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COMPARATIVE STUDY ON THE EFFECTS OF SOME GROWTH PROMOTERS ON FAT DEPOSITION IN MALE NEWZELAND RABBITS

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SUMMARY

The present study was conducted on male Newzeland rabbits to investigate the effects of bovine somatotropin and salbutamol on fat deposition. The recorded findings revealed that both promotors decreased longissimus muscle fat concentration and increased protein: fat ratio and the effects of salbutamol are more pronounced. Growth promotors decreased fat deposition at multiple levels, at gross level by decreasing the weight of selected fat depots and salbutamol is more effective than somatotropin. Growth promotors increased plasma total lipids, triacylglycerols, total cholesterol, high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL) and reduced low-density lipoproteins (LDL) and salbutamol was more potent than somatotropin. Plasma leptin, as an index for whole body fat mass, was decreased in somatotropin injected and salbutamol-fed rabbits. Moreover, bovine somatotropin and salbutamol increased plasma Apo-1/Fas concentration, as an index for programmed cell death or apoptosis. In conclusion, the investigated promotors reduced the fat deposition at multiple levels; gross, biochemical and hormonal levels and these effects were not only confined to internal fat depots but also extended to intramuscular fat.

INTRODUCTION

Increase in live weight is accompanied with an increase in fat deposition that leads to a decline in the rate and efficiency of the growth in virtually all farm animals (Yen et al., 1986). Increased fat deposition inversely affects the feed efficiency because the deposition of one kilogram of triacylglycerol requires three kilogram of food intake above maintenance requirements per kilogram of fat (Clarke, 1993). So, lean tissue gain is four times as efficient as deposition of fat (Owens et

al., 1995).

Somatotropin has been shown to have impressive effect on nutrient partioning between muscles and adipose tissue that leads to a dramatic alteration in the growth of those tissues. Somatotropin treatment increased protein and decreased fat accretion in lambs (Mclaughlin et al., 1993) and cattle (Wagner et al., 1988). Somatotropin treatment in lambs caused a reduction in the major visceral fat depots of the body (Johnsson et al., 1987), subcutaneous fat thickness (Beermann et al., 1990), kidney and pelvic fat and 12th rib fat thickness (Mclaughlin et al., 1993). Moreover, somatotropin decreased back fat depth in cattle (Dalke et al., 1992).

β-adrenergic agonists increased muscle mass and decreased fat mass in lambs (Ricks et al., 1984). β-adrenergic agonists increased protein content and reduced fat content of hind quarter (Baker et al., 1984), longissimus muscle of lambs (del Barrio et al., 1995), and steers (Chikhou et al., 1993 b). The reduction in fat content in beta-adrenergic agonists occurred not only in subcutaneous adipose depot, but also in internal depots (Chikhou et al., 1993a).

The present study was conducted to investigate, in a comparative approach, the effects of bovine somatotropin and salbutamol as a growth promotors on fat deposition at gross, biochemical and hormonal levels (plasma leptin) in male Newzeland rabbits. Furthermore, plasma APO-1/Fas level was estimated as an index for programmed cell death (apoptosis).

MATERIALS AND METHODS

Experimental animals:

This study was conducted on 24 male Newzeland rabbits weighing 1239 ± 39.3 grams at the beginning of the experiment. Rabbits were individually kept in metal batteries and fed commercial pelted balanced growing rabbit's ration (Etmida, Mitghamr).

Rabbits were left one week for adaptation, then vaccinated with pasteurollosis vaccine (Veterinary Serum and Vaccine Research Institute, Egypt) and viral hemorrhagic diarrhea vaccine (Rhone Merieux, France). Furthermore, Monthly prophylactic dose of Baycox as anticoccidial drug (1ml/ 10 liters drinking water) and Ivomec (1 ml of 10% S.C.; Merck Co.; USA) was used.

Experimental design:

Twenty-four male Newzeland rabbits were allocated randomly into three groups of eight rabbits each. First group was kept as control. Second group (Bovine somatotropin administered group; GH): each rabbit in this group was injected S.C. day by day with recombinant bovine somatotropin (rbST; Somatech) (Monsanto Company, Switzerland) in a dose of 0.3 mg/ kg b.w. Somatotropin

was diluted by using bicarbonate buffer (25 mM NaHCO3, 0.154 M Nacl, 25 mM Na2CO3) (Silleace and Etherton, 1991). Third group (Salbutamol supplemented group): each rabbit in this group was fed daily on a diet supplemented with salbutamol in a dose of 1.9 mg /kg b.w. (Miller et al., 1988). The diets of these rabbits were prepared daily. Salbutamol (Salbovent) as sulfate is produced by Alexandria Pharmaceuticals Co.; Egypt. The dose of bovine somatotropin and salbutamol was adjusted according to the weekly changes in body weights. The animals of each group were treated by the previously mentioned regimes for 13 weeks.

Sampling:

At the end of the experiment, rabbits were slaughtered and individual blood samples were collected on heparin as anticoagulant (12 iu/ ml blood). Blood samples were centrifuged at 3000 rpm for 15 minutes and plasma was separated, divided into aliquots and kept in a deep freeze at -20°C until used.

Representative sample from right longissimus dorsi of each rabbit was obtained (~10 grams) and kept in a deep freeze at -20°C for estimation of longissimus muscle protein and lipid concentrations.

Data Collection techniques:

A- Longissimus muscle:

Longissimus muscle protein concentration was determined (Lowry et al., 1951) and lipids were extracted (Folch et al., 1957) and determined (Frings and Dunn, 1970) by kits (Cal-Tech Diagnositics, Inc. Chino, California, USA). The protein fat ratio was calculated.

B- Fat deposition:

Gross measurements were undertaken by weighing selected fat depots including perirenal as the major depot in rabbit and omental fat.

Plasma total lipids concentrations were determined (Frings and Dunn, 1970) by using kit (Cal-Tech Diagnostics, Inc.; Chino, California, USA). Plasma triacylglycerols concentrations (Wahlefeld, 1974) and, plasma total cholesterol estimation (Richmond, 1973) were determined by kit (Stanbio Laboratory Inc.; San Antonio, Texas, USA). Lipoproteins electrophoresis (Henry, 1984) were done in four pooled plasma samples from each group (each is the pooled of two individual samples) by kits (Helena Laboratories, Beaumont, Texas, USA).

Leptin determination was performed in plasma (Ma et al., 1996) by liquid phase radioimmunoassay using a kit purchased from Linco Research, Inc.; St. Louis, USA. The intra- and inter-assay

coefficients of variations were 6% and 4.6%, respectively.

C- Determination of Apo-1/Fas (CD95):

Apo-1/Fas measurement was carried out in plasma according to the method applied by Nagata and Goldstein (1995) by solid phase sandwich Enzyme Linked Immuno-Sorbent Assay (ELISA), using kits purchased from Biosource International, Camarillo, California, USA. The determined intra-assay coefficient of variation was 4.2%.

Statistical analysis:

All data were presented as mean ± SE and were subjected to analysis of variance (ANOVA) test according to Snedecor and Cochran (1980). Treatment means were compared by the least significant difference test (L.S.D) at 5% level of probability. The percentage of lipoproteins was treated according to Bliss' table of conversion of percentage.

RESULTS

Longissimus muscle protein concentration in salbutamol-fed group was greater than that of bovine somatotropin and control groups. There was no significant difference between the last two groups (Table 1). Total lipid concentrations of longissimus muscle of bovine somatotropin injected and salbutamol-fed groups were lower than that of control one. Moreover, the reducing effect of salbutamol was better than that of bovine somatotropin. Both treatments increased longissimus muscle protein/fat ratio when compared with control and the increment effect of salbutamol was higher than that of bovine somatotropin.

Table (1): Effect of recombinant bovine somatotropin administration and salbutamol supplementation on composition of longissmus muscle in Newzeland rabbits. (n=8).

Muscle composition	Contol	Bovine somatotropin	Salbutamol	L.S.D (P<0.05)
Protein (mg/g)	398.80^{a} ±5.40	388.19^{b} ±8.20	$425.92^{a,b}$ ±11.85	25.99
Lipid (mg/g)	$35.30^{a,b}$ ±1.96	$24.72^{a,c}$ ±1.90	$13.31^{b,c}$ ±0.50	4.72
Protein/fat ratio	$11.61^{a,b}$ ±0.5	$18.40^{a,c}$ ±1.8	$27.30^{b,c}$ ±1.3	3.5

* Values having the same letter in the same row are significantly different at P<0.05.

Table (2): Effect of recombinant bovine somatotropin administration and salbutamol supplementation on fat deposition and Apo-1/Fas in male Newzeland rabbits. (n=8).

Fat Deposition	Contol	Bovine somatotropin	Salbutamol	L.S.D (P<0.05)
A- Gross level:-				
Perirenal fat (g)	$104.41^{a.b}$ ±8.0	$84.42^{a.c}$ ±4.5	$49.89^{b.c}$ ±3.6	16.66
Omental fat (g)	$22.84^{a.b}$ ±1.2	$19.71^{a.c}$ ±1.1	$11.04^{b.c}$ ±0.8	3.10
B-Plasma level:-				
Total lipids (mg/dl)	$231.3^{a.b}$ ±5.2	391.7^{a} ±25.9	374.9^{b} ±8.5	48.87
Triacylglycerols (mg/dl)	$79.1^{a.b}$ ±5.1	120.5^{a} ±7.9	133.7^{b} ±6.8	19.56
Total cholesterol (mg/dl)	$79.6^{a.b}$ ±3.4	$173.4^{a.c}$ ±5.7	$156.2^{b.c}$ ±7.1	16.43
HDL	(15.85%) $23.46^{a.b}$ ±0.07	(24.45%) $29.51^{a.c}$ ±1.93	(42.65%) $40.77^{b.c}$ ±0.48	3.98
VLDL	(27.3%) $31.51^{d.c}$ ±0.20	(31.8%) $34.33^{d.f}$ ±0.21	(36.65% $37.24^{e.f}$ ±0.91	1.90
LDL	(56.85%) $49.11^{h.I}$ ±0.05	(43.75%) $41.40^{h.j}$ ±1.50	(20.7%) $27.05^{I.j}$ ±0.49	3.11
C-Hormonal level:-				
Leptin (ng/ml)	$3.4^{a.b}$ ±0.20	$1.7^{a.c}$ ±0.12	$0.8^{b.c}$ ±0.07	0.41
Apo-1/Fas (ng/ml)	$5.8^{a.b}$ ±0.42	$7.4^{a.b}$ ±0.54	$9.7^{b.c}$ ±0.21	1.21

* Values having the same letter in the same row are significantly different at P<0.05.

Data concerning the effect of growth promotors on fat deposition are presented in table (2). The weights of perirenal and omental fat depots in the bovine somatotropin injected and salbutamol-fed groups were significantly lower than those of control one. Moreover, the lowering effect of salbutamol was more obvious than that of bovine somatotropin.

Administration of bovine somatotropin and salbutamol increased plasma total lipids, triacylglycerols and total cholesterol concentrations when compared with those of control. While the differences between the effects of somatotropin and salbutamol on total lipids and triacylglycerols are nonsignificant, the elevating effect of somatotropin on plasma total cholesterol concentration is greater than that of salbutamol.

HDL and VLDL were higher in the treated groups than in control. Moreover, the elevating effects of salbutamol were higher than that of bovine somatotropin. Regarding LDL, it was lower in the treated groups than in control and salbutamol's lowering effect was stronger than that of bovine somatotropin.

Bovine somatotropin administration and salbutamol feeding decreased plasma leptin concentration when compared with control. The lowering effect of salbutamol was more prominent than that of bovine somatotropin.

Plasma Apo-1/Fas (CD95) concentrations in bovine somatotropin and salbutamol groups were higher than that of control group. In addition, that of salbutamol group was higher than that of bovine somatotropin group (Table, 2).

DISCUSSION

Combating of fat deposition is an imperative target for both consumers and animal producers. Growing animals don't store fat and protein without retaining tissue fluid. On wet tissue basis, calories required for unite of protein is less than one fourth of that needed for fat deposition. This means that fat deposition is much less economic (Owens et al., 1995).

In the present study, it was found that the investigated promotors reduced the fat deposition. the decrement effect was examined at multiple levels: gross, biochemical and hormonal levels. At gross level, both promotors decreased the weights of the selected fat depots (perirenal and omental fat depots). The biochemical level is investigated at plasma level (plasma lipid profile) and at compositional level of longissimus muscle (intramuscular lipid concentration).

A decrease in adipose tissue mass might result from either an inhibited deposition of triacylglycerols into adipocytes (lipogenesis) or from an increased in the rate of lipolysis in adipocytes. Both bovine somatotropin and salbutamol decreased fat deposition by increased lipolysis and /or decreased lipogenesis. Regarding lipolysis, there are two proofs in this study that support the notion of

lipolytic potential of salbutamol and bovine somatotropin. The first is the higher very low-density lipoproteins (VLDL), the second is the reduced plasma leptin concentrations. The increased VLDL reflects lipolysis of adipose tissue because nonestrified fatty acids, a product of lipolysis of adipose tissue, are used by the liver for biosynthesis of triacylglycerols fraction of VLDL (Herdt, 1992). The reduced plasma leptin indicates a depletion of triacylglycerols stores in adipose tissue, because leptin is secreted by adipocytes in proportion to their triacylglycerols stores (Jequier and Tappy, 1999).

Salbutamol may induce lipolysis via an increase in the activity of hormone sensitive lipase (HSL), the rate limiting enzyme for adipocyte triacylglycerols degradation, because it was found that HSL activity was increased when phosphorylated by cAMP-dependent protein kinase (Fredrikson and Belfrage, 1983). The cAMP-dependent protein kinase-induced phosphorylation is the characteristic postreceptor event for beta-adrenergic agonists.

somatotropin has dramatic effects on adipose tissue and lipid metabolism. Its effect on lipolysis predominates when animals are at zero or negative energy balance. Moreover, the effects of somatotropin on lipid metabolism are chronic rather than acute (Etherton and Bauman, 1998). lipolysis in rat and mouse adipose tissue was stimulated by growth hormone (Fain and Bahouth, 2000). The somatotropins lipolytic effect may be mediated via an increase in lipolytic response to endogenous catecholamines like that observed in growing cattle (Boisclair et al., 1997) and sheep (Doris et al., 1996). Additionally, somatotropin may reduce the activities of antilipolytic systems of adipocyte, such as adenosine (Lanna and Bauman, 1999) and prostaglandins of E series and also decreased its production (Doris et al., 1996).

The possibility of the existing inhibitory effects of both bovine somatotropin and salbutamol on adipose tissue lipogenesis can't be ignored. These inhibitory influences may be produced via inhibition of lipoprotein lipase activity. Lipoprotein lipase is the enzyme responsible for hydrolysis of chylomicron, triacylglycerols and very low-density lipoprotein (Oscarsson et al., 1999). There are three findings in this study that support the probable inhibition of lipoprotein lipase activity by both bovine somatotropin and salbutamol. Firstly, the observed high VLDL and low LDL. The lowering of LDL fraction may be due to the inhibition of lipoprotein lipase activity; because this enzyme is involved in the conversion of VLDL to LDL (Stein, 1987). Secondly, the increased plasma triacylglycerols concentrations, because this enzyme is responsible for triacylglycerols hydrolysis to provide free fatty acids for adipose tissue utilization or storage (Wang et al.,

1999). Thirdly, the reduced enzyme activity due to decreased adipose tissue mass and / or adipocyte size because lipoprotein lipase activity parallel with fat deposition (Hood, 1982), and adipocyte enlargement (Jamdar et al., 1981).

Salbutamol may inhibit lipoprotein lipase activity via cAMP-dependent mechanisms, since, isoproterenol, a beta-adrenergic agonist, decreased the enzyme activity and its mRNA in rat adipocytes and these effects were associated with reduction in enzyme gene expression (Raynolds et al., 1990). Furthermore, increased beta-adrenergic agonists concentrations or other agents that causes an increase in adipose tissue cAMP concentration tend to cause a decrease in adipose tissue lipoprotein lipase activity (Mersmann, 1998). Besides the inhibition of lipoprotein lipase activity, the probability of inhibition of other lipogenic enzymes activity due to salbutamol can't be refuted because acetyl-CoA carboxylase, the rate limiting enzyme for long chain fatty acid biosynthesis, became inactive when phosphorylated via cAMP-dependent protein kinase A pathway (Liggett and Raymond, 1993).

With respect to somatotropin; somatotropin's suppressive effect on lipoprotein lipase activity was observed in adipose tissue of rat and human both in vivo (Richelsen et al., 1994) and in vitro (Ottosson et al., 1995), bovine (Leisman et al., 1995). The inhibitory effect of somatotropin on lipoprotein lipase activity may be via antagonizing insulin's stimulatory effect on the enzyme (Wang et al., 1999) and/ or the gene that codes for the enzyme during translation and/or posttranslational processing (Ottosson et al., 1995). In addition to inhibition of lipoprotein lipase activity; somatotropin seems to combat lipogenesis via decreasing the activities of the fatty acids synthesizing enzymes, such as acetyl-CoA carboxylase and fatty acid synthase (Harris et al., 1993) and/or lower those enzymes activities by decreasing their mRNA abundance (Donkin et al., 1996).

Regarding hormonal level of fat deposition, leptin offered the most sensitive marker for whole body fat mass. Leptin is a 167 amino acids protein synthesized by adipocytes (Ramsay et al., 1998). Blood leptin level is positively correlated with adipose tissue mass (Ahren et al., 1997), adipocyte number (Shillabeer et al., 1998) and adipocyte size (Houseknecht et al., 1996). In the light of the previous information, it is apparent that both salbutamol and bovine somatotropin decreased whole body fat mass may be via reduction in cell number and / or cell size (increased lipolysis and inhibition of lipogenesis) which is hormonally confirmed by reduced plasma leptin level.

Reduction of plasma leptin level due to beta-adrenergic agonists may be via a decrease in ob mRNA expression and leptin secretion (Donahoo

et al., 1997), leptin release and antagonism of stimulatory effect of insulin and glucocorticoids on leptin mRNA expression (Ricci and Fried, 1999). The possible mechanism by which beta-adrenergic receptor activation abolishes the insulin evoking action (s) on leptin secretion may be via inhibition of glucose uptake by adipose tissue (Carpene et al., 1993) and/ or alteration in insulin binding to its receptor (Lonnroth and Smith, 1983). Moreover, the alteration of insulin binding to its receptor may be due to the impaired receptor affinity, since the elevated cAMP in adipocytes decreased the receptor affinity for insulin and partially uncouple receptor tyrosine kinase activity from activation by insulin (Czech, 1985).

The decrement effect of bovine somatotropin on plasma leptin may be via direct decrease in leptin mRNA expression (Isozaki et al., 1999), and indirect effects may be secondary to the reduction in fat mass (Florkowski et al., 1996; Janssen et al., 1997), or the increase in cAMP in adipocytes (Yip and Goodman, 1999). Additionally, the indirect effect can occur via catecholamines pathway, because it was found that somatotropin caused an increase in maximal catecholamines binding to beta-adrenergic receptors in adipose tissue (Doris et al., 1996).

The reducing effect of investigated promotors on fat deposition is not confined to fat depots, but it further extends to intramuscular fat as indicated by the lower fat concentration of longissimus muscle of treated groups. The reduced longissimus muscle fat concentration due to salbutamol feeding is in accordance with the findings of cimaterol-fed steers (Chikhou et al., 1993b), and salbutamol-supplemented lambs (del Barrio et al., 1995). On the other hand, bovine somatotropin effect on longissimus muscle lipid concentration is in agreement with the findings of rbST-treated bull calves (Holzer et al., 1999).

Furthermore, in the light of data presented in this study, it is clear that the reducing effect of salbutamol on lipid deposition overcame that of bovine somatotropin (as indicated by more reduction in selected depots weights, plasma leptin levels and intramuscular fat concentration). Moreover, the lipolytic activities of the tested promotors is likely to be operated at higher degree than their antilipogenic activities this concept is strengthened by reduced leptin level (reduced adipocytes size), and increased VLDL and Apo-1/ Fas.

Apo-1/Fas (CD95) ligand is a type II membrane protein of 40 KDa that belongs to tumor necrosis factor (TNF) family (Mita and Hayashi, 1996), while Fas antigen (receptor) is a type 1 membrane bound protein also belonging to TNF receptor family (Kobayashi and Koike, 1996). Fas ligand was detected on the cell surface of activated T-cells and induced apoptosis in Fas expressing tar-

get cells (Nagata, 1996). The binding of Fas ligand to Fas antigen induces apoptosis via cross-linking of the antigen with the ligand (Kobayashi and Koike, 1996). Additionally, the unique death domain in the cytoplasmic region of the Fas antigen is essential for apoptosis initiation (Mita and Hayashi, 1996). The increased level of Apo-1/Fas in both treated groups in the current investigation may reflect the apoptosis of adipocytes that undergo lipolysis. The recent findings recorded a close correlation of tumor necrosis factor with the indices of lipolysis and suggested that this cytokine, that is locally produced by adipocytes, participates in a local positive autocrine feedback loop that potentiates lipolysis and inhibits insulin antilipolytic actions (Orban et al., 1999).

In conclusion, the investigated promotors reduced the fat deposition and these effects were not only confined to internal fat depots but also extended to intramuscular fat.

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