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Insulin-like Growth Factor (IGF)-I but Not IGF-II Promotes Lean Growth and Feed Efficiency in Broiler Chickens

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The efficacy of exogenous IGFs to stimulate growth and modulate protein and fat deposition was examined in a number of broiler chicken lines. From around 600 g body weight the chickens received a continuous infusion of vehicle (0.1 M acetic acid), human recombinant IGF-I or [Gly[1]]IGF-II at 300 μg/kg body weight per day, or a combined infusion of 150 μg/kg/day of each IGF for 2 weeks. Experiment 1 used commercial broiler female chickens and included measurements of nitrogen balance, N^{τ}-methylhistidine excretion and muscle protein synthesis rates. In Experiment 2 the same treatments were applied to three experimental lines of chickens selected for high food consumption (relatively fat), high food utilisation efficiency (relatively lean), or at random (control). IGF-I, but not IGF-II, significantly increased growth rate and food utilisation efficiency by around 10–15% in each experiment, an effect which was consistent across all genotypes. Nitrogen balance was significantly increased by IGF-I in Experiment 1 as was carcass nitrogen content in Experiment 2, indicating that the increased growth was in lean tissue. Carcass fat was consistently reduced in chickens receiving IGF-I and was related to the levels of circulating IGF-I ($r^2 = 0.30$, $P < 0.01$) but not triiodothyronine. Protein synthesis rates were unaffected by treatment and could not account for increased growth rate. However, there was a significant reduction in N^{τ}-methylhistidine excretion indicating a reduced rate of muscle protein breakdown in IGF-I-treated chickens (1.56%/day vs 2.05%/day for IGF-I-treated vs controls, $P < 0.05$). The efficiency of feed utilisation was inversely related to the rate of protein breakdown ($r^2 = 0.25$, $P < 0.01$). In conclusion, these experiments are the first to report an enhancement of growth and food utilisation efficiency by broiler chickens receiving exogenous IGF-I. Our results show that IGF-I may be important in controlling the growth and efficiency of food utilisation of young chickens at least in part by modulating the rates of protein breakdown. © 1998 Academic Press

Key Words: IGF-I; IGF-II; growth; protein turnover; chickens.

IGF-I produced by the liver and other tissues is considered to be the prime effector of growth hormone actions on growth and development (Zapf and Hunziker, 1994). The ability of IGF-I to stimulate growth in GH-deficient humans and animals has been well documented (Froesch *et al.*, 1985; Jones and Clemmons, 1995) but the ability of IGF-I to stimulate growth in normal growing animals is less clear. In rats, lean growth is increased by infusion of either exogenous IGF-I (Hizuka *et al.*, 1986; Tomas *et al.*, 1993) or, to a lesser extent, IGF-II (Conlon *et al.*, 1995a). Other species such as the sheep (Cottam *et al.*, 1992; F.M. Tomas, unpublished results), pig (Walton *et al.*, 1996), guinea

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pig (Conlon *et al.*, 1995b), and chicken (McGuinness and Cogburn, 1991; Huybrechts *et al.*, 1992; Spencer *et al.*, 1996) have not shown a growth response when treated with either IGF-I or IGF-II. Adult rats differ from these other species by a relative lack of IGF-II in plasma. Thus, the interactive dynamics between IGF-I, the IGF-binding proteins (IGFBPs), and receptors during IGF-I administration would differ and may contribute to the efficacy of IGFs for growth promotion in this species.

Although IGFs do not appear to stimulate growth in chickens, carcass fat content is decreased by IGF-I (Huybrechts *et al.*, 1992) and increased by IGF-II (Spencer *et al.*, 1996). The latter authors postulated that the changes could be caused by the associated changes in plasma triiodothyronine (T_3) levels which are consistent with the known lipolytic actions of T_3 in poultry (e.g., Cogburn, 1991). However, as is the case for mammals, IGF-I infusion to chickens lowers insulin levels (Tixier-Boichard *et al.*, 1992) and consequent lipogenic activity, thereby reducing fatness (e.g., Simon, 1988).

Chickens have relatively low levels of IGFs and IGFBPs in the plasma (Armstrong *et al.*, 1989; Lee *et al.*, 1989; Johnson *et al.*, 1990; McMurtry *et al.*, 1996) compared to those of mammals. However, the levels of IGF-I and the IGFBPs remain responsive to dietary manipulation (Kita *et al.*, 1996). This is not the case for IGF-II (Kita *et al.*, 1996). This suggests IGF-II plays little part in metabolic regulation, which is consistent with the lack of a recognised specific receptor for IGF-II in the chicken (Goddard, 1991; Matzner *et al.*, 1996). Nonetheless, because it competes with IGF-I for binding both to the IGFBPs and to the type 1 IGF receptor it may be an important modulator of "free" IGF-I levels during IGF infusions.

In this study we have infused IGF-I and IGF-II both singly and in combination to groups of broiler chickens drawn from a range of genotypes showing varied traits relating to food consumption and carcass composition. IGF-I infusion was associated with enhanced growth and reduced carcass fat content.

MATERIALS AND METHODS

Peptide Infusion

Recombinant human IGF-I and human [Gly^1]IGF-II were obtained from GroPep Pty Ltd., Adelaide, South Australia. [Gly^1]IGF-II, an analogue of IGF-II, exhibits similar potency to native IGF-II for binding to type 2 receptors and for stimulation of protein synthesis in rat L6 myoblasts (GroPep specification data). The peptides were dissolved in 0.1 M acetic acid to concentrations required for metered delivery from a miniosmotic pump (Alza Corp., Palo Alto, CA). Delivery rates were planned to average 300 µg/day/kg body weight but the actual rate varied between experiments and over time due to the rapid growth rates of the chickens (see below). The pumps were implanted subcutaneously via a small incision in the dorsal midline at the base of the neck using aseptic procedures. Preliminary experiments were performed to establish competent delivery of each peptide with this procedure.

Experimental Design

Two experiments were performed. The first experiment used commercial broiler stock and included investigation of metabolic effects. The second was a larger study using experimental chicken lines selected for diverse food consumption and food utilisation characteristics.

Experiment 1. Forty-two female broiler chickens from the Cobb sire line were housed in individual cages in a temperature-controlled building. Throughout the study the birds were given free access to water and a commercial broiler starter ration. After acclimatisation, the chickens (621 ± 0.6 g body weight) were distributed among five groups. One group ($n = 10$) was an initial control group to provide starting values for plasma and carcass constituents (see below). The other four groups ($n = 8$) were randomly allocated to receive an osmotic pump (Alza model 2002, 0.65 µl/h at 41°C from a 240-µl reservoir) containing either 0.1 M acetic acid (vehicle), 13.7 mg/ml IGF-I, 13.7 mg/ml [Gly^1]IGF-II (IGF-II), or an equimolar mixture of IGF-I and IGF-II totalling 13.7 mg/ml to deliver 214 µg peptide/day. After 7 days when the average body weight was 1.05 kg, an additional osmotic pump (Alza 2001, 1.03 µl/h at 41°C from a 224-µl reservoir) containing IGF-I and/or IGF-II was implanted to compensate for increased body size and boost actual infusion rates to 315 µg/day for both IGF-I and IGF-II over the remaining 6 days. The dose rate for IGF-I infusions was chosen using results from preliminary experi-

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ments and from reported studies (Tixier-Bouchard *et al.*, 1992). We aimed to increase plasma IGF-I levels by around threefold, a substantial increase within the physiological range and which was below the levels associated with hypoglycaemia. IGF-II infusion rates were chosen to match those of IGF-I.

During the 13-day infusion period body weights and food intakes were measured at 2- to 3-day intervals. Daily excreta were collected over the last 2 days and immediately subsampled and stored at −20°C. At the end of the infusion period a blood sample was taken into a heparinised tube and then each chicken was injected via a wing vein with 10 ml/kg body weight of a solution containing 150 mmol phenylalanine/liter and 20 µCi/ml L-2,6-[^{3}H]phenylalanine. Fifteen minutes after the injection the chicken was killed by cervical dislocation and consistent portions of breast muscle and thigh muscle were immediately removed and clamped in aluminium blocks held in liquid N_2. The contralateral breast muscle, liver, spleen, empty gut, and abdominal fat pad were weighed and returned to the carcass which was stored at −20°C.

Experiment 2. Fifty-one female and 42 male chickens from three experimental selection lines were used. The lines were bred at random (line C; control) or selected for high food consumption (line F) or high food utilisation efficiency (line E) (Pym and Nicholls, 1979). Line F birds are fatter and line E birds are leaner than the controls. The growth rate of these lines was substantially less than that of the commercial broiler chickens used in Experiment 1. The chickens were housed in single cages and given free access to water and a commercial broiler starter ration. At 5 weeks of age (average weight 580, 630, and 695 g for lines C, E, and F, respectively) the chickens were randomised by sex and line to receive a 14-day infusion of either vehicle, IGF-I (225 µg/d), IGF-II (225 µg/d), or combined IGF-I and IGF-II (each 112.5 µg/day). Average daily infusion rates over the 14-day period for lines C, E, and F were 310, 280, and 255 µg IGF/kg body weight, respectively. The peptides were delivered via an osmotic pump as in Experiment 1. Feed intakes and growth rates were measured. After 13 days blood from each chicken was sampled from a wing vein into a heparinised tube. The following day the chickens were weighed and then killed by cervical dislocation. The weights of the abdominal fat pad, empty gut, and breast muscle were recorded and the carcasses were stored at −20°C.

The experiments were approved by the Animal Experimentation Ethics Committees of the South Australian Research and Development Institute and the University of Queensland.

Analytical

Sample preparation. Each carcass was finely minced, mixed to homogeneity, and a sample (around 100 g) was dried under vacuum at room temperature. The dried sample was further minced and a subsample was ground to powder in a liquid nitrogen mill (Spex Inc., NJ) before analysis. Breast and thigh muscles samples were also ground in the freezer mill. The daily excreta collected from each chicken was diluted with water to a total weight of 500 g and homogenised in a Waring blender. Samples of homogenised excreta were dried under vacuum for subsequent measurements of dry matter and nitrogen content. An additional sample of homogenate was centrifuged (35 min at 2000*g*) and the supernatant was taken for N^{τ}-methylhistidine analysis.

Analytical procedures. IGF-I and IGF-II in plasma were assayed on acid-ethanol extracts of plasma using previously described procedures (Johnson *et al.*, 1990; Kita *et al.*, 1996). Chicken and human forms of each IGF showed identical binding in these assays. Plasma levels of insulin were determined using the RIA method described by McMurtry (1983) and those of T_3 were determined using an RIA kit (T3-CTRIA, bioMerieux Vitek-Australia Pty Ltd.). IGFBP in pooled samples of plasma were separated and quantitated using Western ligand binding techniques as described previously (Bastian *et al.*, 1993) with ^{125}I-labelled chicken IGF-II as a probe. Samples were pooled within each treatment by line and sex resulting in one sample per treatment in Experiment 1 and six samples per treatment in Experiment 2. Band densities of proteins near the 30- and 43-kDa molecular weight markers were quantitated and summed. The band near 30 kDa corresponded with a purified bovine IGFBP-2 standard. The 43-kDa protein was assumed to be IGFBP-3. A different gel was used for the pooled samples from within each line in Experiment 2, and since these could not be normalised, line effects could not be compared. Plasma glucose levels were determined using an automated analyser (Cobas; Roche Diagnostics, NY). Total carcass

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fat was determined by chloroform:methanol extraction of the dried carcass and the nitrogen content of carcass, excreta, and feed was assayed using an automated Dumas method (Carlo Erba, Milan, Italy). Protein synthesis rates were determined in breast and thigh muscle samples using established methods (Garlick *et al.*, 1980; Tomas *et al.*, 1991). N^{τ}-methylhistidine levels in hydrolysed feed samples (6N HCl for 18 h at 105°C), hydrolysed excreta extracts (2N HCl for 1 h at 100°C), and in muscle intracellular fluid (2% PCA extract) were determined using an HPLC method essentially as described by Wassner *et al.* (1980) with N^{τ}-ethylhistidine as an internal standard.

Carcass total protein was calculated as total N × 6.25. Protein accretion, synthesis, and degradation rates were expressed as the percentage of the protein pool synthesised per day. For estimation of accretion rates, each chicken was assumed to have the same initial carcass protein content (g/kg body weight) as the average of initial control group. Muscle protein degradation rates derived from N^{τ}-methylhistidine were calculated as described previously (Tomas *et al.*, 1991).

Statistical Procedures

Data were analysed by ANOVA and linear regression using Systat statistical software (SPSS Inc., Chicago, IL). Tabulated means are least-square means from ANOVA presented with the standard error of the difference (SE_{diff}) which is calculated from the error mean square. Bar graphs show least-square means with pooled SE from ANOVA. Elsewhere, data are presented as means ± SEM. Statistical comparisons to the control group or among several means were made using tests with appropriate adjustment of the "*t*" value to control experimentwise error. Correlations between treatment effects in Experiment 2 were calculated from the residuals after controlling for line and sex effects. A probability level of 5% was chosen for assigning differences between means.

RESULTS

Growth Performance

IGF-I infusion significantly increased weight gain in each experiment (Fig. 1), the increase ranging from 8 to 14% above the control chickens. The treatment with combined IGF-I and IGF-II was effective only in the second experiment. Food consumption was not altered by IGF treatment in either experiment ($P \geq 0.29$, data not shown), although it accounted for a significant portion of the overall variance in growth rate and was included as a covariate in the statistical analysis of growth rate. Since growth rate was increased with IGF treatment without a concomitant increase in food intake there was a significant increase in the efficiency of food utilisation expressed as weight gained per food intake (g/g) (Fig. 1). In Experiment 2 both IGF-II and the combined treatment also led to significantly improved feed efficiency. Furthermore, the effects of the IGF treatments on growth performance in this experiment were consistent for each of the lines (Fig. 2). However, the possible influence of the differences in relative infusion rates of the peptides to the different lines must be borne in mind when assessing treatment and/or line effects in this experiment.

Body Composition

The proportional weight of the abdominal fat pad (g/kg body weight) was lowest in IGF-I-treated chickens but statistically different from control chickens only in Experiment 2 (Table 1, Fig. 2). In contrast, IGF-II treatment significantly increased the abdominal fat pad mass above that found in the control (and IGF-I) group in Experiment 1. This may have been an indirect effect since the abdominal fat pad mass was inversely related to plasma IGF-I levels ($r^2 = 0.30$, $P < 0.01$; Fig. 3). There were no significant treatment effects on the total fat or protein content in the carcass nor on the proportional mass of the breast muscle but total carcass fat was inversely related to plasma IGF-I levels in each experiment ($P < 0.05$). As expected, in Experiment 2 there were significant differences in carcass composition between lines with line F birds fatter and line E birds leaner than controls (132.4, 110.9, and 125.5 g/kg body weight, respectively; $SE_{diff} = 3.69$, $P < 0.001$; Fig. 2). However, all these lines were leaner than the commercial broilers used in Experiment 1 (154.7 ± 0.33 g fat/kg) which were also significantly heavier at slaughter. Breast muscle mass (g/kg) was 12% higher in line E birds than in controls and carcass protein concentration was 2% lower in line F birds ($P < 0.05$; Fig. 2). Final total carcass protein (g/bird)

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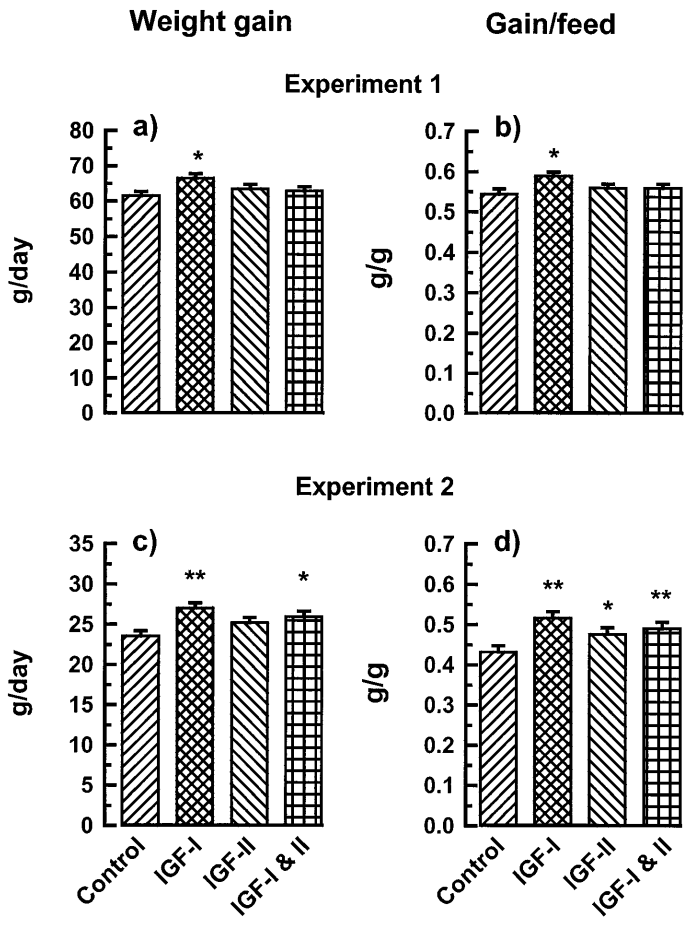

FIG. 1. The weight gain (a and c) and feed utilisation efficiency (gain / feed; b and d) in broiler chickens receiving a continuous 2-week infusion of vehicle (0.1 M acetic acid; control, ▨), recombinant human IGF-I (300 µg / kg / day; ▩), IGF-II (300 µg / kg / day; ▧) or a combination of IGF-I and IGF-II (150 µg / kg / day of each; ▦). Bars show means ± SEM for 7–8 birds in Experiment 1 and least-square means ± pooled SE of treatment effects across lines and sex for 22–23 birds in Experiment 2. Asterisks indicate significant difference from control value. *$P < 0.05$, **$P < 0.01$.

was significantly increased by IGF-I treatment in Experiment 2 (203, 213, 206, and 208 g for control, IGF-I, IGF-II, and combined IGFs, respectively; $SE_{diff} = 3.0$, $P < 0.05$). Proportional gut weight was significantly increased by IGF-I in Experiment 1, with a similar trend seen in Experiment 2 (Table 2, Fig. 2). A similar response pattern to that of the gut was observed for the spleen but liver weights were unaffected by IGF infusion (data not shown).

Plasma Hormones and Glucose

Infusion of IGF-I resulted in a threefold increase in circulating levels of IGF-I without affecting the levels of IGF-II (Table 2, Fig. 4). IGF-II infusion at the same dose rate as for IGF-I led to a relatively smaller increase of up to twofold in circulating levels of IGF-II and an absolute increment only around one fourth that observed for IGF-I. Levels of IGF-I were not significantly affected by IGF-II infusion. During the combined IGF infusion, IGF-I levels reflected the intermediate dosage but IGF-II levels were little changed from those found during IGF-II infusion alone. Relative to control birds combined IGFBP-2 and IGFBP-3 levels in pooled samples of plasma increased 1.8-, 1.1-, and 2.2-fold (Experiment 1) and 1.4-, 1.4-, and 2.0-fold (Experiment 2) for IGF-I, IGF-II, and combined infusions, respectively. Levels of IGFBP-2 showed relatively larger changes than those for IGFBP-3 in each experiment (Table 2). The combined IGF infusion was

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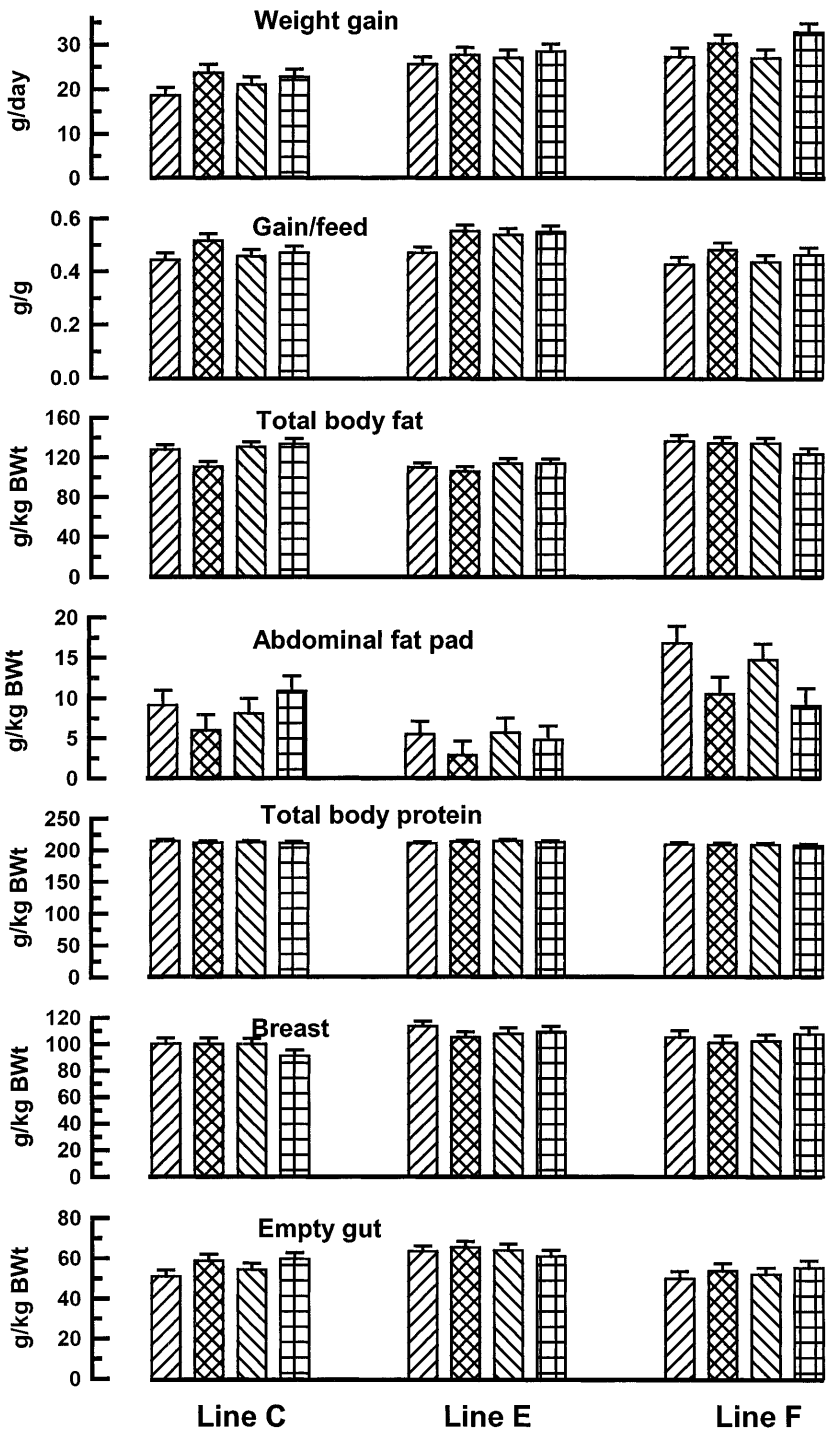

FIG. 2. The weight gain, feed utilisation efficiency (gain / feed), and final body composition of experimental broilers receiving a continuous 2-week infusion of IGF-I and IGF-II. Birds were bred at random (Line C) or selected for either efficient food utilisation (Line E) or high food consumption (Line F). Treatments are given in the legend to Fig. 1. All birds received 225 µg IGF / day and average daily infusion rates for lines C, E, and F were 310, 280, and 255 µg / kg body weight, respectively. Bars show least-square means ± pooled SE of treatment effects. Data for sexes have been combined. Males grew faster and had higher feed efficiency and lower fatness than females ($P < 0.05$) but there were no sex*line interactions. Line effects are indicated in Table 1.

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TABLE 1

Average Content (g/kg body weight) of Total Fat, Abdominal Fat Pad, Total Protein, Breast Muscle, and Empty Gut in the Carcass of Broiler Chickens after 2 Weeks Continuous Infusion Averaging 280–300 μg/kg/day of IGF-I, IGF-II, or a Combination of IGF-I and IGF-II

	Experiment 1					Experiment 2				
Treatment	Total fat	Abdominal fat pad	Total protein	Breast muscle	Empty gut	Total fat	Abdominal fat pad	Total protein	Breast muscle	Empty gut
Control	160.8	10.32	179.0	168.6	40.9	124.9	10.23	211.6	106.6	54.4
IGF-I	142.5	8.95	178.9	170.0	45.8*	117.6	6.41*	211.5	102.0	59.4
IGF-II	160.2	12.36*	183.5	170.9	40.0	125.9	9.51	212.0	103.5	56.6
IGF-I & -II	155.4	10.79	179.1	155.3	43.9	123.2	8.21	210.5	102.4	59.4
SE_{diff}	8.56	0.712	2.50	7.61	1.70	4.11	1.518	2.15	3.61	2.42

Note. Values for Experiment 2 are treatment least-square means from three-way ANOVA of sex, line, and treatment effects. SE_{diff} is calculated from the error mean square obtained from ANOVA. * indicates significant difference from control group with Bonferroni adjusted $P < 0.05$. A significant line effect was found for each of the measures in Experiment 2 ($P < 0.05$).

consistently associated with the highest levels of IGFBP-2 in each of the experiments and selected lines (Table 2, Fig. 4). Both insulin and T_3 concentrations were significantly lowered by IGF-I infusion in each experiment. There was a moderate inverse relationship between plasma levels of IGF-I and insulin in Experiment 1 ($r^2 = 0.19$, $P < 0.02$) but this was much weaker in Experiment 2 (residual $r^2 = 0.08$, $P < 0.02$). In Experiment 2 levels of T_3 were also significantly lower in the IGF-II and combined IGF-treated groups (Table 2, Fig. 4). There was no effect of IGF treatment on plasma glucose levels.

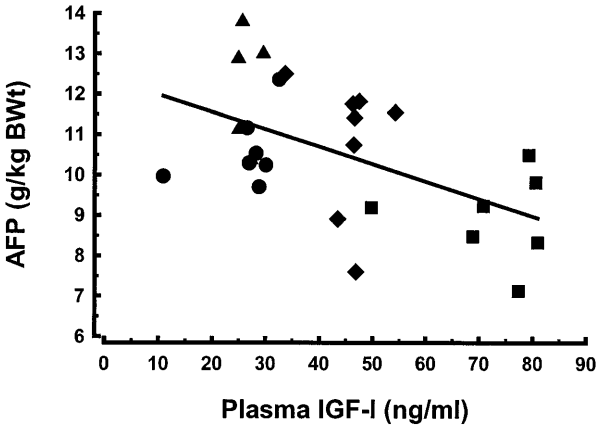

FIG. 3. The relationship between plasma IGF-I levels and the abdominal fat pad (AFP) weight in young chickens after infusion for 2 weeks with vehicle (0.1 mM acetic acid; ●), recombinant human IGF-I (300 μg/kg/day; ■), IGF-II (300 μg/kg/day; ▲), or a combination of IGF-I and IGF-II (150 μg/kg/day of each; ◆). $r^2 = 0.30$, $b = -0.048 \pm 0.014$, $P < 0.01$.

Protein Metabolism

Indicators of protein metabolism were obtained during the last 2 days and at the end of the treatment period in Experiment 1. Nitrogen retention was significantly increased by IGF-I infusion (Table 3), a result that indicates that the increased weight gain was not simply due to fat or water deposition. The rate of muscle protein breakdown calculated from N^{τ}-methylhistidine excretion over the last 2 days of treatment was significantly decreased by IGF-I treatment. However, muscle protein synthesis rates at the end of the infusion period were not affected by treatment. No significant treatment effects were found on the calculated protein accretion or degradation rates which contain large errors of estimation. Nonetheless, the estimates of degradation were in general agreement with the N^{τ}-methylhistidine measures. The concentration of unbound N^{τ}-methylhistidine in the intracellular fluid of muscle was significantly depressed by about 15% in chickens receiving IGF-I either alone or in combination with IGF-II. These measurements support the N^{τ}-methylhistidine excretion data and provide further evidence for a reduced rate of muscle protein degradation during IGF-I treatment.

Metabolic Correlates

In Experiment 1 the abdominal fat pad mass was inversely related to plasma IGF-I levels ($P < 0.01$; Fig. 3). Food utilisation efficiency was positively related to plasma IGF-I levels ($r^2 = 0.14$, $P < 0.02$) and inversely

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TABLE 2

Average IGF-I, IGF-II, T_3, Insulin (ng/ml), IGF-Binding Protein (IGFBP)-2 and IGFBP-3 (Relative Densitometric Units) and Glucose (mmol/liter) Levels in the Plasma of Chickens after 2 Weeks Continuous Infusion Averaging 280–300 µg/kg/day of IGF-I, IGF-II, or a Combination of IGF-I and IGF-II

	Experiment 1							Experiment 2						
Treatment	IGF-I	IGF-II	IGFBP-2	IGFBP-3	T_3	Insulin	Glucose	IGF-I	IGF-II	IGFBP-2	IGFBP-3	T_3	Insulin	Glucose
Control	26.3	7.5	27.3	10.4	1.75	1.17	11.22	15.9	19.3	25.5	18.8	2.79	3.82	13.35
IGF-I	72.5***	5.3	51.6	14.6	1.21*	0.51*	11.04	51.5**	19.2	35.0	25.6	1.88***	2.57*	13.30
IGF-II	26.2	16.4***	28.7	10.5	1.51	1.23	12.64	13.8	31.0**	36.7	27.2	2.11*	3.56	12.96
IGF-I and -II	45.6***	13.7***	63.8	18.4	1.47	0.93	12.09	33.0**	29.5**	61.2*	28.6	1.87***	3.26	13.18
SE_{diff}	3.74	1.54	—	—	0.186	0.264	0.609	3.00†‡	1.64	11.8	7.0	0.277†	0.441	0.254†

Note. Values for Experiment 2 are least-square means from three-way ANOVA of sex, line, and treatment effects. SE_{diff} is calculated from the error mean square obtained from ANOVA. IGFBP data were obtained from plasma samples pooled within treatments. Experiment 2 plasma samples were pooled by line and sex ($n = 6$ for each treatment). *, **, *** indicate significant difference from control group with Bonferroni-adjusted $P < 0.05$, <0.01, and <0.001, respectively. †‡ indicate the presence of significant line effects and [line ∗ treatment] interactions, respectively ($P < 0.05$).

related to the rate of muscle protein breakdown ($r^2 = 0.25$, $P < 0.01$; Figs. 5a and 5b) and total body fat content ($r^2 = 0.45$, $P < 0.001$; data not shown). In Experiment 2 the line and sex effects accounted for most of the variance in these parameters due to their strong correlation with fat content. Nonetheless, removal of these effects by regression analysis showed plasma IGF-I levels were weakly correlated directly with food utilisation efficiency (residual $r^2 = 0.11$, $P < 0.01$) and inversely with the abdominal fat pad mass (residual $r^2 = 0.07$, $P < 0.02$). In neither experiment were the measures of fatness significantly correlated with final levels of total T_3 in plasma.

DISCUSSION

Our data show that continuous infusion of around 300 µg IGF-I/kg body weight into chickens with diverse genetic backgrounds consistently results in enhanced growth rates. These results are at odds with those previously reported. In a similar study Huybrechts *et al.* (1992) infused 300 µg IGF-I/day into young broilers over 14 days but failed to observe a growth response. Other studies which used lower doses of infused or injected IGF-I over 14 days (McGuinness and Cogburn, 1991; Tixier-Bouchard *et al.*, 1992) or a higher dose (450 µg/kg starting weight/day) over only 5 days (Czerwinski *et al.*, in press) also failed to show a growth response. It was noted that the chickens treated with IGF-I have a significantly higher rate of nitrogen gain measured both by nitrogen balance (Experiment 1) and final carcass composition (Experiment 2). This indicates that the growth response is inclusive of a lean tissue response. In contrast, IGF-II was ineffective for promoting growth, which confirms the findings of Spencer *et al.* (1996). Since *in vitro* studies have shown equipotence of IGF-I and IGF-II in adipocytes, hepatocytes, and muscle satellite cells (Widmar *et al.*, 1985; Duclos and Goddard, 1990; Butterwith and Goddard, 1991; Duclos *et al.*, 1991) the relative lack of efficacy of IGF-II must be ascribed to its higher rate of clearance (McMurtry *et al.*, 1996) and resultant lower levels sustained in plasma. Presumably, the mechanism for the higher rate of clearance is not via binding to the IGF-I (type-1) receptors. In addition, our experiments showed that IGF-I treatment leads to a reduction in abdominal fat pad mass which is proportional to plasma IGF-I levels. This confirms and extends the effects of IGF-I infusion on depot fat mass reported by others (Huybrechts *et al.*, 1992; Tixier-Bouchard *et al.*, 1992). Although IGF-I treatment did not significantly reduce total carcass fat compared with that of controls, there was a significant inverse relationship between plasma IGF-I levels and the carcass fat content indicating an overall IGF-I effect. The reduced fat deposition in IGF-I-treated birds shows IGF-I to have an opposite action to that of insulin which leads to increased fat deposition (Simon, 1988). Accordingly we consider that the effects of IGF-I in this experiment are not mediated via the insulin

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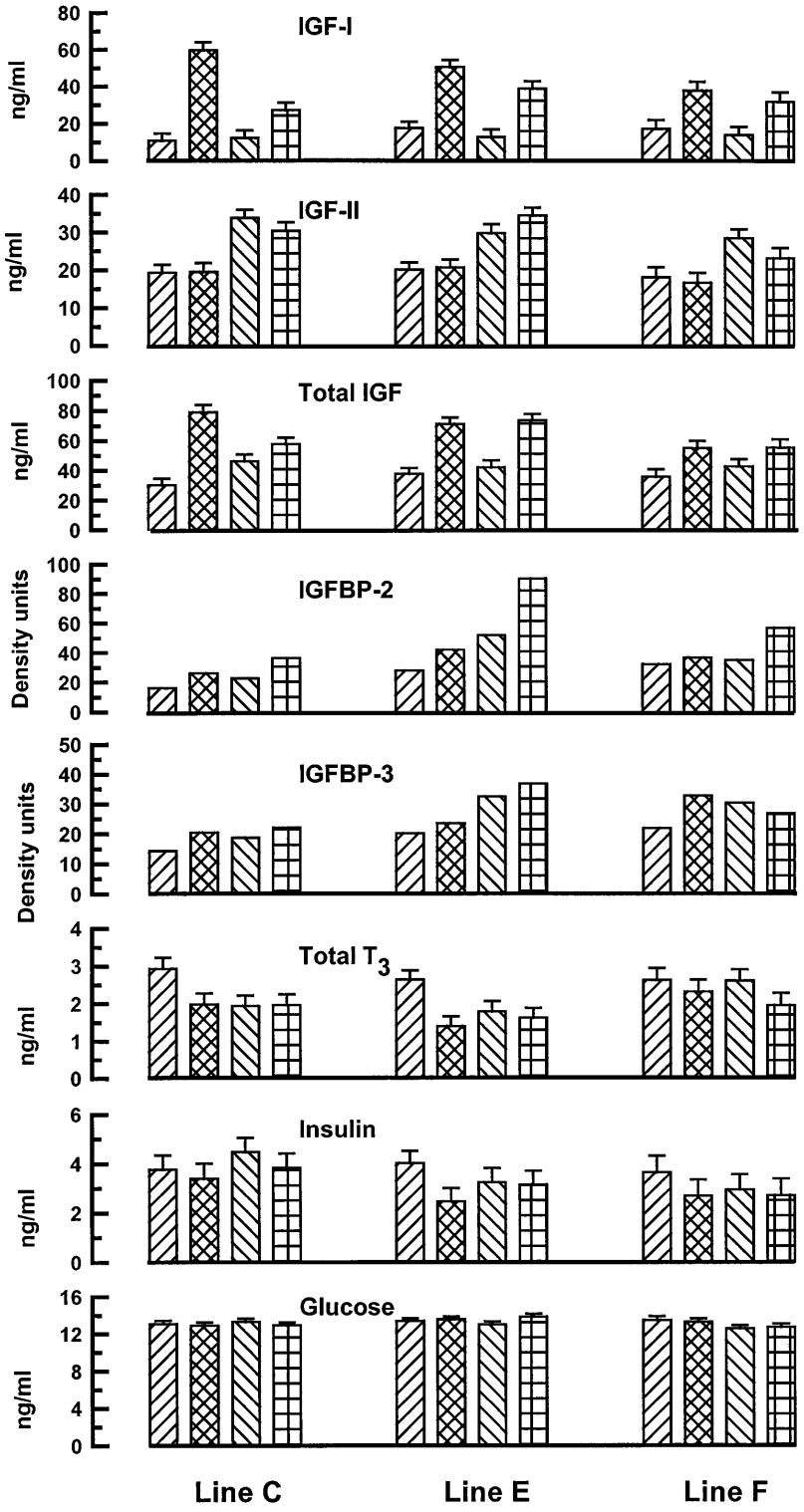

FIG. 4. The levels of hormones, IGF-binding proteins, and glucose in plasma of experimental broilers after receiving a continuous 2-week infusion of IGF-I and IGF-II. Birds were bred at random (Line C) or selected for either efficient food utilisation (Line E) or high food consumption (Line F). Treatments are given in the legend to Fig. 1 and IGF dosage is given in the legend to Fig. 2. Means are least-square means ± pooled SE of treatment effects. Data for sexes were combined since there were no significant differences due to sex. For IGFBP assays the plasma samples for each line were run on a separate gel which precludes comparison between lines. Line effects for other measures are indicated in Table 2.

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TABLE 3

Indicators of Protein Metabolism in Carcass and Muscles of Broiler Chickens after 2 Weeks Continuous Infusion of 300 μg / kg / day of IGF-I, IGF-II, or a Combination of IGF-I and IGF-II (150 + 150 μg / kg / day). Values shown are Total Nitrogen Retention, Fractional Rates of Protein Accretion (k_a), Synthesis (k_s), and Degradation (k_d) and Concentration of Free N^{τ}-Methylhistidine in the Intracellular Fluid of Muscle (N^{τ}-MH_i)

Treatment	Nitrogen retention (g / day)	Carcass k_a (% / day)	Muscle k_s (% / day)	$k_d(k_s - k_a)$† (% / day)	$k_d(N^t MH)$‡ (% / day)	Muscle $N^t MH_i$ (nmol / g protein)
Control	2.24	5.76	10.53	4.66	2.05	128.8
IGF-I	2.54*	5.97	9.90	3.92	1.56*	106.4*
IGF-II	2.30	6.12	11.10	4.98	1.84	122.0
IGF-I and -II	2.37	5.73	11.09	5.35	1.69	107.4*
SE_{diff}	0.069	0.172	0.422	0.476	0.189	1.08

Note. Values are least-square means of seven or eight chickens in each group. Muscle k_s and N^{τ}-MH_i values are averages from separate determinations on breast and thigh muscles. SE_{diff} is calculated from the error mean square obtained from ANOVA.

† Difference between averaged thigh and breast muscle k_s and the estimated total carcass protein k_a.

‡ Fractional excretion of N^{τ}-MH pool which principally reflects myofibrillar protein k_d.

* Significantly different from control group with Bonferroni-adjusted $P < 0.05$.

receptor. This view is reinforced by the higher IGFBP levels observed during IGF treatment as these would limit any rise in the levels of unbound IGF-I in plasma (Logan and Hill, 1992; Lord *et al.*, 1994; Zapf, 1995). Similarly, the increased abdominal fat pad mass resulting from IGF-II infusion in Experiment 1 may have been due to reduced availability of IGF-I resulting from the increased levels of IGFBPs.

Insulin promotes lipogenesis and fat deposition in chickens (Simon, 1988) as it does in mammals (Griffin and Hermier 1987). Thus, the decrease in fat deposition by IGF-I infusion may be partly explained by the associated suppression of insulin levels, presumably due to a direct effect of IGF-I in the pancreas (e.g., Zenobi *et al.*, 1992). We found a significant, albeit weak ($r^2 < 0.15$) positive relationship between plasma insulin levels and total carcass fat in both experiments but in neither case was the relationship significant for the abdominal fat pad weight. Thus the response in abdominal fat pad mass to IGF-I treatment must be partly ascribed to other direct or indirect effects of IGF-I which may be associated with partitioning of nutrients towards lean tissue growth (Tomas *et al.*, 1994). Thyroid hormones are known to enhance lypolysis and reduce carcass fat content (e.g., Cogburn, 1991) and have been suggested as the mechanism for the alteration in fatness induced by IGF-I and IGF-II (Huybrechts *et al.*, 1992; Spencer *et al.*, 1996). However, in our experiments IGF-I treatment consistently lowered plasma T_3 levels, an effect which may be expected to increase fatness (Spencer *et al.*, 1996). Our finding that IGF-I infusion lowered T_3 levels is in agreement with McGuinness and Cogburn (1991) and Czerwinski *et al.* (in press) and consistent with the promotion of hypothalamic somatostatin secretion by IGF-I (e.g., Bermann *et al.*, 1994). We cannot exclude the possibility of either a transient increase in total T_3 levels (Huybrechts *et al.*, 1992) or an increase in free-T_3 levels (e.g., Hussain *et al.*, 1996). We note that IGF-II also depressed plasma T_3 levels although this effect was significant only in Experiment 2. Thus, the key finding here is the lack of any consistent relationship between circulating total T_3 levels and carcass fat content which suggests other mechanisms for the effects of IGF-I on fatness. Also, it is remarkable that the observed effects of IGF-I were consistent across all the selection lines examined.

The combined effects of increased rates of protein gain and decreased rates of fat deposition without an increase in food intake resulted in a more efficient conversion of nutrients to weight gain. This resulted in a substantial increase in food utilisation efficiency (gain:feed ratio). A shift towards deposition of lean tissue and away from deposition of fat is normally associated with improved weight gain per feed intake (Boyd and Bauman, 1989) since lean tissue is less energy dense. Indeed, selection for efficiency of feed utilisation results in selection for a lean faster growing phenotype, such as line E in Experiment 2 (Pym and Solvyns, 1979). Thus, it seems incongruous that Huybrechts *et al.* (1992) were not able to show a change in food utilisation efficiency in chickens which had a significant reduction of the abdominal fat pad mass

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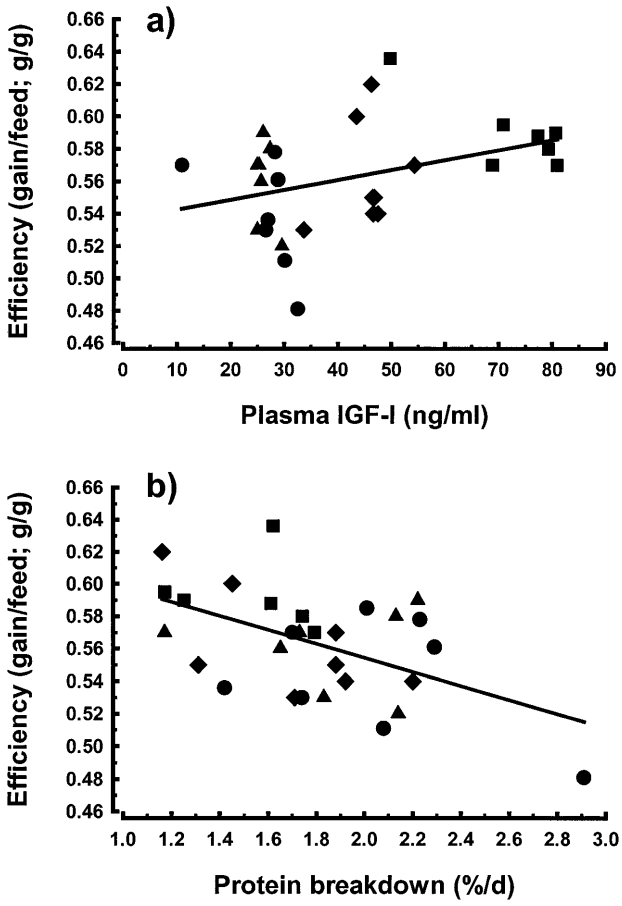

FIG. 5. The relationship food utilisation efficiency (weight gain/feed intake) and plasma IGF-I levels (a) and the rate of muscle protein breakdown (b) in young chickens which had been infused for 2 weeks with vehicle (0.1 mM acetic acid; ●), recombinant human IGF-I (300 µg/kg/day; ■), IGF-II (300 µg/kg/day; ▲) or a combination of IGF-I and IGF-II (150 µg/kg/day of each; ◆). For a, $r^2 = 0.14$, $b = 0.0006 \pm 0.0003$, $P < 0.05$. For b, $r^2 = 0.25$, $b = -0.042 \pm 0.014$, $P < 0.01$.

during IGF-I infusion, but this was possibly due to the lack of a concomitant growth response, as occurred in our study.

The continuing metabolic processes associated with protein turnover require a substantial input of energy, with estimates varying from 15 to 40% of maintenance energy requirements (e.g., Millward *et al.*, 1976; Webster, 1981; Muramatsu *et al.*, 1985, 1987). Since both synthesis and breakdown processes require energy, an increase in protein mass is achieved most efficiently with a reduction in rates of protein breakdown, rather than by simply increasing rates of protein synthesis or turnover. In addition, reduced rates of protein degradation allow an increase in lean body mass without a proportionate increase in maintenance energy needs. We have previously reported that efficiency of feed utilisation in selected lines of broilers (Tomas *et al.*, 1988, 1991) and rats (Tomas and Pym, 1995) is inversely related to rates of muscle protein breakdown. Our results from Experiment 1 confirm those findings and indicate that about 25% of the variation in feed use efficiency was associated with the variation in the rate of muscle protein breakdown (Fig. 5b). The association with plasma IGF-I levels was weaker ($r^2 = 0.14$, $P < 0.05$), possibly because increased feed use efficiency was secondary to the IGF-I-mediated changes in fat content and protein turnover which accounted for some 62% of the variation in the gain:feed ratio.

It is important to note that the increased rate of protein gain during IGF-I infusion could not be attributed to an increase in synthesis rates and appeared entirely due to reduced rates of protein breakdown (Table 3). Our finding of increased growth and reduced rates of protein breakdown in these experiments differs from our earlier report (Czerwinski *et al.*, in press) in which chicken IGF-I was infused at a 1.5-fold higher dose for only 5 days. An explanation for the different response is not immediately apparent because of the marked differences in the experimental protocol. However, the lack of stimulation of protein synthesis is consistent between these studies and may be attributed to the depression in plasma insulin levels upon IGF-I administration (e.g., Cottam *et al.*, 1992; Lo and Ney, 1996; McMurtry *et al.*, in press). Indeed, it was recently reported that maintenance of plasma insulin and/or amino acid levels in rats during IGF-I administration resulted in stimulation of protein synthesis (Jacob *et al.*, 1996). It is interesting to note that, whereas IGF-I infusion does not stimulate rates of protein synthesis in normal growing rats (Tomas *et al.*, 1993), it can return these rates to near normal levels in diabetic rats (Tomas *et al.*, 1991, 1993, 1996). Furthermore, if insulin levels are the prime determinant of protein metabolism, we would expect the rates of protein breakdown, which are tonically suppressed by insulin (e.g., Bennet *et al.*, 1991) to increase during IGF-I treatment because insulin levels are lower. In fact, the reverse was observed. This suggests that the action of IGF-I more than replaces the insulin loss and indicates

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that IGF-I and insulin have convergent metabolic actions.

Although infused at the same rate, IGF-II levels in the plasma remained well below those for IGF-I. This is consistent with the 1.75-fold greater metabolic clearance rate of IGF-II relative to IGF-I (McMurtry *et al.*, 1996). The mechanism for this difference is unclear since no type 2 IGF receptor has been identified in chickens (Goddard and Boswell, 1991). Nonetheless, the IGF-II infusion was at least equipotent with IGF-I for stimulation of IGFBP levels. The consistently enhanced effects of the combined IGFs on IGFBP levels did not reflect either the total IGF infusion rates or their levels in plasma (Table 2, Fig. 4). The fact that IGF-II levels during the combined IGF infusion were almost the same as during IGF-II infusion alone suggests altered pharmacokinetics for this IGF when coinfused with IGF-I. A corresponding effect was not apparent for IGF-I and suggests that IGF-I interfered in some way with the clearance of IGF-II, perhaps by competition for the same receptor or by changing the fraction of plasma IGF-II bound to the 70-kDa protein which specifically binds IGF-II (McMurtry *et al.*, 1996).

In conclusion, these experiments are the first to report an enhancement of growth and food utilisation efficiency by broilers receiving exogenous IGF-I. The increased growth rate was consistent across sexes and several lines with diverse feed efficiency and body composition characteristics. It reflected an increased accumulation of lean tissue resulting largely from a reduction in the rate of muscle protein breakdown. We also report that IGF-I infusion reduces carcass fat, in agreement with published data. The reduction in fat was not related to plasma T_3 levels as reported elsewhere (Huybrechts *et al.*, 1992) but rather appeared to be mediated by depressed insulin levels. Our results show that IGF-I may be important in controlling the growth of young broilers at least in part by modulating the rates of protein breakdown.

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