

STUDY OF THE BIOLOGICAL EFFECT OF SUBSTITUTION SOYABEAN MEAL WITH *Lupinus albus* ON SOME BIOCHEMICAL PARAMETERS IN AWASSI LAMBS FROM WEANING TO PUBERTY

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ABSTRACT

This study was carried out at the farm of the Animal Resources Dept. / College of Agriculture / University of Tikrit during the period from 15/2/2017 to 15/6/2017. Twelve Awassi lambs at the age of 4-5 months of age with an average weight of 24.65 ± 2.49 kg, were distributed into 3 groups (4 lamb/group) to study the effect of replacement of soybean with *Lupinus albus* at levels of 0, 6 and 12% for the T1 (control), T2 and T3 respectively and its effect on some biochemical indicators including cholesterol, HDL, LDL, total protein, albumin, globulin, AST, ALT, glucose and calcium Ca^{+2} concentrations for four age periods, 4-5, 5-6, 6-7 and 7-8 month for first, second, third and fourth periods respectively. The results showed significant decrease ($P < 0.01$) in cholesterol level in T2 during the second period, while the third group showed a significant increase ($P < 0.01$) in HDL at the first period and significant decrease ($P < 0.01$) in LDL at the second and third periods. The level of globulin was increased significantly ($P < 0.01$) in T2 at the second period and significant increased ($P < 0.01$) in AST and ALT for T3 at first and second periods while serum glucose was significantly increase in T3 at the third period and Ca^{+2} levels was significantly increased ($p < 0.01$) at the same period.

Keywords: *Lupinus albus*, , Soybean meal, lamb , puberty

دراسة التأثير البيولوجي لاحتلال الترمس الابيض محل كسبة فول الصويا على بعض المعايير الكيمو حيوية في

الحملان العواسي من الفطام إلى سن البلوغ

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الخلاصة

اجريت الدراسة في حقل الانتاج الحيواني - كلية الزراعة/جامعة تكريت للفترة من 15/2/2017 إلى 15/6/2017. اثنا عشر حملا عواسيا بعمر 4-5 اشهر بمعدل وزن ابتدائي 24.65 ± 2.49 كغم، وزعت الحملان على 3 مجاميع (4 حملان/ مجموعة) لدراسة تأثير احتلال بذور الترمس الابيض *Lupinus albus* L. بمستوى 0 و 6 و 12% محل كسبة فول الصويا للمعاملة الاولى (سيطرة) والثانية والثالثة على التوالي واثرا في بعض المؤشرات الكيموحيوية والتي اشتملت على الكوليستيرول، البروتينات الدهنية واطئة الكثافة LDL، البروتينات الدهنية عالية الكثافة HDL، البروتين الكلي TP، الالبومين Alb، الكلوبولين Glob، انزيم اسبارتات امينو ترانسفيراز AST و انزيم الالانين امينو ترانسفيراز ALT، الكلوكونز Glucose و الكالسيوم Ca^{+2} بأربع فترات 4-5، 5-6، 6-7 و 7-8 للفترة الاولى والثانية والثالثة والرابعة على التوالي. اظهرت النتائج حصول انخفاض معنوي ($P \leq 0.01$) في مستوى الكوليستيرول في المعاملة الثانية وخلال الفترة الثانية بينما سجلت المعاملة الثالثة ارتفاع معنوي ($P \leq 0.01$) في مستوى HDL خلال الفترة الثانية وانخفاض معنوي ($P \leq 0.01$) في مستوى LDL خلال الفترتين الثانية والثالثة. وارتفع مستوى الكلوبولين معنويا ($P \leq 0.01$) في المعاملة الثانية خلال الفترة الثانية وتحققت زيادة معنوية ($P \leq 0.01$) في مستوى AST و ALT في المعاملة الثالثة خلال الفترة الاولى والثانية وانخفاض معنوي ($P \leq 0.01$) في مستوى كلوكونز الدم في المعاملة الثالثة في الفترة الثالثة وارتفع مستوى الكالسيوم Ca^{+2} بشكل معنوي ($P \leq 0.01$) لنفس الفترة.

كلمات مفتاحية: الترمس الابيض، كسبة فول الصويا، حملان ، بلوغ

INTRODUCTION

In many animal production systems (APS), nutrition is one of the largest breeding requirements, cost dependent on the availability of nutrients and their nutrient content (19). Provision of animal feed one of the main constraining factors for increase of livestock production, and which is an obstacle for small-scale farmers in arid and semi-arid regions to increase profitability, occurs especially during the dry season, when the quality of fodder decreases (15).

Lupinus albus L. is one of the agricultural crops used for supporting protein content and improving feeding efficiency of animals, followed by *Lupinus* (*Lupinus* spp.) where its most important legume crop is grown in winter, with three species: Blue lupins (*Lupinus angustifolius* L.) , *Yellow Lupin* (*Lupinus luteus* L.) and *White Lupin* (*Lupinus albus* L.). Lupins (*Lupinus* spp.) which are a potential significant alternative to soybean: their seed protein content is high (up to 44%) and the quality of the protein is good (3).

Lupins offer potential health benefits, and they contribute to the sustainability of cropping systems as well as increase in essential amino acids especially lysine, and increase protein digestion within the rumen (21,16). Lupine seeds contain 5.95% fat, 5-6.5% fiber, 2.65-3.7% ash and 43% carbohydrates (8, 9), 13.55% saturated fatty acids, 55.4% monounsaturated fatty acids, 31.1% unsaturated fatty acids, 55.4% oleic acid, 22.4% linoleic and 8.7% linolenic acid from unsaturated fatty acids (20). The constituent chain of Lupins is characterized by its ability to retain

water and adherence to fat (10). Thus, the cholesterol-lowering process is associated with the stimulation of LDL receptors and this has an active role in lowering blood glucose (24). It also has an effective role in reducing cholesterol, whether it is cholesterol within the body or not within the body (absorbed cholesterol) in which low cholesterol is associated with stimulation of low-density lipoprotein LDL receptors. It also has an active role in reducing blood glucose (24).

White lupine seeds are characterized by an increase in maximum protein efficiency ratio (PER) expressed by the amino acids leucine and tyrosine (1) which are, therefore, considered as protein substitutes to soybeans for functional efficiency in many diets (17). However, with low content of vitamin B1 and B2 as compared to soybeans (9) the content of protein and the balance of the amino acid play a role in improving digestion coefficient and have a chemical effect in which the normal level of total protein and albumin is maintained in blood plasma (18). On the contrary, it reduces the activity of liver enzymes such as AST and ALT by 58% and 21% respectively (11). In addition, it has a positive effect in improving the growth rate of the body by enhancing the function of the thyroid gland (4).

Therefore, the aim of this study was to identify the effect of *Lupinus albus* L. seeds as an alternative to soybeans in some biochemical indicators from weaning to puberty for Awassi lamb.

MATERIALS AND METHODS

The study was conducted from 15/2/2017 to 15/6/2017 in Constantine region at the location of the animal production farm of the Collage of Agriculture /University of Tikrit. A total of Twelve Awassi lambs were divided in to three groups (each group consisted of 4 lambs), aged between 4 - 5 months with an average control

body weight of 24.65 ± 2.49 kgs. The control group (T1) was fed on a diet* containing 12% soybean and 0% *Lupinus albus*, the 2nd group (T2) was fed with a diet containing 6% soybean and 6% *Lupinus albus*, and the 3rd group (T3) was fed on 0% Soybean and 12% *Lupinus albus*.

Table 1: Ingredient percentages of the experimental diets of Awassi lambs

Ingredients (%)	Diets		
	1	2	3
Barley	48	43	38
Wheat	19	17	18
Wheat bran	19	26	30
Soybean	12	6	0
<i>Lupinus albus</i> L.	0	6	12
Salt	1	1	1
Minerals and vitamins	0.5	0.5	0.5
Limestone	0.5	0.5	0.5
Total	100	100	100
CP%	15.8	15.6	15.4

*The chemical analysis has been calculated as the ratio of crude protein to all components of the chemical analysis tables of the Iraqi feed material (Alkhawaja et al., 1978).

Animals were housed in an individual cage 1.35m², supplied with container for diets, minerals, salt and water. Blood was collected once every 15 days from jugular vein. Samples were collected into a 10 ml serum clot tube from each lamb in the morning before feeding, and blood samples were centrifuged at 3000 rpm for 10 minutes, for separation of serum. The serum samples were stored at -20°C before analysis. Then, they were performed according to the manufacturers' instructions as follows : The method of Richmond (22) to determine the cholesterol concentration (mg/100ml) and the method of Warnick(26) to determine concentration of high density lipoprotein HDL mg /100ml and low-density lipoproteins LDL (mg /100ml). Total protein (TP) concentration (g/100ml) was determined by method of Henry(12). The method for the detection of albumin (g/100 ml) was adopted from Douma(6). The concentration of globulin (g/100ml) was calculated by subtracting albumin concentration from total protein concentration according to Kilpi(14). Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentrations U/L were determined by method of Kessler (13), and the method of Coles(5) was used to determined concentration of Glucose (mg/100ml) and calcium Ca⁺² (mg/100ml) was determined by method of Kessler(13).

STATISTICAL ANALYSIS

All data were expressed as means \pm standard error (SE). Differences between group means were

estimated using a one-way analysis of variance (ANOVA) and a Duncan (7). The test was performed to test the significance of differences among all groups using the software SAS (23), as a complete randomized design (CRD). Results were considered as statistically significant at ($P \leq 0.01$).

Data from experiment were analyzed according to the following model: $Y_{ij} = \mu + T_i + e_{ij}$

Where: Y_{ij} = the dependent variable, μ = mean, T_i = effect of the treatment (i = control, 6% *Lupinus albus*, 12% *Lupinus albus*), e_{ij} = random residual error.

RESULTS AND DISCUSSION

The results of the statistical analysis in Table (2) showed that there were no significant differences between the treatments at different levels of substitution of the Lupine seeds at ages of 6-7 and 7-8 months: Although the level of 6% of the Lupine seeds was the best, giving a significant decline ($P < 0.01$) in blood cholesterol at age 5-6 months as compared with control 44.67 (mg/100) regarding as compared with 50.66 and 48.88(mg/100) for the first and second groups respectively. The concentration of high-density fats, the results of the statistical analysis indicated that there were no significant differences between the three group as animal age was progressed.

Table 2: Effect of substitution of soybean meal with *Lupinus albus* for on the concentration of cholesterol, HDL and LDL in Awassi lambs (as appeared \pm S.E.)

	Groups	Age (months)			
		4-5	5-6	6-7	7-8
Cholesterol mg/100ml	T1	33.22 \pm 1.50b	50.66 \pm 0.79a	52.10 \pm 2.32	61.85 \pm 7.79
	T2	34.97 \pm 1.53b	44.67 \pm 0.55b	49.64 \pm 7.60	59.40 \pm 9.45
	T3	45.56 \pm 1.35a	48.88 \pm 0.14a	49.64 \pm 4.54	52.94 \pm 3.30
High-density lipoproteins (HDL) mg/ml	T1	19.87 \pm 1.22b	25.07 \pm 0.28	32.12 \pm 1.87	35.56 \pm 7.08
	T2	22.52 \pm 0.79b	25.98 \pm 1.83	30.33 \pm 3.03	32.24 \pm 4.82
	T3	27.54 \pm 1.20a	28.61 \pm 1.28	33.63 \pm 4.70	31.48 \pm 1.07
Low-density lipoproteins LDL mg /100ml	T1	34.54 \pm 0.65a	34.65 \pm 0.89a	23.11 \pm 1.07 ab	22.15 \pm 2.40
	T2	35.04 \pm 0.60a	14.88 \pm 0.94b	28.32 \pm 2.48 a	22.83 \pm 4.41
	T3	28.02 \pm 1.13b	15.36 \pm 2.23b	17.57 \pm 0.67 b	18.02 \pm 4.07

- Mean having different letters at the same column are significantly differed at level of 0.01.

-T1: control group 0% *Lupinus albus* L. 12% soya bean.

-T2: 6% *Lupinus albus* L. 6% soya bean.

. -T3: 12% *Lupinus albus* L. 0% soya bean

While low-density fats have been significantly ($P < 0.01$) reduced at the age of animals and feeding 12% lupine seeds + 0% soya bean in the third group at the age of 4-5 months 28.02 mg/100ml as compared with to the control group 34.5 (mg/100ml) at age 5-6 months which amounted to 15.36 mg/100ml compared to the control group 34.65 (mg/100ml). This decrease was due to the far matrix of long cohesive chain, water retention and fat adhesion of the Thermos protein plant (10).

In Table (3), the results of the statistical analysis showed that there were no significant differences among animal groups in which soybean was substituted *Lupinus albus* at the

levels 0%, 6% and 12% respectively, regarding the characteristic of total protein and globulin as the animal age progresses, although the second group at 4-5 months of age of was significantly superior to the third one at the same of age, value were 6.78 and 4.25 (g/100ml) for total protein and globulin for T2 at age of 4-5 months compared with 5.68 and 2.27 (g/100 ml) for T3 at the same age. This can be explained by the functional protein efficiency of the thermos seeds and the balance of essential amino acids that run on improved digestion (25) which chemically affected the maintenance of the normal level of total protein, albumin and globulin in blood plasma.

Table 3: effect of level substitution of soybean meal with *Lupinus albus* on concentration of serum total protein, albumin and globulin (g/100ml±S.E.)

	Groups	Age (months)			
		4-5	5-6	6-7	7-8
Total protein g/100ml	T1	5.65±0.13b	5.80±0.11	6.05±0.19	6.33±0.25
	T2	6.78±0.34a	5.65±0.12	6.18±0.25	6.33±0.30
	T3	5.68±0.09b	5.39±0.18	5.23±0.31	5.69±0.25
Albumin g/100ml	T1	2.40±0.27	2.80±0.06	2.69±0.13	3.13±0.14
	T2	2.53±0.10	2.68±0.20	2.80±0.07	2.98±0.14
	T3	2.95±0.06	2.61±0.07	2.39±0.19	2.61±0.09
Globulin g/100ml	T1	3.25±0.16ab	3.01±0.16	3.36±0.28	3.20±0.26
	T2	4.25±0.40a	2.98±0.22	3.38±0.26	3.35±0.30
	T3	2.72±0.05b	2.78±0.11	2.83±0.21	3.08±0.16

- Mean having different letters at the same column are significantly differed at level of 0.01.

-T1: control group 0% *Lupinus albus* L. 12% soya bean.

-T2: 6% *Lupinus albus* L. 6% soya bean.

. -T3: 12% *Lupinus albus* L. 0% soya bean

Table 4 shows the effect of feeding *Lupinus albus* on some liver enzymes. Higher ($P<0.01$) AST and ALT concentration were detected in third group of lambs as compared with other groups during the first month. As a result of the cumulative effect of feeding thermos seeds, AST and ALT, values were decreased in the third group (12% lupinus + 0% soya beans) at 6-7 months as compared with the control group (0% lupinus + 12% soy) and second group (6% Lupinus + 6% soya beans) due to the effective role of the chemical effect of thermos protein as affected by the balance of amino acids and that have an effective role in the lack of activity level of liver enzymes such as AST and ALT. This was seen in the third group that gave the lowest level to AST and ALT liver enzymes at the age of months. At 7-8 months at age higher ($P<0.01$) AST concentration was recorded in T3 as compared with that of T1 and T2, values were 217.89, 133.53 and 95.70 U/L respectively.

As shown in Table (5), there was a significant decline in the blood glucose concentration over a period of 90 days in third group (12% lupines ± 0% soybeans) associated with the highest significant ($P<0.01$) decline as compared to the control group (0% Lupines ± 12% soybeans), while the second group was associated during age, of 4-5 and 5-6 months with a significant ($P<0.01$) decline as compared with control group. In the last two periods, there were no significant ($P<0.01$) differences between the first and second groups. The reason for the lower blood glucose concentration in the third group as compared with the other groups is the effective role of the white Lupines seeds alkaloids 13- α -hydroxy lupanine, 17-oxo-lupanine, and 2-thionosparteine that have been reported to enhance insulin secretion however, the former two compounds showed effects at elevated glucose concentrations in lowering the blood glucose concentrations (24). As shown in Table 5 result of effect of feeding thermos seeds at 4-5 months, calcium concentration was increased significantly ($P<0.01$) in the third

group (12% lupinus + 0% soya beans) 11.51 as compared with the control group (0% lupinus + 12% soy) and second group (6% Lupinus + 6% soya beans) 9.37 and 10.38 (mg/100ml)

respectively. While in the last three periods 5-6, 6-7 and 7-8 months there were no significant ($P<0.01$) differences in calcium concentration.

Table 4 : The effect of level of substitution of soybean meal with *Lupinus albus* on concentration of ALT and AST (U/L \pm S.E.)

	Groups	Age (months)			
		4-5	5-6	6-7	7-8
Aspartate aminotransferase (AST) U/L	T1	55.93 \pm 2.48b	73.23 \pm 0.70b	85.95 \pm 2.35 a	113.53 \pm 14.99b
	T2	60.80 \pm 3.66ab	73.25 \pm 2.5b	88.05 \pm 2.34 a	95.70 \pm 4.99b
	T3	72.15 \pm 1.88a	94.69 \pm 1.43a	68.23 \pm 2.72 b	217.89 \pm 56.76a
Alanine aminotransferase (ALT) U/L	T1	9.45 \pm 0.16b	12.83 \pm 0.69	11.25 \pm 0.69 a	16.18 \pm 0.55
	T2	12.23 \pm 0.74b	13.83 \pm 0.80	12.58 \pm 0.55 a	16.15 \pm 0.67
	T3	15.85 \pm 0.93a	13.93 \pm 0.72	5.48 \pm 0.28 b	16.25 \pm 0.78

- Mean having different letters at the same column are significantly differed at level of 0.01.

-T1: control group 0% *Lupinus albus* L. 12% soya bean.

-T2: 6% *Lupinus albus* L. 6% soya bean.

-T3: 12% *Lupinus albus* L. 0% soya bean.

Table 5: Effect of level of substitution of soybean meal with *Lupinus albus* on the concentration of glucose and Ca⁺²

	Groups	Age (months)			
		4-5	5-6	6-7	7-8
Glucose mg/100ml	T1	94.20 \pm 1.95a	90.32 \pm 3.76a	80.08 \pm 1.29 a	60.92 \pm 3.11
	T2	87.88 \pm 3.30ab	73.25 \pm 1.06b	76.09 \pm 3.10 a	74.44 \pm 6.34
	T3	79.20 \pm 2.55b	83.13 \pm 0.54ab	53.39 \pm 4.95 b	67.26 \pm 2.53
Ca ⁺² mg/100ml	T1	9.37 \pm 0.17c	10.12 \pm 0.35	10.11 \pm 0.37	10.74 \pm 0.28
	T2	10.38 \pm 0.14b	10.49 \pm 0.26	10.68 \pm 0.14	11.54 \pm 0.24
	T3	11.51 \pm 0.26a	10.29 \pm 0.10	9.57 \pm 0.44	10.33 \pm 0.34

- Mean having different letters at the same column are significantly differed at level of 0.01.

-T1: control group 0% *Lupinus albus* L. 12% soya bean.

-T2: 6% *Lupinus albus* L. 6% soya bean.

-T3: 12% *Lupinus albus* L. 0% soya bean

In conclusion from this study the higher levels of *lupinus albus* substitution caused an increase in proteins, cholesterol and high-density lipoproteins in the blood at the fourth to the seventh month, and *lupinus albus* reduce

blood glucose concentration, but increased AST enzyme starting from the fourth month to the eighth month.

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