GROWTH, HEMATOLOGICAL AND BIOCHEMICAL RESPONSES OF GROWING LAMBS INJECTED WITH GROWTH HORMONE AND SUPPLEMENTED WITH CALCIUM SOAPS OF FATTY ACIDS

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ABSTRACT

Physiological and productive responses to recombinant bovine somatotropin (rbST) injection and calcium soap of fatty acids (CSFA) supplementation were studied in male Rahmani crossbred lambs. Twenty male lambs of similar body weight (27.9kg) and age (162d) were randomly assigned to four equal groups of 5 animals each. The first group was fed the basal diet and served as control; the second group (GH) was biweekly injected with 100mg rbST; the third group (CSFA) was supplemented with 50g/d of calcium soap of fatty acids; and the fourth group (GH+CSFA) was injected biweekly with 100mg rbST plus 50g/d of CSFA. Lambs were weighed biweekly at mornings before access to feed and water. Blood samples were collected by jugular vein puncture one week after each rbST injection. Treatments with rbST, CSFA or a combination of rbST and CSFA increased ($P < 0.05$) final body weight and average daily gain compared with the control group. Concentration of insulin-like growth factor-1 (IGF-1) in lambs treated with (GH) or (GH+CSFA) was higher ($P < 0.01$) than that in lambs treated with CSFA or control group. Hematological parameters (RBC, WBC, and hemoglobin concentrations) did not change among treatment groups. Lambs treated with rbST showed higher ($P < 0.05$) serum total protein concentration than other treated and control lambs. Control lambs showed the least serum albumin concentration in comparison with other treated groups. Injection of rbST or supplementation of CSFA let to an increase in serum glucose concentration compared with control lambs. Serum urea concentration was not affected by injection of GH, but their values were lower ($P < 0.05$) in the CSFA-supplemented group compared to the control group. Serum triglyceride concentrations decreased ($P < 0.05$) in the rbST-injected lambs compared to other treatment groups. Lambs supplemented with CSFA only or in combination with rbST injection had higher ($P < 0.05$) concentration of serum cholesterol than control or rbST-injected lambs. Results of the present study suggest that rbST and CSFA may increase the average daily gain and improve the physiological status of the growing lambs.

Key words: Lambs, rbST, calcium soap of fatty acids, IGF-1, average daily gain, hematological and biochemical parameters

INTRODUCTION

Technological developments in a variety of scientific and engineering disciplines will be needed to support the growing world population, which is expected to double in the next 40 years (Etherton and Bauman, 1998). To meet this need, it will be essential for scientists to continue developing newer technologies to increase production-efficiency of food production. Exogenously administered somatotropin is one biotechnology tool that increases growth rate, improves feed efficiency, and increases the proportion of lean to fat tissue in the carcasses of sheep (McLaughlin *et al.* 1991) and cattle (Rausch *et al.* 2002). Growth hormone exerts some of its actions on skeletal muscle growth via IGF-1 (Florini and Ewton, 1995) and, thus, the concentration of IGF-1 in blood is generally increased by somatotropin treatment (Vestergaard *et al.* 2003; Govoni *et al.* 2004).

Commercial products based on calcium soaps of fatty acids (CSFA) are widely used in dairy rations, while these products are less used for growing steers or growing lamb diets (El-Bedway *et al.* 2004). Bypass fats are hydrolyzed to their polyunsaturated fatty acids and glycerol, which is a precursor for glucose (Morsy, 2008). Moreover, Moallem *et al.* (1997) reported that plasma urea concentrations were decreased in high producing dairy cows fed calcium soaps of fatty acids, reflecting better utilization of amino acids as reviewed by Etherton and Bauman (1998). Furthermore, fat supplementation has also been shown to increase concentration of circulating growth hormone (Williams and Stanko, 1999).

The objectives of this study were to evaluate the effects of rbST administration and calcium soaps of fatty acids on growth rate, serum IGF-1 concentration, hematological and serum biochemical parameters in growing Rahmani crossbred lambs.

MATERIALS AND METHODS

The present study was conducted at the Experimental Research Station of the Department of Animal Production, Faculty of Agriculture, Alexandria University. The goal of the study was to improve growth performance of growing lambs by feeding diets containing protected fat sources (calcium soaps of fatty acids, CSFA) and/or biweekly injections of recombinant bovine somatotropin (rbST) for a period of approximately 6 months.

Animal Management: Twenty weaned Rahmani crossbred lambs (initial live weight 27.9 ±1.8 kg and 162 ±3 days of age) were randomly assigned to four equal groups of five animals each. All animals were fed basal diet containing Berseem hay *ad libitum* and commercial concentrate mixture containing at least

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14% crude protein. The amounts of concentrate were adjusted according to body weight starting with 0.5kg/ head/ day and reached 1.0kg/ head/ day at the end of the experiment. Fresh drinking water and salts were freely available. Animals were de-wormed and vaccinated before the initiation of the experiment, and were housed in shaded barns in separate groups.

Treatments: The first group was fed the basal diet and received no further treatment and served as a control. The second group was fed basal diet and was injected biweekly with 100mg/ head of sustained release formulation of recombinant bovine somatotropin (rbST; Somatec, Elanco-Eli Lilly export, S.A. Geneva). The third group was fed basal diet plus 50g/ head/ day of protected fat (calcium soaps of fatty acids; CSFA; Megafat, Alfa Chemical Co, Alexandria, Egypt). The fourth group was fed the basal diet plus 50g/ head/ day of CSFA and was injected with 100mg/ head/ 14 days rbST. Growth hormone was administered subcutaneously posterior to the forelegs on alternating sites each time after morning feedings. The treatments were continued for 6 months. Chemical analysis of the protected fats supplement is shown in Table 1.

Data collected: Lambs were weighed biweekly at mornings (8:00 am) before access to feed and water. Feed and water were withheld the night before weighing. Blood was collected by jugular vein puncture one week after each rbST injection. Collected blood samples were immediately withheld into heparinized tubes for fresh hematological blood analysis. Non-heparinized blood samples were centrifuged at 3000rpm for 20min and serum was harvested and stored at -20°C for later analyses.

Non-coagulated blood was tested shortly after collection for hemoglobin (Hb), red blood cell (RBC) counts, white blood cell (WBC) counts and packed cell volume (PCV) using conventional methods (Hepler, 1966). Concentrations of serum insulin-like growth factor (IGF-1) were determined using a commercial kit (IGF-1- ELISA, Biosource, Europe SA, B-1400 Nivelles, Belgium). Serum total protein, albumin, glucose, urea and cholesterol concentrations were determined using commercial enzymatic colorimetric kits (Diamond Diagnostics, Egypt). Serum triglyceride concentrations were determined using commercial kits (BioSystems S.A. Costa Brava 30, Barcelona, Spain). Globulin concentrations were calculated as the difference between total protein and albumin.

Statistical analyses: A 2x2 factorial experimental design was analyzed by the general linear model (GLM) procedure using SAS statistical package (SAS, 2002). The model included the rbST and CSFA as main effects and their interaction. Duncan's Multiple Range Test was used to test any variation between means.

Table (1): Chemical composition of calcium soaps of palm oil fatty acids (CSFA)

Components	g/kg
Total fat	840
Myristic (C14:0)	12.6
Palmitic (C16:0)	369.6
Stearic (C18:0)	42
Oleic (C18:1)	336
Linoleic (C18:2)	79.8
Ash	110
Ca++	9.9
moisture	50

RESULTS

Initial age and weight of lambs were almost similar among treatment groups, averaging 162 days of age and 27.9 kg of body weight. The effect of GH injection and calcium soaps of fatty acid (CSFA) supplementation on body weight of growing lambs is presented in Figure 1. Mean final weights were 41.5, 46, 48 and 47 kg for lambs of control, GH injected, CSFA supplemented and GH injected plus CSFA supplemented groups, respectively (Table 2). Mean final body weights and average daily gain (ADG) values were greater ($P < 0.05$) in all treated groups compared to the control group, but differences in final weights among the treated groups were not significant. In addition, average daily gain was lower ($P < 0.05$) in control group compared to all treated groups. The daily gains were improved by 26.9, 47.2 and 40.8 % for GH, CSFA and GH + CSFA treated groups, respectively.

Table (2): Effect of GH and calcium soaps of fatty acids on live body weight and average daily gain of growing lambs (means ± SE)

Variables	Groups			
	Control	GH	CSFA	GH + CSFA
Number of lambs	5	5	5	5
Initial age (day)	164.0 ± 6.0	159.0 ± 5.0	161.0 ± 6.0	165.0 ± 2.0
Initial body weight (kg)	27.8 ± 1.3	28.5 ± 1.8	27.7 ± 2.5	27.7 ± 2.5
Final age (day)	333.0 ± 6.0	329.0 ± 5.0	331.0 ± 6.0	334.0 ± 2.0
Final body weight (kg)	41.5 ± 3.3^{b}	46.0 ± 1.2^{a}	48.0 ± 3.4^{a}	47.0 ± 2.5^{a}
Average daily gain (g/d)	81.1 ± 19.7^{b}	102.9 ± 14.0^{a}	119.4 ± 15.6^{a}	114.2 ± 20.7^{a}
Daily gain improvement (%)	-	26.9	47.2	40.8

$^{a, b}$ Means with different superscripts within rows differ significantly ($p<0.05$)

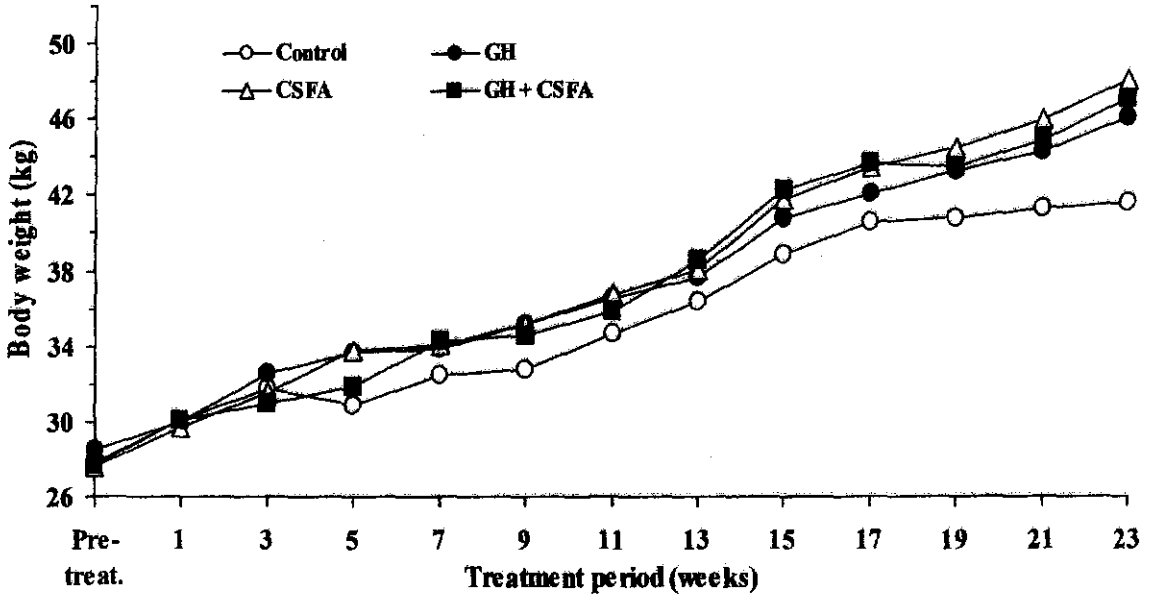

Fig. (1): Changes in body weight of growing Rahmani crossbred lambs treated with GH and/or CSFA for 6 months.

Table (3): Effect of GH injection and CSFA supplementation on blood hematological parameters of growing lambs (means ± SE)

Parameters	Groups			
	Control	GH	CSFA	GH + CSFA
RBC (x 10^{6}/ ml)	13.70 ± 0.37	14.21 ± 0.22	13.79 ± 0.34	13.82 ± 0.32
WBC (x 10^{3}/ ml)	16.65 ± 0.31	16.33 ± 0.53	16.87 ± 0.30	16.47 ± 0.31
PCV %	32.81 ± 0.44	32.82 ± 0.40	32.75 ± 0.32	32.40 ± 0.41
Hb (g/100 ml)	10.45 ± 0.18	10.60 ± 0.14	10.58 ± 0.11	10.63 ± 0.19

Renaults presented in Table 3 indicate that hemoglobin concentrations (Hb), red blood cell (RBC) counts, white blood cell (WBC) counts, and packed cells volume (PCV) did not differ significantly in lambs injected with GH, supplemented with CSFA or the combination of both two treatments compared to control lambs. Serum concentrations of insulin-like growth factor-1 (IGF-1) in lambs treated with GH or GH + CSFA were higher ($P < 0.01$) than those treated with CSFA and control groups (Figure 2). During the treatment period there was a gradual increase in serum IGF-1, particularly in lambs treated with GH alone or in combination with CSFA.

Serum total protein in GH-treated group was higher ($P < 0.05$) than all other treatment groups (table 4). The control group had lower ($P < 0.05$) serum albumin concentration compared to all treated groups. Moreover, the group supplemented with CSFA alone had higher ($P < 0.05$) albumin concentration than groups injected with GH only or in combination with CSFA supplementation. However, serum globulin concentration was extremely variable among all treatment groups. The group injected with GH had the highest concentration of serum globulin concentration compared to all other groups. Glucose concentrations were increased ($P < 0.05$) by GH injection or CSFA supplementation compared to control animals. Serum urea concentrations were not affected by injection of GH, while CSFA supplementation decreased ($P < 0.01$) serum urea concentrations compared to controls. On the other hand, serum triglyceride concentrations were lower ($P < 0.01$) in GH injected lambs than the remaining treatment groups, but there was no significant difference between lambs supplemented with CSFA and controls. Lambs supplemented with CSFA only or in combination with GH injection had higher ($p<0.05$) concentration of serum cholesterol than the control or GH-injected lambs. There were no significant differences between GH-injected group and control animals.

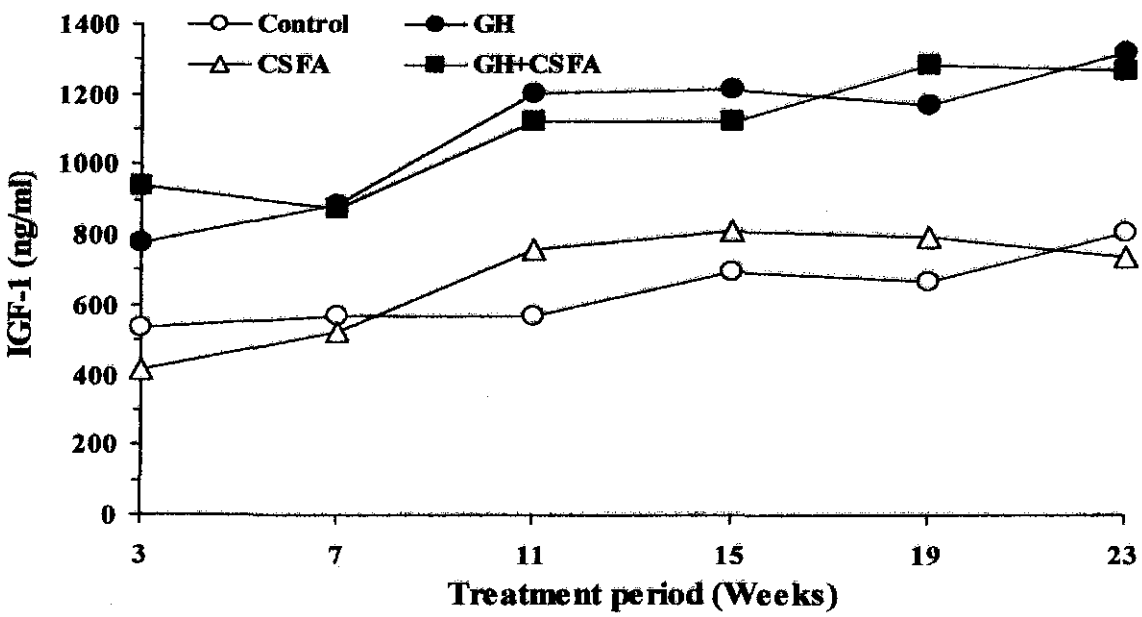

Fig. (2): Changes in serum insulin-like growth factor-1 (IGF-1) concentrations in growing lambs treated with GH injection and/or calcium soaps of fatty acid (CSFA) supplementation for 6 months.

Table (4) changes in serum biochemical parameters of growing lambs treated with GH and calcium soaps of fatty acids (CSFA) (means ± SE)

Parameters	Groups			
	Control	GH	CSFA	GH + CSFA
Total protein (g/ 100 ml)*	6.69 ± 0.06^{b}	7.30 ± 0.05^{a}	6.58 ± 0.04^{b}	6.58 ± 0.06^{b}
Albumin (g/ 100 ml)*	3.12 ± 0.06^{c}	3.26 ± 0.03^{b}	3.54 ± 0.04^{a}	3.33 ± 0.05^{b}
Globulin (g/ 100 ml)*	3.57 ± 0.05^{b}	4.03 ± 0.05^{a}	3.04 ± 0.05^{d}	3.25 ± 0.05^{c}
Glucose (mg/ 100 ml)*	53.95 ± 1.49^{b}	60.92 ± 1.70^{a}	63.63 ± 1.26^{a}	62.71 ± 1.19^{a}
Urea (mg/ 100 ml)**	53.37 ± 1.41^{a}	53.99 ± 1.03^{a}	37.79 ± 0.79^{b}	39.88 ± 0.70^{b}
Triglycerides (mg/ 100 ml)**	53.04 ± 1.50^{a}	26.75 ± 1.12^{b}	57.45 ± 1.45^{a}	57.74 ± 1.39^{a}
Cholesterol (mg/ 100 ml)*	14.72 ± 0.86^{b}	12.58 ± 0.78^{b}	24.84 ± 1.42^{a}	24.80 ± 1.61^{a}

*Means with different superscripts within rows differ significantly ($p<0.05$)
**Means with different superscripts within rows differ significantly ($p<0.01$)

DISCUSSION

Results of the present study showed that average daily gain was significantly lower in the control group compared to all treated groups. The daily gain improvements were 26.9, 47.2 and 40.8 % for GH, CSFA and GH + CSFA treated groups, respectively. These results are much higher than those in earlier studies, which indicated that growth rate of lambs increased by 16% (McLaughlin *et al.* 1991), 18% (Beermann *et al.* 1990 after 7 weeks of treatment), and 17% than control wethers (Godfredson *et al.* 1990).

On the other hand, Hegazy et al. (1999) found that live weight, body condition score (BCS) and their net increases were higher ($p<0.01$) in Barki ewes fed the basal diet supplemented with about 50gm/ head/day (3% of DM) of CSFA than control group. However, growth performance or ADG of Pelibuey lambs were not influenced by the inclusion of different levels of calcium soaps of tallow in diets (Salinas *et al.* 2006). Horton *et al.* (1992) also reported that CSFA had no effect on BW of ewes fed different levels (0, 75, 150 and 300g/ head/ day) of CSFA. Moreover, Rotunno et al. (1998) suggested that the addition of rumen- protected fat (4 and 8% of DM) in diets of lactating ewes did not change body weights and it was similar across all diets. The present results showed that the effects of CSFA on ADG were more pronounced that those of GH. Similar effects were reported by McFadden *et al.* (1990) who demonstrated that lambs supplemented with unsaturated fat were heavier in final body weight than lambs injected with growth hormone.

The present data revealed that hematological parameters did not differ significantly among the different experimental groups. These results are in accordance with the findings of Sallam *et al.* (2005) who reported that hematological parameters did not change in lactating Rahmani ewes injected with rbST. Additionally, fat supplementation had no effect on total white blood cells (WBC's) as well as blood hematocrit (Gartiner *et al.* 1969; Lane and Campbell, 1969). Mc Dowell *et al.* (1964) also indicated that high fat feeding either in the form of oil or hard fat had no significant effect on RBC or WBC counts. However, Hussien. (1995) noted a significant decrease in RBCs but no effect on WBCs, PCV % or hemoglobin (Hb %) in crossbreed rams fed (6% of DM) CSFA. Additionally, El-Bedawy *et al.* (2005) in their study on Friesian bulls found that feeding (4 or 8% of DM) CSFA significantly increased RBC counts with no change in WBCs.

The results of the current study indicated that injection of rbST either alone or in combination with CASF increased serum IGF-1 concentrations. These results are in agreement with the findings of earlier studies in wether lambs (Godfreson *et al.* 1990), in prepubertal heifers (Vestergaad *et al.* 2003), and Hereford calves (Govoni *et al.* 2004). Recently, Schlegel *et al.* (2006) found that Holstein steers treated with rbST had greater serum IGF-1 concentrations than control steers. Vestergaard *et al.* (2003) suggested that some of the effects of exogenously administered GH on tissue metabolism, including skeletal muscle, are mediated though GH – IGF-1 axis. On the other hand, supplementation of different levels of calcium salts of palm fatty acids did not exert significant effect on plasma IGF-1 concentration in dairy cows (Kokkonen *et al.* 2004).

Treatment of growing lambs with GH markedly increased serum total protein, albumin, globulin and glucose concentrations compared to controls (Table 4). Serum urea and cholesterol

concentrations were not affected, while serum triglyceride concentrations decreased significantly due to GH injection. The present results are in part similar to those reported by McLaughlin *et al.* (1991) who found that lambs treated with rbST had greater serum glucose and albumin concentrations than control lambs. In contrast, the same investigators reported that serum total protein and globulin did not change, while blood urea nitrogen was decreased in the rbST-treated lambs compared with control lambs. Treatment of dairy cows with rbST increased rate of gluconeogensis and decreased whole body glucose oxidation resulting in increased serum glucose concentration (Etherton and Bauman, 1998). Also, GH is diabetogenic due to reduction of tissue sensitivity to insulin (Scanes, 2003). Moreover, Sallam *et al.* (2005) found that plasma urea was lower in ewes treated with rbST, while cholesterol concentration was not affected compared to untreated control ewes. In addition, somstotropin administration dramatically reduced fatty acid synthesis in adipose tissue and decreased adipocyte hypertrophy (Dunshea *et al.* 1992). This may explain the reduction in serum triglyceride concentrations in lambs treated with rbST.

Present results demonstrated that CSFA-supplemented lambs had marked elevation in serum albumin, glucose and cholesterol concentrations compared to untreated control lambs. Serum triglyceride concentrations were not affected, while serum urea concentration decreased significantly in CSFA-supplemented animals. On the other hand, Shabaan (2002) noted that does fed 4% of CSFA supplementation had higher blood total protein compared to controls. Hussien (1995) also reported that crossbreed rams fed CSFA (6% of DM) had higher glucose concentration than control rams. Espinoza et al. (1997) observed that mean concentration of cholesterol was higher in Pelibuey ewes fed two levels of CSFA (2.5% or 5.0%) than in ewes fed the control diet. Also, Hegazy *et al.* (1999) showed that increased lipid intake in Barki ewes supplemented with 50gm CSFA in their diet significantly increased serum concentration of cholesterol by 67% when compared to the control group.

Schneider *et al.* (1988) found that plasma triglyceride concentrations were not affected by feeding 0.5kg CSFA /day to Friesian cows. Also Sklan *et al.* (1991) reported that plasma triglyceride concentrations of cows were not affected by feeding CSFA (2.6 % of DM) during lactation. Similar trend was noted in the present study. Urea concentrations decreased in cows fed calcium soaps of fatty acids (Moallem *et al.* 1997). In addition, Metwally *et al.* (2006) found that protected fat and oil supplementation with ration of Friesian cows decreased blood urea concentration. Thus, the decrease of urea concentration may be explained by improved efficiency of amino acid utilization and increased protein accretion.

In conclusion, both injection of bovine somstotropin and supplementation of calcium soaps of fatty acids improved significantly average daily gain of growing Rahmani lambs. Injection of GH alone or in combination with CSFA resulted in increased serum concentration of IGF-1. All treated lambs had increased serum albumin and glucose. CSFA-supplementation decreased serum urea concentration indicative of increased protein utilization efficiency, while rbST-injection decreased serum cholesterol and triglyceride concentrations.

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الملخص العربي

النمو والاستجابات الهيماتولوجية والبيوكيميائية في الحملان النامية المعاملة بهرمون النمو أو المغذاة علي الأحماض الدهنية المحمية بأملاح الكالسيوم

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أجريت هذه الدراسة بهدف معرفة مظاهر النمو والإستجابات الفسيولوجية لذكور حملان أغنام الرحماني المعاملة بالحقن بهرمون النمو أو المغذاة علي كمية إضافية من الأحماض الدهنية المحمية ، وفي هذه الدراسة تم إستخدام عدد ٢٠ ذكر من حملان الرحماني متوسط عمرها ١٦٢ يوم ومتوسط وزنها ٢٧,٩ كجم بعد الفطام مباشرة. وتم تقسيمها عشوائيا الي أربعة مجاميع متساوية. المجموعة الأولي غذيت علي عليقة أساسية وأعتبرت كمجموعة ضابطة ، المجموعة الثانية غذيت علي العليقة الأساسية وتم حقنها بهرمون النمو بمعدل ١٠٠ مجم / رأس / إسبوعين ، المجموعة الثالثة غذيت علي العليقة الأساسية مضاف لها ٥٠ جم / راس/ يوم من الأحماض الدهنية المحمية بأملاح الكالسيوم ، المجموعة الرابعة غذيت علي العليقة الأساسية مع حقنها بـــ ١٠٠ مجم من هرمون النمو كل إسبوعين وكذلك إضافة ٥٠جم / رأس/ يوم من الأحماض الدهنية المحمية بأملاح الكالسيوم . ووزنت الحيوانات كل إسبوعين صباحاً وقبل تناول الغذاء أو الماء ، وتم جمع عينات الدم من الوريد الوداجي بعد إسبوع من كل حقنة بهرمون النمو ، وأوضحت النتائج أن المعاملة بهرمون النمو أو بإ ضافة الأحماض الدهنية المحمية أو بكلتا المعاملتين معا أدت إلي زيادة الوزن النهائي وكذلك معدل النمو اليومي لهذه الحملان بالمقارنة بالضابطة . مستوي عامل النمو الشبيه للأنسولين لزداد بصورة معنوية في الحيوانات المعاملة بهرمون النمو بالمقارنة بالحيوانات المعاملة بالدهون المحمية أو المجموعة الضابطة ، المعايير الهيماتولوجية لم تتغير نتيجة أي من المعاملات السابقة ، أدت المعاملة بهرمون النمو إلي زيادة مستوي البروتين الكلي في الدم وذلك بالمقارنة بباقي المعاملات أوالمجموعة الضابطة . والمجموعة الضابطة كانت تحتوي علي أقل مستوي من الألبيومين بالمقارنة بباقي مجاميع المعاملات . والحقن بهرمون النمو أو التغذية الإضافية علي الأحماض الدهنية المحمية أدت إلي زيادة مستوي الجلوكوز في سيرم هذه الحملان بالمقارنة بالحملان الضابطة . مستوي اليوريا بالسيرم لم يتأثر بالحقن بهرمون النمو بينما المعاملة بالدهون المحمية أدت الي إنخفاض مستوي اليوريا في السيرم بالمقارنة بالمجموعة الضابطة . مستوي الجلسريدات الثلاثية إنخفضت بصورة معنوية عند المعاملة بهرمون النمو بالمقارنة بباقي المجاميع المعاملة.، هذا في حين أن مستوى كوليستيرول الدم إزداد عند المعاملة بالأحماض الدهنية المحمية فقط أو مع الحقن بهرمون النمو بالمقارنة بالمجموعة الضابطة أو المجاميع المعاملة بهرمون النمو. وعليه فإن نتائج هذه الدراسة توضح أن معاملة الحملان النامية بهرمون النمو أو إضافة الأحماض الدهنية المحمية للغذاء تؤدي الي تحسين معدلات النمو اليومية وتحسين الحالة الفسيولوجية للحيوانات.

الكلمات الدالة: الحملان – هرمون النمو – الأحماض الدهنية المحمية بالكالسيوم – معدل النمو اليومي – عامل النمـــو المـشابه للأنـــسولين – المعايير الهيماتولوجية والبيوكيميائية