or availability in the animal. Their biological effects can range from a mild reduction in animal performance to death (Saini, 1989). Studies with animals have demonstrated that the anti-nutritional factors in raw, unprocessed oil seeds, in general, produce adverse physiological effects when ingested and, lower nutrient utilization and animal performance, and where ten major anti-nutritional factors were defined as non-protein amino acids, quinolizidine alkaloids, cyanogenic glycosides, isoflavones, tannins, oligosaccharides, saponins, phytate, lectins and protease inhibitors (Enneking and Wink,2000 and Nalle, 2009). The decrease in amino acid digestibility in diets containing tannins is attributed to the binding of dietary tannins and feed proteins, and the complexation of tannins with digestive enzymes (Bressani *et al*., 1988 and Nalle, 2009).

A maximum daily feed intake of 1.03 g/kg DM tannins for the two-bird experimental unit, there was no evidence to suggest that tannins had significant adverse effects on broiler performance (Wareham *et al,*.1993). It has been noted, however, that tannins negatively affected duckling growth, poultry egg production and nitrogen digestibility (Aramananious *et*

al., 1973; Kantar, 1994) and reduced live-weight gain in chicks as observed by Ward *et al*. (1977) and Kantar (1994).

Ingale and Shrivastava (2007) found that the Cyanide content of two safflower varieties varied from 3.46 mg/100g in PBNS-12 to 3.730 mg/100g in PBNS-40; oxalate content varied from 0.079 g/100g in PBNS-12 to 0.085 g/100g in PBNS-40; tannin content varied from 0.511 g/100g in PBNS-12 to 0.530 g/100g in PBNS-40 while no inhibition of trypsine and haemagglutinating activity was observed in PBNS-12 and PBNS-40. These values were closely similar to each other and were found to be similar to other oil seeds (Dominguez *et al*., 1993; Montgomery 1969; Chubb 1982)

Ingale and Shrivastava (2011) found that the safflower oil seeds when compared to the sunflower and groundnut seeds had the least Cyanide content (3.458%) whereas it was maximum (4.818%) in sunflower. The tannin content of safflower seeds was found to be in the range from 0.51 to 0.53 %. The lowest content of oxalate was (0.079%) in safflower seeds. Furthermore, no trypsine inhibitor activity was observed in the three varieties of oil seeds. Hemagglutinin activity was observed in the range from 1:16 to 1:8 in sunflower seeds, while it has not been reported in safflower seeds when tested on chicken or goat bloods. Also the workers found that safflower seeds presented a significantly better feed efficiency ratio and nitrogen utilization percentage than sunflower seeds when fed to the rats. The protein fraction of the meal contains two phenolic glucosides, the bitter-flavoured matairesinol-β-glucoside and the purgative 2-hydroxyarctiin-β-glucoside. They can be removed by extraction with water or methanol, by the addition of β-glucosidase (Darroch, 1990), or by a combination of physical and enzymatic treatments (Jin *et al*., 2010).

8. Poultry Research on Safflower Meal

a. Broilers

In 1947, Kratzer and William prepared a safflower meal which was fed to chicks as the only source of protein with addition of amino acids to determine specific deficiencies. They found that the omission of arginine, methionine and lysine singly or glycine and cystein together resulted in a significant decrease in growth (Kratzer and Williams, 1947).

The combination of 2 parts of safflower protein to 1 part of soybean protein gave significantly poorer growth than soybean alone or 1 part of safflower and 2 parts of soybean, also safflower alone gave poor growth (Kratzer and Williams, 1951).

Safflower meal can substitute 50% of the soybean meal in a corn-soybean diet. Also, safflower can replace all of the soybean meal if the diet is supplemented with lysine. Valadez *et al*. (1964) also found that, plasma lysine concentrations of birds fed various diets, were reflecting the lysine content of the diet. While Kohler *et al*. (1968) found out that chick growth rate from lysine supplemented safflower rations exceeded that from soy rations.

Lysine supplemented safflower rations produced better chick growth but poorer feed efficiency than the soy rations. Feed efficiency was maintained the same when the chicks were fed iso-caloric safflower and soybean meals. Also the chick weight gains from 18% CP SFM were equal to those from the 22% CP SBM (Kohler *et al*., 1968).

Farran *et al*. (2010b) prepared a de-hulled clean extruded (SFM) safflower meal with 58.4% CP, 11.7% crude fat, 2.59% crude fiber, and 2564 kcal Apparent MEn/kg. The trial showed that extensive de-hulling of safflower seeds followed by cold extrusion resulted in a low-fiber CSM that is rich in both energy and protein. Compared with SBM 44, this CSM is

higher in arginine, slightly richer in TSAA and tryptophan, but deficient in lysine (Farran *et al.*, 2010b).

Farran *et al*.(2010b) suggested that the de-hulled extruded safflower meal can replace up to 67% soybean meal in a practical diet without affecting broiler performance and thus considered as a promising feed ingredient for the poultry industry.

b. Layers

White Leghorn pullets fed a diet containing 50% safflower oil during the first 2 weeks of egg production, produced consistently greater egg weight than control groups fed tallow where the amount of linoleic acid in the diets was 4.4 and 0.6% respectively (March and Macmillan, 1990). Results of preliminary feeding trials indicate that at least 15% of safflower seed oil meal can be fed in place of soybean oil meal in an all-mash ration for laying hens (Grau and Zweigart, 1953).

9. Effects of Feeding Safflower on Chicken Immune System

The standard chicken diet supplemented with 0.1% safflower leaves fed to coccidial parasite-infected chickens exhibited body weight gains, identical to those of uninfected controls, and significantly reduced fecal oocyst shedding, compared to animals that were given a non-supplemented standard diet (Lee *et al*., 2009). Furthermore, there were increased splenic lymphocytes proliferation as well as greater percentages of CD4+ T cells; however, decreased CD8+ cells were observed in animals fed a 0.1% safflower leaves-supplemented diet, which suggests a protective function of these cells in innate immune response against *Eimeria acervulina* (Lee *et al*., 2009). Similar results were obtained by Yun et al., 2003 who

demonstrated that a treatment of mice with oat ß-glucan decreased the percentage of CD8+ cells and increased CD4+ cells concomitant with enhanced disease resistance against S*taphylococcus aureus* and *Eimeria vermiformis* infections.

In addition, IFN-γ, IL-8, IL-15, and IL-17 transcripts in the 0.1% safflower-supplemented group were higher than the non-supplemented controls. These results indicate that safflower leaf, when given as a dietary supplement, possesses immunity enhancing properties and protective immunity improvement against experimental coccidiosis infection (Lee *et al*., 2009).

Another investigation was conducted to examine the effects of methanol extracts of 3 Korean indigenous plants (dandelion root, mustard leaf, and safflower leaf) on various in-vitro parameters of innate immunity (peripheral blood lymphocyte proliferation, nitric oxide production by HD11 macrophages, and free radical scavenging activity) and tumor cell growth (Lee *et al*., 2007).. All plant extracts inhibited tumor cell growth and exerted antioxidant effects compared with the control samples. In addition, safflower leaf extracts stimulated lymphocyte proliferation while mustard leaf induced nitric oxide production. These results demonstrate, for the first time, that traditional Korean medicinal plant extracts are effective in enhancing innate immunity and suppressing tumor cell growth (Lee *et al*., 2007).

Furthermore, sunflower oil, palm oil and safflower oil can be used as sources of oil for broiler diets without having any effect on performance, immune responses or the activity of anti-oxidizing enzymes (Rama Rao *et al*., 2011).

10. Feed Mixing Based on Digestible Amino Acids

For a dietary amino acid to be retained in tissue protein in an animal, the amino acid needs to be ingested by the animal and absorbed from the intestinal tract (Stein, 2003). The digestibility is defined as the difference between the amount of a certain amino acid ingested by the animal and the amount that is excreted in the feces or ileal fluids of the animal divided by the amount that is ingested (Sauer and Ozimek, 1986). It is assumed that the digestible amount of dietary amino acids equals the amount that was absorbed. By multiplying the fraction calculated by 100, the digestibility coefficient is calculated. Thus, digestibility coefficients are calculated by measuring the undigested quantity of dietary amino acids rather than the portion that was digested (Stein, 2003).

CHAPTER III

MATERIALS AND METHODS

A. General Procedure

Two experiments were performed in the Bekaa at the Agricultural Research and Education Center (AREC) of the American University of Beirut to determine the effects of feeding various levels of extruded safflower meal included in a starter practical corn-soybean meal diet on the performance of male broilers. In the first experiment, six graded levels (by an increment of 20%) were used in a starter diet formulated on digestible amino acids basis using male broilers. Performance and internal organ weights of birds fed various levels of SFM were compared to birds fed the practical corn-soybean control diet.

The second experiment was designed to test and compare the performance of male broilers fed nine levels of SFM (0, 35, 40, 45, 50, 55, 60, 65 and 70 %) included in a starter corn-soybean diet prepared on digestible amino acids basis to those fed the practical starter Corn- Soybean diet. In addition, the serum antibody titers were analyzed to determine the effects of feeding safflower meal on the vaccination immune response of the birds.

The purpose of these experiments was to find the best level of extruded safflower meal that can replace or substitute a definite level of the conventional used soybean meal in broiler starter diets without affecting the bird's health and performance.

B. Preparation of the Safflower Meal

Safflower seeds (PI 603207) obtained from AREC were decorticated in a centrifugal mill and hulls were removed partially through a column seed cleaner (Agriculex, Guelph, Ontario, Canada). Oil from the partially dehulled kernels was cold-extruded (CA59G, IBG Monforts Oekotec GmbH and Co., Monchengladbach, Germany) and was subjected to further hull removal and cleaning when ran through the above-mentioned column seed cleaner at a higher speed to produce extensively clean kernels that were extruded to obtain clean safflower meal.

C. Proximate Analysis of the Feed Ingredients

Proximate analysis methods (AOAC, 1998) were applied to analyze test feed ingredients (Corn, Safflower and Soybean) for moisture, crude protein, crude fat, crude fiber, and ash.

D. Amino Acids Analysis

The amino acid profiles of the feed ingredients (corn, soybean, safflower) and the final feeds were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories according to AOAC (1998) (Tables 4 and 10).

The concentration of amino acids, including TSAA and tryptophan in the test feed samples, were determined using HPLC 2690 (Waters Co., Milford, MA). Except for tryptophan, all amino acids in feed samples were quantified after acid hydrolysis in 6 N HCl using the 982.30E and 982.30Ea methods of AOAC (1998) in the presence of phenol at 110°C for 24 h. For TSAA determination, samples were subjected to performic acid oxidation before acid hydrolysis as in AOAC (1998) official method 982.30Eb. Tryptophan was quantified after sample hydrolysis in barium hydroxide at 120°C for 16 h according to AOAC (1998)

official method 982.30Ec. All amino acids, except for tryptophan, were derivatized using the AccQTag method of Waters, whereas all amino acids were separated by Waters HPLC column (AccQ-Tag 3.9 × 150) and then identified and quantified using Waters 474 scanning fluorescence detector at a range between 285 and 345 nm for tryptophan and 250 to 395 nm for the other amino acids.

Table 4. Amino Acids Composition and CP of Feed Ingredients (% as is basis)

Amino Acid	SFM	SBM	Corn
Aspartic Acid	4.38	5.53	0.48
Threonine	1.4	1.92	0.26
Serine	1.65	2.14	0.31
Glutamic Acid	8.6	8.69	1.23
Proline	1.98	2.64	0.63
Glycine	2.54	2.14	0.29
Alanine	1.95	2.2	0.52
Cysteine	0.66	0.69	0.15
Valine	2.13	2.2	0.31
Methionine	0.71	0.67	0.15
Isoleucine	1.62	2.24	0.24
Leucine	2.88	3.81	0.81
Tyrosine	1.29	1.72	0.2
Phenylalanine	2.05	2.5	0.34
Lysine	1.52	3.13	0.25
Histidine	1.13	1.27	0.21
Arginine	4.32	3.55	0.35
Tryptophan	0.8	0.76	0.06
CP %	**49.18**	**49.53**	**6.69**

E. Experiment 1

An experiment was conducted as a complete randomized design to test the effects of feeding different levels of SFM in addition to a practical corn-soybean diet on the live weight, weight gain, feed intake, feed conversion and internal organs such as RTC, liver, heart, gizzard and spleen.

A total of 500 one day old Ross 308 male broilers were raised in Petersime battery brooders for 1 week and offered a control soybean meal diet. At the end of the week, 252 birds were selected according to their body weights, wing-banded and distributed in groups of 7 birds per pen, with 6 pens per treatment, where all the replicates had similar mean initial body weight range.

Six diet treatments were formulated using the least cost program, based on digestible amino acids, to be iso-caloric (3150 Kcal/kg) and iso-nitrogenous (23% CP) and to meet the NRC (1994) and the Ross Broiler Nutrition Specifications (2007) requirements. The diets were fed to the birds for a period of two weeks with 6 replicates per treatment. The six treatments were mixed in such a way using a combination of SFM and SBM where 0, 20, 40, 60, 80 and 100% of the dietary protein was provided by SFM (table 5). All the diets were formulated using least cost computerized program.

At the age of 3weeks, all the birds were individually weighed and a sample of two birds per pen representing the average weight of the pen were selected and slaughtered to determine RTC, heart, gizzard, liver and spleen weights. All the data were analyzed using the General Linear Model (GLM procedure) and means were separated using Duncan's multiple range test (SAS, 1992).

Table 5. Percentage Calculated Feed Composition of the First Experimental Diets

Ingredients	SFM100	SFM80	SFM60	SFM40	SFM20	SBM
Yellow Corn	60.836	60.339	59.843	59.346	58.85	58.353
Safflower Meal	34.236	27.389	20.542	13.694	6.847	0
Soy bean 49	0	7.135	14.269	21.404	28.538	35.673
Salt	0.419	0.424	0.429	0.434	0.439	0.444
Limestone	1.194	1.191	1.188	1.184	1.181	1.178
Dicalcium-Phosphate	1.659	1.688	1.717	1.746	1.775	1.804
DL Methionine	0.232	0.226	0.219	0.213	0.206	0.2
Lysine	0.65	0.537	0.425	0.312	0.2	0.087
Threonine	0.202	0.162	0.121	0.081	0.04	0
Soybean Oil	0.237	0.572	0.906	1.241	1.575	1.91
Amprol HI-E	0.1	0.1	0.1	0.1	0.1	0.1
Vit. Min. Premix	0.25	0.25	0.25	0.25	0.25	0.25
Calculated Analysis						
ME, (Kcal/Kg)	3150	3150	3150	3150	3150	3150
Crude Protein (%)	23	23	23	23	23	23
Calcium (%)	1	1	1	1	1	1
Available P(%)	0.45	0.45	0.45	0.45	0.45	0.45
Lysine (%)	1.2	1.2	1.2	1.2	1.2	1.2
Methionine (%)	0.5	0.5	0.5	0.5	0.5	0.5
Meth + Cyst (%)	0.7	0.7	0.7	0.7	0.7	0.7

*Provided per kilogram diet:vitamin A (retinyl acetate), 12,500 IU; vitamin D3, (cholecalciferol), 2,500 ICU; vitamin E (dl-α-tocopheryl acetate), 30 IU; vitamin K (menadione sodium bisulfide), 3.0 mg; vitamin B1, 2.7 mg; vitamin B2, 12.6 mg; vitamin B6, 6.6 mg; vitamin B12, 13.2 µg; Niacin, 53.1 mg; Folic acid, 1.65 mg; pantothenic acid (calcium-D-pantothenate), 15.9 mg; D-Biotin, 55.2 µg; Choline, 300 mg; vitamin C, 100 mg; BHT, 150 mg; manganese, 108 mg; iron, 102 mg; zinc, 77.4 mg; copper, 16.1 mg; cobalt, 0.16 mg; iodine, 0.60 mg; selenium, 0.46 mg.

The amino acid analysis of the feed ingredients (Table 4) and the treatment diets (Table 5) were performed at the University of Missouri Agriculture Experiment Station Chemical Laboratories according to AOAC (2006) and the amount of tannins in the SFM were determined at AUB, using the method of Price *et al.* (1978).

All the birds were provided feed and water ad-libitum and 24 hours continuous light. Also litter-trays and waterers were cleaned on daily basis. Mortality and unusual behavior were also recorded.

F. Experiment 2

Another experiment was conducted in a Petersime battery brooder using a complete randomized design, to test the effects of feeding various levels of SFM in addition to a practical corn-soybean diet on the live weight, weight gain, feed intake, feed conversion and serum antibody titers.

A total of 800 one day old Ross 308 male broilers were raised for 1 week and fed the same control diet. At the end of the first week, 350 birds were selected according to their body weights, wing-banded and distributed in 50 groups of 7 birds per pen, where all the replicates have similar mean initial body weight. In addition, a total of 50 birds (5 birds/treatment), one bird from each cage, were selected for blood sampling at days 14 and 28 in order to analyze the sera antibody titers to Infectious Bursal Disease, Infectious Bronchitis, and NewCastle Disease virus using Idexx Elisa plates, to test if there is any immunological effect on the birds fed the experimental diets.

Ten diet treatments were formulated using the least cost program, based on digestible amino acids, to be iso-caloric (3150 Kcal/kg) and iso-nitrogenous (23% CP) and to meet the

NRC (1994) and the Ross Broiler Nutrition Specifications (2007) requirements. Diets were offered to the birds for a period of two weeks with 5 replicates per treatment. All the treatments were mixed in such a way using a combination of SFM and SBM where 0 (control), 30, 35, 40, 45, 50, 55, 60, 65 and 70% of the dietary protein was provided by SFM (table 6). All the diets were formulated using least cost computerized program.

At the age of three weeks, all the birds were weighed individually to calculate the live weight and weight gain; in addition feed intake and feed conversion were obtained. All the resulted data were subjected to the General Linear Module for analysis, and means were separated using Duncan's multiple range test (SAS, 1992).

The amino acid analysis of the feed ingredients (Table 4), were performed at the University of Missouri Agriculture Experiment Station Chemical Laboratories according to AOAC (2006), and the amount of tannins in the SFM was determined at AUB, using the method of Price *et al.* (1978).

All the birds were provided ad-libitum feed and water and 24 hours continuous light, also litter-trays and waterers were cleaned on daily basis. Mortality and unusual behaviors were also recorded.

Table 6. Percentage Calculated Composition of the Second Experimental Diets

Ingredients	SBM	SFM30	SFM35	SFM40	SFM45	SFM50	SFM55	SFM60	SFM65	SFM70
Yellow Corn	54.201	55.009	55.149	55.288	55.43	55.571	55.711	55.851	55.996	56.136
Safflower Meal	0	11.131	13.063	14.983	16.929	18.875	20.808	22.738	24.73	26.66
Soy bean 49	38.467	26.838	24.818	22.812	20.779	18.746	16.726	14.709	12.629	10.612
Salt	0.445	0.437	0.436	0.434	0.433	0.432	0.43	0.429	0.427	0.426
Limestone	1.177	1.182	1.183	1.184	1.185	1.185	1.186	1.187	1.188	1.189
Dicalcium-Phosphate	1.788	1.741	1.733	1.725	1.717	1.709	1.701	1.692	1.684	1.676
DL Methionine	0.253	0.269	0.272	0.274	0.277	0.28	0.283	0.285	0.288	0.291
Lysine	0.1	0.3	0.335	0.37	0.405	0.44	0.475	0.509	0.545	0.58
Threonine	0.048	0.108	0.118	0.128	0.139	0.149	0.16	0.17	0.181	0.191
Soybean Oil	3.221	2.686	2.593	2.501	2.407	2.313	2.22	2.128	2.032	1.939
Amprol HI-E	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vit. Min. Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated Analysis										
ME, (Kcal/Kg)	3150	3150	3150	3150	3150	3150	3150	3150	3150	3150
Crude Prot. (%)	23	23	23	23	23	23	23	23	23	23
Calcium (%)	1	1	1	1	1	1	1	1	1	1
Available Prot. (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine (%)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Methionine (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Meth + Cyst (%)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7

*Provided per kilogram diet: vitamin A (retinyl acetate), 12,500 IU; vitamin D3, (cholecalciferol), 2,500 ICU; vitamin E (dl-α-tocopheryl acetate), 30 IU; vitamin K (menadione sodium bisulfide), 3.0 mg; vitamin B1, 2.7 mg; vitamin B2, 12.6 mg; vitamin B6, 6.6 mg; vitamin B12, 13.2 µg; Niacin, 53.1 mg; Folic acid, 1.65 mg; pantothenic acid (calcium-D-pantothenate), 15.9 mg; D-Biotin, 55.2 µg; Choline, 300 mg; vitamin C, 100 mg; BHT, 150 mg; manganese, 108 mg; iron, 102 mg; zinc, 77.4 mg; copper, 16.1 mg; cobalt, 0.16 mg; iodine, 0.60 mg; selenium, 0.46 mg.

G. Vaccination Program

The Vaccination program used for the first and second experiment is shown in table 7. In addition, the experimental practices followed the guidelines and regulations set by the Institutional Animal Care and Use Committee of the American University of Beirut.

Table 7. The Vaccination Program Used in the First and Second Experiments

Age (Day)	Vaccine	Administration Route
6	IB-MA5/Clone	Eye drop
9	Gumboro	Eye drop
15	Clone 30/ IB 491	Eye drop
18	Gumboro	Eye drop

H. Tannic Acid Analysis

The amounts of tannins in the safflower meal were determined using the method of Price *et al.* (1978), using a Jasco V-570 UV/VIS/NIR spectrophotometer.

Two grams of SFM was extracted with 10 mL of methanol in capped, rotating test tubes for 20 min. The tubes are then centrifuged in a desk top centrifuge at 3000 xg for 10 minutes. Assays were performed on the supernatant at 30 ^{o}C with reagents previously warmed to this temperature. The supernatant is dispensed in 1ml aliquots in 10 ml screw capped-glass tubes. A volume of 5 ml of Vanillin reagent was freshly prepared by mixing equal volumes of 1% vanillin in methanol and 8% concentrated HC1 in methanol, is added to one-mL aliquot of the sample. Five milliliters of Vanillin reagent is added to one-mL of methanol (the blank). A first

absorbance reading (A1) at 500 nm is performed after 20 min, using a spectrophotometer whose reading cells temperature was previously adjusted to 30 degrees Celsius. Five more readings were done at intervals of 1 min (A2, A3, A4, A5 and A6) and the absorbance of the blank is subtracted. The average of six ΔAbs is calculated for each sample. A standard curve is constructed using catechin concentrations of 0, 0.6, 1.2, and 1.6 mg/mL (Figure 2) showing a correlation coefficient of 0.9998.

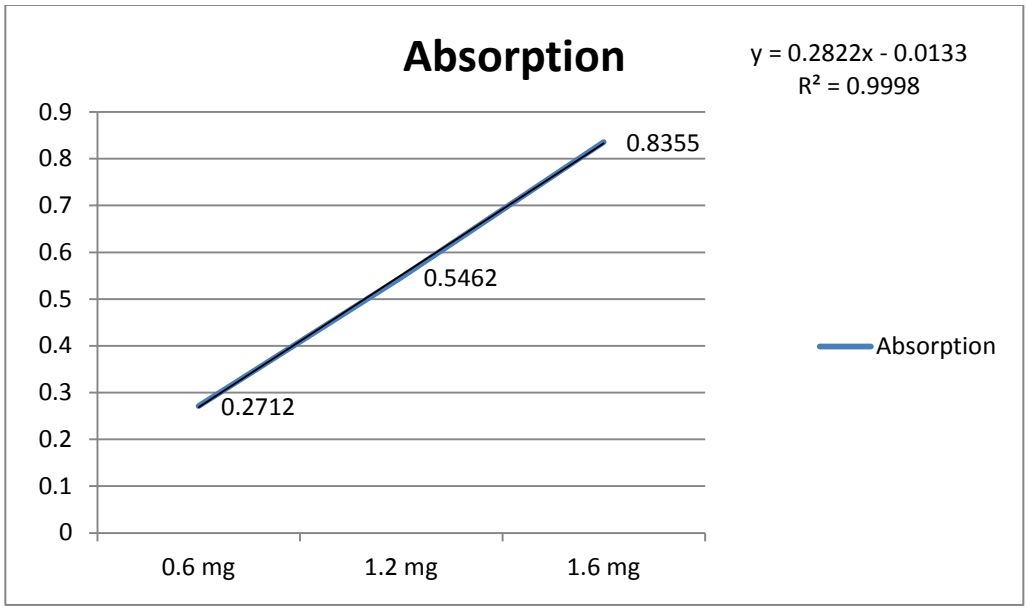

Fig. 2. Tannic Acid Absorption Calibration Curve

CHAPTER IV

RESULTS AND DISCUSSION

A. First Experiment

1. Proximate Analysis of the Feed Ingredients

The proximate analysis of the feed ingredients for testing the protein, fat, fiber, moisture and ash contents shown in Table 8, demonstrates that the corn has relatively less crude protein (6.27%) and crude fiber (1.6%), but more crude fat (5.38%) when compared with the value provided by the NRC (1994) respectively (8.5%, 2.2% and 3.8%). In general, this corn is considered of low quality because of its low protein content.

Table 8. Crude Protein, Crude Fiber, Crude Fat, Moisture and Ash of Feed Ingredients (% as is basis)

Feed Ingredient	Crude Protein (%)	Crude Fiber (%)	Crude Fat (%)	Moisture (%)	Ash (%)
Corn	6.27	1.60	5.38	6.21	1.11
Safflower	48.7	7.83	12.1	4.25	5.86
Soy Bean 48	47.5	3.23	1.82	5.23	7.05

Similarly, soybean 48 has a lower crude protein % (47.5%) and crude fiber (3.23%) but higher crude fat (1.82%) when compared to the NRC (1994) values (48.5, 3.9 and 1% respectively).

The safflower meal presented the highest crude protein content (48.7%) which was higher than the value 43% provided by NRC (1994) but lower than the value 55% obtained by Farran *et al*. (2010) . This difference in CP % between the same cold extruded safflower meal prepared by Farran *et al*. (2010) and the currently used meal is due to the partial cleaning of the seeds and applying less pressure while extracting the oil, thus resulting in higher crude fat and fiber 12.1 and 7.83% respectively when compared to the values obtained by Farran *et al*. 11 and 2.44%, respectively.

2. Amino Acids Analysis

The results of the amino acids analysis are shown in table 9. The leucine (1.52%) and isoleucine (0.74%) contents in SFM 100 were slightly lower than the other experimental diets; also the arginine (1.37%) and Methionine (0.43%) contents of SBM-control diet were the lowest among the treatments. However, we can notice a decline in the percentages of 12 different amino acids (Aspartic Acid, Threonine, Serine, Glutamic Acid, Proline, Alanine, Isoleucine, Leucine, Phenylalanine, Lysine and Histidine) when increasing the SFM levels from 20% to 100%, but all these values are still meeting the NRC (1994) requirements except of isoleucine (0.74%) in SFM 100 which is slightly below the NRC requirements (0.8%).

Table 9. Proportion Analyzed Amino Acids Composition of the First Experimental Diets

Treatments	SFM 20	SFM 40	SFM 60	SFM 80	SFM 100	SBM*
Aspartic Acid	2.13	2.02	2.13	1.89	1.74	2.12
Threonine	0.82	0.8	0.85	0.81	0.75	0.78
Serine	0.88	0.82	0.87	0.77	0.67	0.87
Glutamic Acid	3.51	3.45	3.81	3.57	3.42	3.41
Proline	1.25	1.13	1.16	1.05	1.06	1.3
Glycine	0.92	0.95	1.04	1	1.01	0.87
Alanine	1.06	1.03	1.07	1	0.98	1.04
Cysteine	0.31	0.32	0.33	0.32	0.31	0.32
Valine	1.06	1.05	1.14	1.05	1.07	1.06
Methionine	0.46	0.5	0.52	0.51	0.52	0.43
Isoleucine	0.9	0.83	0.89	0.77	0.74	0.92
Leucine	1.81	1.71	1.78	1.64	1.52	1.81
Tyrosine	0.72	0.68	0.68	0.6	0.59	0.71
Phenylalanine	1.06	1.01	1.04	0.93	0.9	1.05
Lysine	1.22	1.22	1.3	1.14	1.21	1.26
Histidine	0.55	0.53	0.57	0.52	0.51	0.55
Arginine	1.47	1.52	1.69	1.62	1.62	1.37
Tryptophan	0.29	0.27	0.32	0.28	0.28	0.26

*Birds of control group were fed a conventional soybean/corn diet

3. Performance Parameters of Birds From Various Experimental Groups

The performance parameters of birds of the experimental groups are shown in Table 10. Initial weight of birds of various experimental groups was recorded at the beginning of the experiment, and showed no significant differences among the treatments.

SFM 40 resulted in the highest live weight (969g) at day 21 which was significantly different $P<0.05$ from SFM 100 that showed the lowest live weight (912 g). In addition, SFM 40 had the highest body weight gain of 788g which was significantly higher than SBM (754g) and SFM 100 (731g) but comparable with SFM 20, SFM 60 and SFM 80. Additionally, SFM 100 presented the lowest body weight gain with a significant difference from SFM 20, SFM 40 and SFM 60. The mortality rate was within the normal range which indicates that the SFM does not increased the mortality rate of the birds.

Table 10. Initial Weight, Live Weight, Body Weight Gain, Feed Conversion and Frequency of Mortality among Birds of Different Experimental Groups of the First Trial

Treatment	Initial Weight (g)	Live weight (g)	Body Weight Gain (g)	FC	Mortality
SBM*	181.5	937^{ab}	754^{bc}	1.37^{c}	1\42
SFM20	181.1	947^{a}	765^{ab}	1.40^{bc}	1\42
SFM40	180.6	969^{a}	788^{a}	1.39^{bc}	0\42
SFM60	181.4	953^{a}	771^{ab}	1.41^{bc}	1\42
SFM80	181.5	942^{ab}	761^{abc}	1.42^{b}	0\42
SFM100	181.3	912^{b}	731^{c}	1.49^{a}	0\42
SEM[1]	**0.6**	**10.4**	**10.3**	**0.015**	**NA**

[1] Pooled standard error of means.
[a-c] Means within a row with no common superscripts differ significantly ($P < 0.05$).
*Birds of control group were fed a conventional soybean/corn diet

SBM presented the best feed conversion ratio of 1.37 which was significantly different from SFM 100 (1.49) and SFM 80 (1.42) and which agrees with the data of Farran *et al*. (2008). The feed conversion values of SFM 20, 40 and 60% were comparable to that of the control. The current results are not in agreement with those reported earlier by Kratzer and Williams (1951), and Valadez *et al*. (1965) who showed no significant change in weight gain and feed efficiency when decorticated safflower meal partially replaced SBM at levels of 25-75% in lysine balanced diets. On the other side, the current results are in total agreement with the ones obtained by Farran *et al*. (2008) which indicated that the SFM 100 resulted in the highest FCR and lowest body weight gain when compared to SFM 50 and SBM. In addition, the relatively poor performance of birds on the 100% SFM in this trial and that reported by Farran et al. (2008) could not be attributed to diet palatability since feed intake was not affected by the SFM inclusion rate. This reduction in performance, however, could be associated with a dietary imbalance related mainly to a marginal level and/or availability of essential amino acid(s) such as Iso-leucine and other amino acids.

The highest ready to cook carcass percentage (Table 11) was obtained by SFM40 and SFM80 diets, averaging 65.4%, which was not significantly different with SFM 60 and SFM 100 (both 65%) but significantly different from that of SFM20 and SBM ($P<0.05$). No significant differences were detected among all treatments for the liver, spleen, gizzard and heart as percentages of the live body weight.

Table 11. Live Weight (LW) and Ready-To-Cook (RTC), Liver, Gizzard, Heart, Spleen Percentages of Live Weight among Birds of Different Experimental Groups of the First Trial

		% of Live Weight				
Treatment	**Live WT (g)**	**RTC**	**Liver**	**Gizzard**	**Heart**	**Spleen**
SBM*	937	63.7[b]	2.5	2.1	0.6	0.09
SFM20	947	63.9[b]	2.4	2.1	0.6	0.08
SFM40	969	65.3[a]	2.6	2	0.6	0.07
SFM60	953	65.0[ab]	2.4	1.8	0.6	0.08
SFM80	942	65.5[a]	2.6	1.9	0.6	0.08
SFM100	912	65.0[ab]	2.5	2	0.6	0.08
SEM[1]	**10.4**	**0.43**	**0.13**	**0.08**	**0.02**	**0.007**

[1] Pooled standard error of means.
[a-b] Means within a row with no common superscripts differ significantly ($P < 0.05$).
*Birds of control group were fed a conventional soybean/corn diet

Results of the present work indicate that the SFM inclusion in the diet does not have any negative effects on the internal organs. In addition, an SFM inclusion of 40% resulted in higher weight gain and RTC carcass yield but in feed conversion similar to that of the control treatment during the starter period. It is worth mentioning that no other related studies investigating the effect of dietary SFM on ready to cook carcass and internal organs were found in the literature.

B. Second Experiment

1. Performance data

Based on the outcome of the first trial, the inclusion rate of SFM was limited between 30 and 70%, with an interval change of 5% between treatments.

The results of the second experiment further confirmed the results obtained in the first trial. The initial weight of the birds at 7 days was very similar among treatments with no significant difference (Table 12) but different from the initial bird's weight of the first experiment most probably due to the age of the breeders that we obtained the chicks from. In addition, birds fed SFM between 35 and 70 percent inclusion had weight gain and live weight values comparable to those of the control birds. It is worth mentioning that the SFM 30 resulted in the least weight gain (720g) and live weight (866g) values, while SFM 40 resulted in the greatest live weight (920g) and weight gain (773g).

Table 12. Initial Weight, Live Weight, Weight Gain, Feed Intake, Feed Conversion and Mortality of the Second Trial

Treatments	Initial wt(g)	Live wt(g)	Weight gain(g)	Feed Intake(g)	FC	Mortality
SBM*	147.1	886^{ab}	739^{ab}	6803	1.34	1\35
SFM30	146.6	866^{b}	720^{b}	6545	1.32	0\35
SFM35	147.3	917^{a}	770^{a}	6967	1.32	0\35
SFM40	146.9	920^{a}	773^{a}	6969	1.29	0\35
SFM45	147.7	901^{ab}	753^{ab}	7006	1.28	0\35
SFM50	146.7	909^{a}	762^{a}	6769	1.31	0\35
SFM55	146.9	916^{a}	768^{a}	6984	1.33	0\35
SFM60	146.2	899^{ab}	751^{ab}	6804	1.33	1\35
SFM65	146.1	915^{a}	769^{a}	7026	1.34	1\35
SFM70	146.8	908^{a}	761^{a}	7014	1.32	0\35
SEM[1]	**1.30**	**12.0**	**11.8**	**144.1**	**0.022**	**NA**

[1] Pooled standard error of means.
[a-b] Means within a row with no common superscripts differ significantly ($P < 0.05$).
*Birds of control group were fed a conventional soybean/corn diet

No significant differences were detected within feed intake or feed conversion among different groups. However, SFM 45 gave the least FC (1.28) followed by SFM 40 (1.29) and the highest FCs were obtained by SBM (1.34) and SFM 65 (1.34). These results in addition to the results obtained by Farran *et al.* (2010b), confirm that the SFM inclusion rate between 40 and 50 % will give feed conversion values that are comparable to those of birds fed a practical corn-soybean meal diet.

Again in this trial, the mortality rate was within the normal range which indicates that the inclusion of SFM in broiler diets is safe and does not interfere with the bird's health.

The 70% SFM diets in the current trials is probably sufficient in lysine since Valadez *et al*, (1965) reported that a corn - safflower meal diet supplemented with adequate lysine level resulted in broiler performance comparable to that of 100% SBM diet.

2. Analyzed Sera ELISA Titers

The analyzed sera titers didn't differ significantly among the different dietary treatments at the same bird's age. Consequently, SFM didn't interfere with the immunity status of the birds as birds on all SFM diets had titers similar to those of birds fed the control diet (table 13). Moreover, the low tannins level of the safflower seeds (figure 3) did not affect the bird's immunity which agrees with Saini (1989).

Table 13. Sera ELISA Titers, at Day 14 and 28, to IBDV, IB, and NDV of Birds of Different Experimental Groups of the Second Trial

Treatments	ELISA titers to IBDV		ELISA titers to IBV		ELISA titers to NDV	
	14 days	28 days	14 days	28 days	14 days	28 days
SBM*	38	818	549	733	133	626
SFM30	9	820	192	236	67	427
SFM35	10	686	208	555	81	595
SFM40	35	528	176	444	243	561
SFM45	18	633	127	558	266	772
SFM50	36	1057	344	452	42	483
SFM55	7.6	603	369	520	64	376
SFM60	36	879	247	335	83	621
SFM65	21	968	459	464	35	427
SFM70	8	708	136	463	278	549

*Birds of control group were fed a conventional soybean/corn diet

It was obvious that the titers increased at day 28 mostly due to the response to the vaccination. In addition, the low titers observed in the experimental birds at day 14 could be probably due to the age of breeders that might have had low titers as well. In addition, the storage conditions of the vaccine or other undefined reasons may lead to the same results.

Also it is worth to mention that this was the first time that the IBDV, IBD and NDV sera titers have been tested to detect the effects of feeding SFM on the broilers immune system.

3. Tannic Acid Analysis

The Tannin calibraton curve (Figure 2), using spectrophotometry, showed a high positive correlation between the concentration of Tannins and the Absorbance values between 0 and 1.6 mg of catechin equivalents/ml.

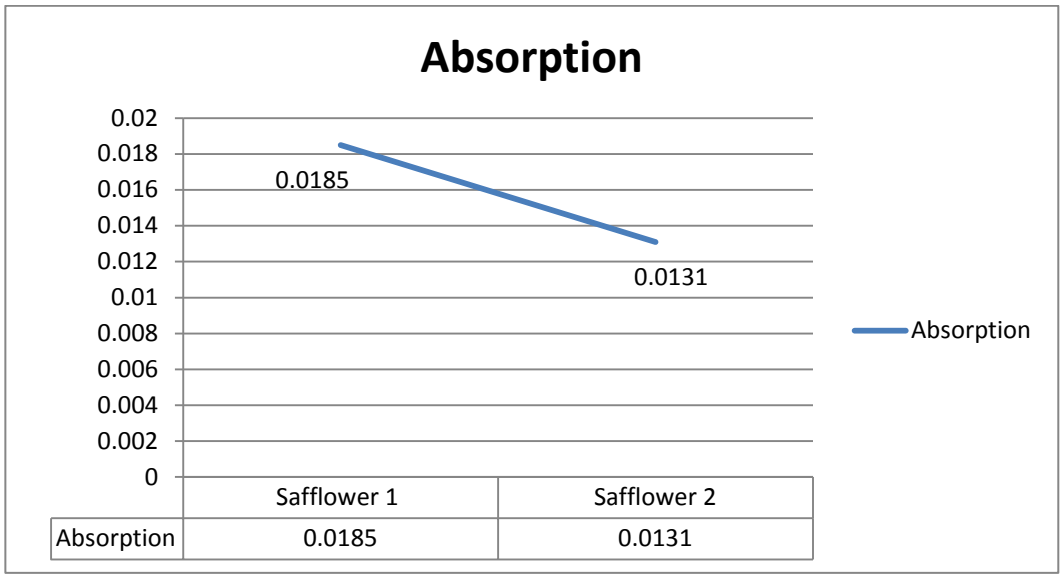

Fig. 3. Tannic Acid Absorption Curve

Tannin determination was conducted in duplicates (Figure 3), where it was detected in an average of 0.0158mg/g of dry Safflower meal. And according to Wareham *et al.*(1993), Ingale and Shrivastava (2007) and Ingale and Shrivastava (2011) it is considered as low tannin content and do not have any negative effects on bird's health and performance.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

The increasing demand for alternative poultry feed ingredients, other than corn and soybean meal, lead scientists to start investigating other available feed components which can totally or partially replace one of the conventional used ingredients. For this purpose, two experiments were conducted to test the effects of inclusion of different levels of the cold extruded locally produced safflower meal in the broiler starter diet, on the performance of day old broilers fed different treatment diets.

The first experiment was designed to test the effects of feeding different levels of SFM (20, 40, 60, 80 and 100%) in addition to a practical corn-soybean diet on the live weight, weight gain, feed intake, feed conversion, RTC and internal organs such as liver, heart, gizzard and spleen. The results showed that the treatment SFM40 presented a higher weight gain and RTC carcass yield but a feed conversion value comparable to that of the SBM control treatment. In addition, no significant differences were detected for the relative weights of the liver, heart, spleen and gizzard.

The second experiment was also designed to test the effects of feeding various proportions of SFM (30, 35, 40, 45, 50, 55, 60, 65 and 70%) in addition to a practical corn-soybean diet on the live weight, weight gain, feed intake, feed conversion and sera anti-bodies titers for ND, IBDV and IB. Again, SFM 40 presented the greatest numerical live weight and weight gain and both SFM 40 and SFM 45 presented the lowest numerical feed conversion values.

Also, no significant differences were detected among the different dietary treatments for the serum antibody titers for ND, IBDV and IB at the same bird's age.

The two conducted experiments lead to several conclusions. An inclusion rate of 40-45% SFM will increase the bird's live weight and weight gain when compared to the practical SBM starter diet, and the feed conversion may be even improved especially at an inclusion rate of 45%. In addition, the inclusion of safflower meal in the starter broiler diets has not shown negative effects on the bird's mortality rate and the anti-bodies titers for NDV, IBDV and IB. At the end, Safflower meal is a potentiated replacement crop for the soybean meal used in the broiler's commercial diets

According to the obtained data, the 40% inclusion rate is recommended to obtain the highest weight gain while the SFM 45% is the best for FCR. In addition, more studies should be conducted to test the effects of including different levels of safflower meal in broiler grower and finisher rations on the birds general performance, as well as to correct the decreasing level of the twelve amino acids that may alter the performance (higher FCR and lower weight gain) when increasing the inclusion rate of SFM. Also, it is recommended that graded levels of dietary SFM be fed to laying hens and their effects on egg performance and egg quality parameters be investigated.

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