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EFFECT OF LONG-TERM INSULIN INJECTION ON GROWTH PERFORMANCE, SERUM CONSTITUENTS AND SLAUGHTER MEASURMENTS IN SHEEP

(With 2 Tables & 2 Figuers)

By

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تأثير الحقن بالانسيولين لفتره طويله على النمو ومكونات السيرم ومقاييس الذبيحه فى الأغنام

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تم دراسة تأثير الحقن لمده طويله بالانسيولين على النمو ومكونات الدم ومقاييس الذبيحه فى الأغنام . تم تقسيم ١٢ حمل صعيدى (عمر ١٢ اسبوع) على معاملتين الاولى كنترول والثانيه تم حقنها بالانسيولين بواقع ٧٥ر٠ وحده دوليه لكل كيلو جرام من وزن الجسم خلال فترة التجربه (٢٨ أسبوع). الحقن بالانسيولين أدى الى زياده معنويه ($P < 0.01$) لوزن الجسم . أنخفض معدل الجلوكوز فى الدم معنويا ($P < 0.01$) عند الأسبوع الثالث من التجربه . أدى الحقن بالانسيولين الى نقص فى تركيز كل من بروتين السيرم . الجلوبيولين والترايجليسرايدز بمقدار ٦٪ ($P < 0.05$) ، ١٣٪ ($P < 0.01$) ، ١٨٪ على التوالى . لا يوجد هناك فروق معنويه بين المعاملتين فى تركيز اليوريا - نيتروجين و الكوليسترول و GOT فى السيرم . المعامله بالانسيولين أدت الى زياده فى وزن الجسم عند الذبح ووزن الجسم بدون محتويات القناه الهضميه بنسبه ١٠٪ و ٩٪ على التوالى. ادى الحقن بالانسيولين الى زياده فى وزن كل من الطحال والكبد والرنتين ودهن الكليتين والمعده والأمعاء بينما لم يؤثر على وزن الكليتين .

SUMMARY

Growth performance, some serum constituents and slaughter measurements were studied following long-term insulin injection in sheep. Twelve Saidi lambs (12 weeks old) were divided into two groups, a control group with no insulin treatment and the other group was injected daily with 0.75 IU insulin per Kg body weight during the experimental period (28 weeks). Insulin injection increased significantly ($P<0.01$) body weight. Lower ($P<0.01$) serun glucose concentration was observed in insulin treated animals than in control at wk 3 of the experimental peroid. Insulin injection decreased serum protein, globulin and triglycerides concentrations by about 6% ($P<0.05$), 13% ($P<0.01$) and 18%, respectively. There were no significant differences between the two

groups of animals in serum urea-nitrogen, cholesterol and GOT concentrations. Insulin treatment increased slaughter body weight and empty body weight by about 10% and 9%, respectively. Among viseral organs, insulin produces anabolic effects on spleen, liver and lungs, but not on kidney. Insulin treatment increased the weights of kidney fat, caul and omental fats.

Keywords: Sheep, insulin, growth, blood, slaughter measurments.

INTRODUCTION

Insulin is the only hormone that directly lowers blood glucose *(HADLEY, 1984)*. In addition, insulin, plays important roles in the synthesis of fat *(GREEN, 1983; MAY and MIKULECKY, 1983; WALTON & ETHERTON,1986)* and protein *(HARVEY and KAYE, 1991; LEWIS et al., 1992)*. In sheep, *LOBLEY (1992)* found that exogenous insulin had no anabolic effect when the animal fed above maintenance intake. However, the anabolic effect of insulin may be more affected by the dose injected. In rats, *JEPSON et al. (1988)* suggested that insulin action on protein metabolism may be more related to plasma insulin concentration. Similarly *KOBEISY (1994)* found that insulin (0.75 IU/kg per day) administration increased ($P<0.02$) fresh tissue weight of the total digestive tract by about 24% in sheep. Also, *SAKATA et al. (1980)* found higher mitotec index of rumen epithelium as a result of infusion (6 h) of insulin (0.125 IU/kg per h) in sheep. Apart from this, there is no available information on the long-term effect of insulin on blood metabolities and slaughter measurements in ruminants. The objective of this investigation was to examine the long-term effects of insulin (0.75 iu/kg per day) on: a) growth performance, b) serum constituents, particularly serum glucose, and c) slaughter measurements in sheep.

MATERIAL AND METHODS

This study was carried out during the winter and spring seasons in Animal Production Experimental Farm of the Faculty of Agriculture, Assiut University.Twelve, 6 males and 6 females, Egyptian native lambs (Saidi) at the age of 12 wk were randomly assigned to two treatment groups, similar in average body weight, a control group (A) with no insulin administration and an insulin-treated group (B), injected with 0.75 IU insulin per kg body weight daily. Insulin dose was calculated weekly according to body weight. Insulin (Nova Industria, Denmark)was administred subcutaneously at about 10.00 h daily, 2 h after morning feeding. Animals were fed 60% of their requirements as concentrate while the rest was given as roughage (containing 1:1 wheat straw and

bersem). The concentrate diet was consisted of corn (40%), cotton-seed meal (25%), wheat bran (32%), limestone (2%) and sodium chloride (1%). The daily requirements of growing lambs were calculated according to *GRAHAM (1982)*. Body weight was recorded weekly, before morning feeding. The trial conducted 28 weeks. Blood samples were taken from each animal at selected weeks (3, 6,9,12,16,20 & 27) of the experimental period. Samples were collected at 09.00 h before insulin injection. Serum was then separated by centrifugation at 3000 rpm for 15 min and stored at -20 °C until analyzed. Serum glucose and urea nitrogen were determined using kits supplied by Diamond Diagnostics (Egypt). Total protein was determined using supplied by bio merieux (France). Albumin was determined using kits supplied by Bio Analytics (USA). Triglycerides and cholesterol were determined using kits supplied by Sclavo Diagnostics (Italy). Glutamic oxaloacetic transaminase (GOT) was determined using kits supplied by Boehringer Mannheim (Germany).

At the end of the experiment, four animals (2 males and 2 females) from each treatment were slaughtered, 2 h after morning feeding. Slaughter and empty body weights, the weights of head, feet, pelt, spleen, liver, kidney, lungs, kidney fat, caul and omental fats were recorded. Body weight and serum constituants were statisticaly analyzed according to *HARVEY (1987)* computer program. Slaughter measurements were analyzed using general linear model (GLM) procedures of *SAS (1982)*.

RESULTS

Results are presented in (Tables 1 and 2) and Figures (1 and 2).

DISCUSSION

Growth performance:

Injection of insulin resulted in a 9% increase ($P<0.01$) in body weight of lambs (Fig. 1). Average daily gain were 91.17 and 111.12 g/d in control and insulin-treated lambs, respectively. Such effect could mainly due to the anabolic effect of insulin. *GARDNER and KAY (1991)* stated that insulin has both long-term growth effects and short-term anabolic effects on various cell types in vivo. Similar anabolic effect was reported in rumen *(KOBEISY, 1994; SAKATA et al. 1980)*, colon *(KOBEISY, 1994)*, mouse embryos *(CARO et al. 1987)* and bovine granulosa cells *(SPICER et al. 1993)*. Indeed, insulin increased both number and size of cells. *GARDNER and KAY (1991)* found that insulin increased cleavage by 10-20% when added to medium. On the other hand, insulin stimulated growth of cells through promoted incorporation of amino acids into protein *(KRAHL, 1961; CARO et al. 1987; HEYNER et al. 1989)*.

increased fatty acid synthesis, from glucose or from acetate or pyrovate, and inhibits libolosyis *(KRAHL, 1961; OLEFSKY, 1977; GREEN, 1983; MAY and MIKULECKY, 1983; WALTON and ETHERTON, 1986).*

Serum Constituents:

Long-term insulin injection decreased ($P<0.01$) serum glucose by about 48% at the third wk of the experimental period. Such effect was observed at 9 and 12 wk of the experimental period, but it was not significant. Otherwise serum glucose was not affected by insulin injection or even slightly increased at the end of the experimental period in insulin-treated animals (Fig. 2).

At the initiation of insulin treatment serum glucose rapidly decreased, this effect is a principal immediate response to insulin, where it is the only hormone that directly lowers blood glucose level *(HADLEY, 1984). KRAHL (1961)* stated that insulin increased the rate entrance of various sugars into the intracellular phase of muscle. However, insulin stimulated glucose metabolism independently of effects on transport *(MAY and MIKULECKY, 1982)* by enhancing certain enzymatic steps of glucose metabolism *(DENTON et al. 1981). MAY and MIKULECKY (1983)* suggested many approaches to show the effect of insulin on glucose metabolism. 1, insulin lowered intracellular glucose 6-phosphate concentrations, due to activation of phosphofructokinase. 2, insulin increased CO_2 production from glucose. 3, insulin decreased glucose level in the intracellular phase to a value less than expected if transport alone was stimulated by using H_2O_2, which means increased metabolic flux.

On the other hand, a possible explanation for the apparent decrease insulin-sensitivity in cells of insulin-treated lambs towards the end of the experimental period might be due to, first, decreased insulin receptors as insulin concentration increased *(HADLEY, 1984)*, second, long-term insulin injection (28 wk in the present work) might caused B-cell degranulation (this needs further study), third, the nonovin insulin used in this experiment might be immunogenic in lambs. For this reason, in human, the human sources of insulin must be used when the nonhuman sources of insulin prove to be immunogenic, and now the DNA recombinant technology, using sequences of DNA encoding for the A and B peptide chains of insulin which ligated separately into the Escherichia coli B-galactosidase gene, provide a plentiful supply of human insulin *(HADLEY, 1984)*.

Serum total protein concentration was significantly ($P<0.05$) lower in insulin-treated lambs than in control. Insulin injection decreased serum total protein by about 6% and this decrease was mainly due

to the decrease ($P<0.01$) in serum globulin concentration rather than serum albumin (Table 1). Insulin plays an important role in the control of protein synthesis in muscle. In the intact animals, the administration of exogenous insulin decreased plasma amino acid concentration *(AHMED et al., 1983; LOBLEY, 1992)* through active transport of amino acids into muscle, consequntly increased protein synthesis*(HADLEY,1984;HARVEY and KAYE, 1988; HARVEY and KAYE, 1991; LEWIS et al., 1992)* which in turn a positive nitrogen balance *(KRAHL, 1961)*. In addition, the lower serum protein of treated animals in this experiment may be due to not only increased protein synthesis but also decreased the rate of protein breakdown. *HADLEY (1984)* reported that amino acids and glucose cannot enter muscle cells in the absence of insulin, which results in protein catabolism. Consistent with this during starvation, lower insulin level, BROCKMAN (1984) reviewed that protein synthesis decreased and proteolysis increased in the hindquarters of sheep.

Serum urea nitrogen was slightly lower in insulin-treated lambs than in control (Table 1). Similarly, PRIOR and *CHRISTENSON (1978)* found that infusion of rapid injection of insulin into ewes did not alter plasma urea-N.

Serum cholesterol concentration was not affected by insulin administration (Table 1). *KRAHL (1961)* reported that in the case of insulin deficincy the synthesis of cholesterol is not impaired.

Insulin injection decreased serum triglycerides concentration from 59.25 to 48.61 mg/dl (Table 1). Such decrease may be due to the increase of lipid synthesis in treated lambs *(GREEN, 1983; MAY and MIKULECKY, 1983; WALTON and ETHERTON, 1986)*. Glutamic oxaloacetic transaminase (GOT) was not affected by insulin injection (Table 1).

Slaughter Measurments:

Insulin injection increased slaughter body weight and empty body weight by about 10 and 9% respectively. Also, head, feet and pelt were heavier in treated animals, but not significant (Table 2). Among visceral organs, insulin produces anabolic effects in sleen, liver and lungs, but not in kidney. Similar result was reviwed by *KRAHL (1961)*.

The weight of kidney fat, caul and omental fats showed always higher values in insulin-treated lambs. Such effect could mainly due to the increase in lipogenic rate as a result of insulin injection. In the absence of insulin, lipogenic capacity was decreased approximately 75% after 48h of culture *(ETHERTON and EVOCK, 1986)*. In addition, insulin can inhibit epinephrine-induced lipolysis *(BROCKMAN, 1984)*.

In conclusion, long-term insulin injection increased body weight

and this result may suggest that insulin increased fat and protein synthesis in sheep. Insulin decreased blood glucose, but long-term injection may decrease insulin-sensitivity in cells, so that the own sources of insulin must be used in similar case.

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Table 1. Effect of insulin injection on some serum constituents in sheep.

Item	Treatment a,b		
	A	B	S. E
Total Protein (g/dl)	8.12 c	7.63 d	0.17
Albumin (g/dl)	3.85	3.92	0.10
Globulin (g/dl)	4.27 e	3.71 f	0.20
Urea-nitrogen(mg/dl)	16.30	15.84	0.47
Cholesterol (mg/dl)	75.82	77.82	2.69
Triglycerides (mg/dl)	59.25	48.61	3.01
GOT (u/l)	25.04	24.10	0.93

a, Values are least-squares mean and SE = standard error;
b, Treatments : A = control; B = .75 iu insulin /kg BW daily.
c,d ($P<0.05$); e,f ($P<0.01$).

Table 2. Effect of insulin injection on weight (kg) of body components in sheep.

Item	Treatment a.b	
	A	B
Slaughter body weight	35.35 ± 3.15	38.90 ± 2.17
Empty body weigh	30.42 ± 2.93	33.11 ± 1.51
Head	2.05 ± 0.17	2.16 ± 0.16
Feet	0.93 ± 0.07	0.83 ± 0.03
Pelt	4.99 ± 0.28	5.30 ± 0.16
Spleen	0.05 ± 0.00 a	0.06 ± 0.00b
Liver	0.62 ± 0.06	0.68 ± 0.07
Kidney	0.10 ± 0.01	0.10 ± 0.01
Kidney fat	0.19 ± 0.04	0.31 ± 0.13
Caul fat	0.18 ± 0.03	0.43 ± 0.19
Omental fat	0.23 ± 0.05	0.29 ± 0.08
Lungs	0.45 ± 0.03	0.50 ± 0.02

a, Values are least-squares mean + standard error,
b, Treatments; A = contrl, B = .75 iu insulin/kg BW daily.
c,d ($P<0.05$).

Fig. 1. Effect of insulin injection on body weight of sheep

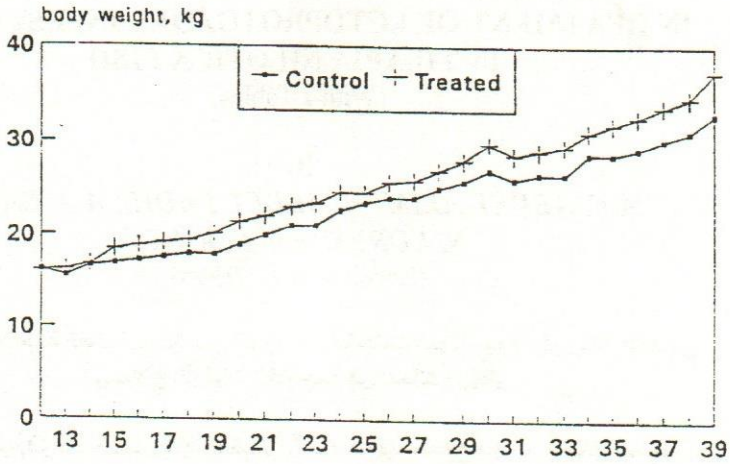

Fig 2. Serum glucose concentration in lambs as influenced by long-term insulin administration.