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Running Head: Retinoic acid and Broiler Carcass Lipid Feeding Retinoic Acid Reduces Total Carcass Fat in Growing Broiler Chickens

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Abstract

All-trans retinoic acid (RA), the carboxylic form of vitamin A, inhibits fat cell differentiation *in vitro* in multiple mammalian species. However, the ability of RA to limit adipose tissue accretion in monogastric meat-producing animals *in vivo* has not been investigated. The objective of this study was to determine the effect of feeding RA on total carcass lipid and tissue concentrations of RA in growing broilers. In the first experiment, 48 one-day-old birds were fed either a commercial starter-grower diet or the commercial diet supplemented with 14 mg/kg RA for six weeks. Feeding RA decreased carcass weight (12%; $P<0.0001$), feed intake (9%; $P<0.0001$), and average daily gain (12%; $P<0.0001$) while tending to increase feed efficiency (4%, $P<0.07$). Retinoic acid significantly decreased total carcass lipid was by 14% ($P<0.008$) though abdominal fat mass only tended to be decreased in RA-treated birds (12%; $P<0.07$). In the second experiment, 36 one-day-old chicks were randomly assigned to either the control or RA-supplemented diet (14 mg/kg) and liver and adipose tissue RA concentrations were determined using HPLC. Liver RA concentrations were three-fold higher in birds consuming the RA-supplemented diet ($P<0.02$) diet did not significantly affect RA concentration in adipose tissue ($P<0.21$). However, RA concentrations were numerically lower in birds fed the RA-supplemented diet on each day measured. Expression of retinoic acid receptors, retinoic acid receptor alpha (RARα) and retinoid X receptor alpha (RXRα) was verified in adipose tissue by semi-quantitative PCR. These results suggest that feeding RA can reduce carcass adiposity. However, this approach may be limited by physiological mechanisms that constrain retinoic acid accumulation in adipose tissue. Better characterization of these mechanisms may reveal targets that could be useful for reducing carcass fat in growing broilers either through selection programs or the administration of exogenous effectors.

Keywords: Broiler; Retinoic acid; Adiposity; Feed efficiency

Abbreviations: 4-[(E)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid (TTNPB), Cellular Retinol-Binding Proteins (CRBPs), Cytochrome P450 enzyme; P450AI (CYP26), Peroxisome Proliferator-Activated Receptor Gamma (PPARγ), Retinoic Acid (RA), Retinoic Acid Receptor Alpha (RARα) and Retinoid X Receptor Alpha (RXRα), White Adipose Tissue (WAT)

Introduction

Traditional selection practices have emphasized rapid growth without consideration of carcass composition leading to broilers that are comparatively fatter [1,2]. Though fat depots in the neck and legs are more pronounced in broilers at hatch, the abdominal fat pad is the most economically important because it displays the greatest growth rate among fat depots post-hatch and accounts for the greatest percentage of carcass fat at market weight [3]. Given the prevalence of further processed poultry products, limiting carcass fat has become a priority due to its negative impact on feed efficiency and its low commodity value.

Adiposity in vertebrates is dictated both by hyperplasia (adipocyte number) and hypertrophy (average adipocyte volume). Several studies have examined adipocyte cellularity in abdominal fat of growing broilers to clarify the roles of adipocyte number and size in the development of this depot. Generally, adipocyte number increases in the abdominal fat pad of broilers from a few days post-hatch until 4-7 weeks of age [4-6]. However, some studies have documented increases in adipocyte number in the abdominal fat pad as late as 12-14 weeks post-hatch [7-9]. Conversely, adipocyte size becomes a progressively dominant influence on abdominal fat mass with age [2]. These data suggest that inhibiting the differentiation of adipocyte precursors (adipogenesis) may be a strategy to limit carcass lipid in growing broilers.

Retinoic acid (RA), the carboxylic acid form of vitamin A, potently inhibits the differentiation of 3T3-L1 preadipocytes [10,11] and primary preadipocytes from both swine and steers [12-14]. This inhibitory action on primary preadipocytes suggests that feeding RA may reduce fat cell differentiation in growing animals. Although the potential of RA to limit adipose tissue accretion in monogastric meat-producing animals is largely unexplored, this possibility is supported by the observation that intramuscular adipose tissue is increased in steers that are deliberately fed vitamin A-deficient diets [13,15,16-24]. The objective of this study was to determine the effect of feeding RA on total carcass lipid and tissue concentrations of RA in growing broilers.

Materials and Methods

Animals

All animals were handled according to guidelines approved by the Animal Care and Use Committee at Oregon State University. One-day-old chicks were purchased from a commercial supplier (Jenks Hatchery Inc, Tangent, OR.) and housed in groups for seven days with ad libitum access to water and Standard Purina Mills Start and Grow MP (Table 1; Purina Mills, St Louis, MO). On day 8, chicks

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were weighed, housed in individual cages, and randomly assigned to treatments. Room temperature was maintained at 32°C during week 1 and lowered 2.8°C each week until finally maintained at 24°C. Birds were maintained in continuous light (23 hours light: 1 hour dark per day) for weeks 1 and 2 and switched to 12 hours of light per day from weeks 3 through 6. Necropsies were performed by staff veterinarians at the College of Veterinary Medicine Diagnostic Laboratory at Oregon State University.

Retinoic acid

Retinoic acid was purchased from BIOMOL (Plymouth Meeting, PA) and was weighed and handled under yellow light, dissolved in 100 ml corn oil and then mixed into the commercial ration. Since Retinoic acid is highly labile, rations were mixed weekly and stored in the absence of light at 0°C until distributed to birds.

Experimental plan

In the first experiment, forty 7-day-old chicks were transferred to individual cages and were randomly assigned to one of five rations (0, 3.5, 7, 14, 28 mg RA/kg feed; n=8 per level) corresponding to feeding RA at 0, 8, 16, 32 and 64 times the suggested NRC vitamin A requirements for growing broilers. Birds were given ad libitum access to water and feed. Body weight gain and feed intake were recorded biweekly until day 42 when all birds humanely euthanized. Broilers were randomly selected from each treatment group to undergo necropsy in order to determine if feeding RA was associated with toxicity.

In the second experiment, forty-eight 7-day-day old chicks were transferred to individual cages and randomly assigned to either the control ration or the control ration supplemented with 14 mg RA/kg feed (n=24). Birds were given ad libitum access to water and feed. Body weight gain and feed intake were recorded biweekly until day 42 when all birds were euthanized. Carcasses were scalded for 1 minute and defeathered. Abdominal fat (considered to be fat extending within the ischium, surrounding the cloaca, and adjacent to the abdominal muscle) was immediately dissected from the viscera and abdominal fat mass was recorded. Internal organs were removed from the viscera and examined for potential lesions related to retinoic acid toxicity. Birds were sexed based upon the presence or absence of testis. The feet and head were removed from the carcass and carcass weight was recorded. Abdominal fat and organs were replaced in the carcass and the processed carcass was stored at -20°C until lipids were extracted for determination of total carcass lipid.

In the third experiment, thirty six 7-day-old chicks were randomly assigned to either the control or RA-supplemented ration as above. A serial slaughter protocol was utilized in which six chicks from each treatment were euthanized on days 0 (baseline), 28 and 42 of treatment administration. Feed intake and body weight were recorded for each time point. Liver and adipose tissue were rapidly collected following euthanasia and minced, quickly frozen in liquid nitrogen, and subsequently stored at -80°C until utilized for determination of retinoic acid concentrations via HPLC or for RNA studies.

Ingredient	g/100g diet
Crude Protein	17.00
Lysine	0.85
Methionine	0.30
Crude Fat	3.00
Crude Fiber	5.00
Calcium (Ca)	1.00
Phosphorus (P)	0.60
Salt (NaCl)	0.60

Table 1: Composition of broiler diets.

HPLC procedures

Retinoic acid was analyzed using reverse-phase HPLC following the procedures of Wang et al. (1991). Briefly, liver and adipose tissues were extracted under yellow light without saponification with the following slight modifications. One hundred milligrams of tissue was extracted by adding 150 µl water, 107 µl 2 M NaOH, 50 µL of the internal standard, Ro 13-6307 (100 ng), and 500 µl hexane. Samples were centrifuged for 5 minutes at 1500 X g and the organic layer was re-extracted by adding 150 µL 2 M HCL, 215 µL ETOH, and 500 µL hexane followed by centrifugation for 5 minutes at 1500 X g. The organic layer was evaporated to dryness under N_2 at 37°C and the residue was re-dissolved in 200 µl methanol. A 50-µl aliquot of the final extract was injected into the HPLC system. The HPLC system consists of a Waters 2690 Separation Module (Waters Chromatography, Milford, MA), a Waters Nova-Pak C18 3.9 x 150 mm steel column and a Waters 996 Photodiode array detector set at wavelength of 350 nm. The column was a Waters Nova-Pak C18. The mobile phase was 100% methanol (solvent B) and H_2O (solvent A). The gradient procedure was as follows: flow rate was .8 ml/min with 90% solvent B and 10% solvent A followed by a one minute linear gradient to 1.5 ml/min 100% solvent B, a 18 minute hold at 100% solvent B then a one minute linear gradient back to 10% solvent A, 90% solvent B and a final 5 minute hold. Column temperature was 35 ° C. Retinoic acid was quantified by determining peak areas calibrated against known amounts of standards as calculated by the Millenium32 software (version 3.05.01). The lowest limit of detection was .2 pmol.

Total carcass lipid

Total carcass lipid was determined using the method of Cherian et al., (2005). Briefly, carcasses were thawed and tightly sealed in a 3 liter beaker containing 1 liter of water, and then autoclaved at 121°C (15psi) for 2.5 h. When cooled, the contents were homogenized in a 4 L Waring Heavy Duty Laboratory blender at 20,000 rpm for 5 min. The resulting slurry was stored at -20°C until lipid analysis. Approximately 2-g samples were weighed into 50-mL test tubes, 18 ml of Folch solution (chloroform:methanol=2:1 w/v, Folch et al., 1957) was added, and the samples were incubated at room temperature for 6 hours. Then 4 ml 0.88% NaCl was added and tubes were gently inverted followed by centrifugation at 2000 rpm for ten minutes. Subsequently, 3 ml of the chloroform layer was pipetted onto pre-weighed aluminum pans in duplicate. The chloroform was gently evaporated off and pans reweighed. Percent total lipid was calculated as follows [(pan +sample weight-pan weight) * 12 ml chloroform* 100]/ [3 ml *sample weight].

RNA isolation

Total RNA was extracted from tissues using the quanidinium-acid phenol method [25]. Total RNA concentration was determined spectrophotometrically using A260 and A280 measurements. The ratio of A260 / A280 was between 1.9 and 2.1 for all samples. Five micrograms of total RNA from each sample was separated on a 1.2% denaturing formaldehyde gel and stained with ethidium bromide. The RNA integrity was assessed visually by judging the quality of 18 and 28s rRNA bands.

Semi-quantitative RT-PCR

Reverse transcription (RT) reactions (20 µL) consisted of 4 µg of total RNA, 50 U SuperScript II reverse transcriptase (Invitrogen/

Life Technologies), 40 U of an RNAse inhibitor (Invitrogen/Life Technologies), 0.5 mmol/L d NTP, and 100 ng random hexamer primers. Multiplex polymerase chain reaction (PCR) was performed in 50 µL containing 20 mmol/L Tris-HCL, pH 8.4, 50 mmol/L KCL, 1.0 µL of RT reaction, 2.5 U of Platinum Taq DNA polymerase (Hot Start, Invitrogen/Life Technologies), 0.2 mmol/L dNTP, 2 mmol/L Mg^{2+} (Invitrogen/Life Technologies), and 10 pmol each of gene-specific primers for β-actin (forward: 5'- CGTGGGCCGCCCT AGGCACCA-3'; reverse: 5'-TTGGCCTTAGGGTTCAGGGGGG-3'), RARα (forward: 5'-GCATCCAGAAGAACATGGTGT-3'; reverse: 5'-CCTGCTTGGCGAACTCCAC -3') and RXRα (forward: 5'-GGACACACCCATTGACACCT-3'; reverse: 5'-AAGACCCTGTAG TCGCCCAA-3'). Thermal cycling parameters were as follows: 1 cycle 94°C for 4 min, followed by 26-30 cycles, 94°C for 1 min, 56°C for 2 min, 72°C for 2 min with a final extension at 72°C for 8 min. Primers were synthesized at the Center for Gene Research at Oregon State University. Identity of PCR products was verified by DNA sequencing. Cycle number for the multiplex PCR reaction was selected by experimentally determining the highest cycle number in which the amplification of both cDNA products was within a linear range. The optimal cycle number was then considered to be two cycles lower than the highest cycle of linearity. RT-PCR products were visualized by separating DNA on a 3% agarose gel and staining with SyberGreen according to the manufacturer's directions (Molecular BioProbes, Eugene, OR). Data for each replicate represented the mean of three individual RT-PCR reactions.

Statistical analysis

Data are expressed as the mean +/- SEM. Broilers were individually housed in cages. Thus, the experimental unit is individual bird. Data were analyzed by using the analysis of variance procedure followed by multiple comparisons of means with Fisher's least significant difference using SAS (SAS institute, Cary, NC). Differences were considered significant if $P<.05$.

Results

Effect of retinoic acid supplementation on growth performance and adiposity in broilers

To establish an appropriate level of retinoic acid that would not adversely affect broiler health, forty 7-d-old chicks were randomly assigned to one of five rations (0, 3.5, 7, 14, 28 mg RA/kg feed). After 42 days on the test rations, no parameter of growth performance (weight gain, feed intake, average daily gain, or feed conversion) was significantly affected by diet. However, post hoc analysis indicated feeding 28 mg/kg RA-supplemented ration tended ($P<0.10$) to decrease feed intake versus the control ration. No broilers randomly selected from any dietary group demonstrated signs of retinoic acid toxicity as determined by necropsy at the conclusion of the experiment. Consequently 14 mg/kg was chosen as the appropriate level of RA for the subsequent growth studies.

To determine the effect of retinoic acid on growth performance and carcass adiposity, forty-eight 7-d-old broiler chicks were randomly assigned to either a control ration or a retinoic acid supplemented ration (14 mg RA/kg feed). Rations were fed for six weeks and body weights and feed intake measured biweekly (Table 2). As expected, female broilers had more abdominal fat than their male counterparts (21%; $P<0.003$) and abdominal fat was a higher percentage of carcass weight in female broilers versus males (25%; $P<0.0003$). However, there were no sex effects on any other growth parameter therefore data for males and females were pooled when determining the effect of RA on growth performance. The RA-supplemented ration decreased carcass weight (12%; $P<0.0001$), feed intake (9%; $P<0.0001$) and average daily gain (12%; $P<0.0001$) while tending to increase feed efficiency (4%; $P<0.07$) (Table 2). Feeding the RA-supplemented ration decreased total carcass lipid 14% versus control broilers ($P<0.008$; Figure 1). Sex did not influence the effect of RA on total carcass lipid. Feeding RA tended to decrease dissected abdominal fat mass versus control birds (12%; $P<0.07$). These data suggest that feeding RA can significantly decrease carcass adiposity in growing broilers.

Tissue concentrations of retinoic acid in the liver and adipose tissue of growing broilers fed either the control or RA-supplemented ration

To evaluate RA absorption in growing broilers, thirty-six 7-day-old chicks were randomly divided between the control and the RA-supplemented ration (14 mg/kg) and a serial sampling strategy was utilized in which six chicks from each treatment were euthanized on days 0 (baseline), 28 and 42 of treatment administration. Hepatic and adipose tissue retinoic acid concentrations were determined using HPLC (Table 3). Representative chromatographs are shown in Figure 2. On day 28, hepatic retinoic acid levels were 2.4-fold higher in broilers fed the RA-supplemented ration versus the control ration ($P<0.03$) with retinoic acid levels reaching 3.7-fold higher in the

Variable	Control	Retinoic Acid	SEM	P-value
Initial BW[3], kg	0.119	0.114	0.003	0.11
Carcass weight, kg	1.46	1.28	0.02	0.0001
Feed intake, kg	2.09	1.89	0.034	0.0001
ADG[4], kg	0.041	0.036	0.001	0.0001
Feed conversion[5]	1.47	1.52	0.005	0.07

Table 2: Growth performance of broilers fed control and retinoic acid supplemented diets.

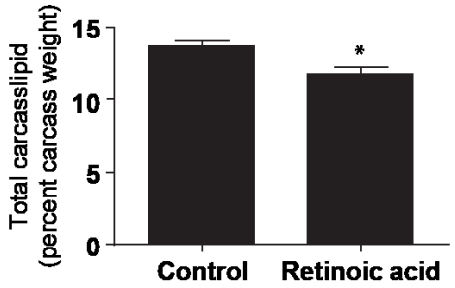

Figure 1: Total carcass lipid in broilers fed either a control diet or a diet supplemented with 14 mg/kg all-trans retinoic acid for 42 d. Feeding retinoic acid daily decreased total carcass lipid by 14% in broilers. Total carcass lipid was determined as described in Materials and Methods and expressed as a percentage of final carcass weight. Values are means +/- SEM, n= 12. Data was analyzed using one-way analysis of variance with treatment as the main effect. Treatments with an asterisk were significantly different than control, P < .01.

Sample	Control	Retinoic Acid	P-value
Day 0	165 ± 41	128 ± 45	0.61
Day 28	459 ± 168	1088 ± 168	0.03
Day 42	610 ± 521	2239 ± 672	0.01
Overall Response	429 ± 223	1241 ± 264	0.02

Table 3: Concentration of retinoic acid in liver of broilers fed control or retinoic acid supplemented diets.

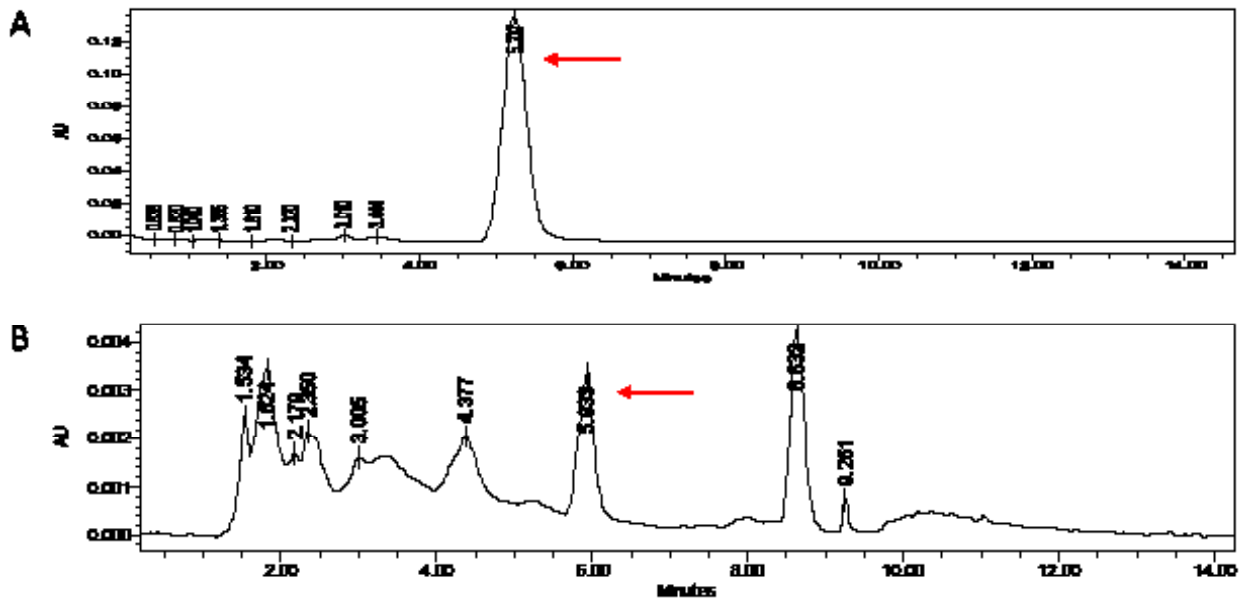

Figure 2: Representative chromatographs depicting elution time of the 4-[(*E*)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid (TTNPB) standard (A), and all-trans retinoic acid (B) extracted from liver samples. Arrows indicate relevant peaks. Extraction and detection were performed as indicated in the Materials and Methods.

livers of treated broilers by day 42 ($P<0.01$). The overall response for hepatic retinoic acid concentration was significantly ($P<0.02$) higher in broilers fed the RA-supplemented ration versus broilers fed the control ration indicating that dietary RA was absorbed by broilers in sufficient quantities to enrich tissue concentrations. Retinoic acid supplementation did not affect the total RA concentration in adipose tissue (Table 4; $P<0.21$). However, RA concentrations were numerically lower in broilers fed the RA-supplemented diet on each day measured. Collectively, these data indicate that broilers fed supplemented diets absorbed significant amounts of retinoic acid.

Expression of retinoic acid receptor in abdominal adipose tissue of broilers

Since retinoic acid primarily acts through regulating gene expression in a process involving activation of nuclear retinoic acid receptors, it is essential that retinoic acid receptors (RAR) and retinoid X receptors (RXR) be present in the target tissue. To verify the expression of RAR and RXRα mRNA in abdominal adipose tissue of 42 day-old broilers, semi-quantitative RT-PCR was performed (Figure 3). Transcripts for both RARα and RXRα mRNA were detected in abdominal fat of broilers supporting the hypothesis that abdominal fat is responsive to RA. However, feeding RA-supplemented rations did not affect the expression of either nuclear receptor gene in abdominal adipose tissue versus the control ration.

Discussion

Retinoic acid potently inhibits preadipocyte differentiation in primary culture models encompassing pigs [12,14], sheep [26] and cattle [13] suggesting that feeding RA may inhibit fat cell differentiation in growing animals. Consistent with an inhibitory action on adipose tissue hyperplasia, it is now firmly established that feeding vitamin A-deficient diets to cattle promotes increased intramuscular fat deposition [15-23,27]. However, the effect of RA supplementation upon adiposity in monogastric production animals has never been directly investigated. This study suggests that feeding RA to growing broilers for six weeks decreases carcass adiposity as evidenced by an ability to elevate liver RA concentration and to reduce total carcass lipid by 14%.

These results are consistent with several studies that have examined the effect of vitamin A upon adiposity in rodents and cattle. For instance, feeding elevated levels of vitamin A has been shown to decrease adiposity in rodents. A 9% decrease of adiposity was reported in F-344xBN rats following supplementation of their diets with 50 fold higher levels of vitamin A [24,28]. A 46% decrease in body fat content was observed in male NMRI mice fed 100 mg RA/kg body weight for 4 days versus control mice [29] and these changes were correlated with the down-regulation of a key transcription factor responsible for stimulating adipogenesis, peroxisome proliferator-activated receptor gamma (PPARγ), in white adipose tissue (WAT). Several studies have also examined the effect of vitamin A upon adiposity by feeding vitamin A deficient diets. Ribot et al. (2001) reported that mice fed a diet containing vitamin A at less than 7% of daily minimum requirements had 63% greater epididymal and inguinal WAT masses versus control animals. In Japan, beef cattle that have been fed vitamin-A deficient diets have greater marbling and low serum retinol concentrations have been correlated with higher marbling in beef [8,27,30]. These results have been firmly established in finishing cattle encompassing a wide range of restriction levels, timing and durations [8,15-23]. Given that RA can inhibit preadipocyte differentiation in both clonal and primary culture cell models and the manipulation of vitamin A levels in growing animals can influence adiposity and intramuscular adipose tissue cellularity, it seems likely that vitamin A and its metabolite, RA, regulate adiposity through direct effects upon adipocyte hyperplasia. Though adipose tissue cellularity as not measured in the current study, our results suggest for the first time that feeding elevated levels of RA to growing broilers can decrease carcass adiposity.

The depression of several indices of growth performance such as feed intake, ADG, and carcass weight is general undesirable and raises

Sample	Control	Retinoic Acid	P-value
Day 0	205 ± 41	193 ± 45	0.84
Day 28	110 ± 20	78 ± 21	0.30
Day 42	231 ± 46	142 ± 50	0.22
Overall Response	182 ± 23	138 ± 26	0.21

Table 4: Concentration of retinoic acid in adipose tissue of broilers fed control or retinoic acid supplemented diets.

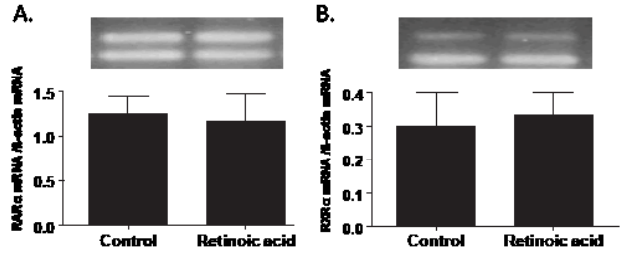

Figure 3: Expression of retinoic acid receptor alpha (RARα) and retinoid X receptor alpha (RXRα) mRNA in abdominal adipose tissue of broilers fed either a control diet or a diet supplemented with 14 mg/kg all-trans retinoic acid. Abdominal adipose tissue was collected following 42 d of treatment and total RNA isolated as described in the Material and Methods. The mRNA expression was measured using semi-quantitative RT-PCR. Values are means +/- SEM, n=5 for control and n=4 for treated group with each replicate for RT-PCR data representing the mean of 3 repeated PCR reactions.Reaction products were resolved on 2% agarose gels and stained with SyBR green dye. Representative multiplex PCR reactions are shown in insets with RARα and RXRα amplicons resolving as the top band and the β- actin amplicon resolving as the bottom band in respective photomicrographs. Data was analyzed using one-way analysis of variance with treatment as the main effect.

the possibility that chronically feeding 14 mg/kg RA was associated with toxicity. However, no gross signs of severe toxicity were observed in the current study. Furthermore, while any feed additive that negatively impacts important growth performance parameters would be avoided by the broiler industry, the observed decreases in feed intake and carcass weight were accompanied by a significant decrease in total carcass lipid and abdominal fat mass concomitant with a modest increase in feed efficiency. It is possible that feeding a dose of RA lower than 14 mg/kg could effectively reduce carcass adiposity while avoiding negative side effects. Thus, further trials investigating the impact of RA in growing broilers are warranted.

Given that the efficiency of RA absorption is poorly characterized in growing broilers, the second objective of this study was to determine how supplementing diets with RA effected the concentrations of retinoic acid in liver and adipose tissue. Retinoic acid primarily acts through regulating gene expression in a process involving activation of nuclear retinoic acid receptors which then bind DNA and regulate gene transcription [31,32]. Thus in order for retinoic acid to exert an effect upon a tissue, it is essential that retinoic acid be present in the target cell. Studies in ruminants have indicated that the efficiency by which vitamin A is absorbed can dramatically decrease as levels of vitamin A increases in a ration [33-35]. This suggests the possibility that the poor absorption of retinoic acid could be a limiting factor in feeding studies. However in the present study, overall retinoic acid concentrations were three-fold higher in livers of birds consuming the RA-supplemented diet versus birds fed the control diet. Since droppings were not collected, it is not possible to directly quantify the absorption efficiency of retinoic acid in the present study. However, the three-fold enrichment of the liver RA concentrations indicates that birds fed the supplemented diets indeed absorbed significant amounts of RA suggesting that absorption was not a limiting factor. Furthermore, the significant effects of dietary RA observed upon growth performance supports the conclusion that birds were able to absorb significant amounts of RA from the supplemented diet. Interestingly, while retinoic acid levels were elevated in the livers of treated birds, adipose tissue concentrations were not enriched by RA supplementation. In fact, RA concentrations were numerically lower in birds fed the RA-supplemented diet on each day measured. This suggests that the potential for RA to inhibit fat accretion in growing broilers may have been limited by a physiological mechanism that regulates retinoic acid levels in adipose tissue.

Clinical studies suggest that barriers may exist which could limit the effect of supplementing retinoic acid in broiler diets by creating a state of "retinoic acid resistance" in adipose tissue. The activity of retinoic acid in a given tissue is determined at a number of levels [36]. The expression of retinoic acid receptor levels is vital to the activity of RA, thus, alterations in the levels of retinoic acid receptors could have significant effects on the ability of RA to regulate processes in adipose tissue. Importantly, mRNA was detected in abdominal adipose tissue for both the RAR and RXR genes while RA supplementation did not affect the expression of either gene suggesting retinoid receptor expression was not a limiting factor in the ability of broiler adipose tissue to respond to RA. Cellular retinol-binding proteins (CRBPs) also play a major role in regulating tissue responsiveness to RA as CRBPs are required for the efficient conversion of retinol to retinoic acid in peripheral tissues. Once inside the cell, CRBP proteins rapidly bind retinol. Noy and Blaner (1991) have demonstrated that cellular uptake is actually dictated by the intracellular content of apo-CRBP as decreases in CRBP expression limits tissue uptake of retinol ultimately limiting tissue retinoic acid levels. Finally, the control of RA levels in a peripheral tissue is further regulated by a balance between RA synthesizing and catabolizing enzymes. Since retinol is irreversibly oxidized to retinoic acid in cells, retinoic acid catabolism governs tissue sensitivity to RA. Several studies indicate that RA catabolism is induced by high levels of RA and this can result in the development of retinoic acid resistance in peripheral tissues and cell lines such as 3T3-L1 preadipocytes [36,38-41]. This effect is primarily believed to occur via a retinoic acid-induced increase in CYP26 (cytochrome P450 enzyme; P450AI) expression and activity [36]. While neither CRPB nor CYP26 were measured in the current study, it is possible that they played a role in limiting RA concentrations in abdominal adipose tissue. Further study would be necessary to address these issues.

Better characterization of the mechanisms that limit RA accumulation in the adipose tissue of growing broilers may reveal targets that could be useful for reducing carcass fat either through selection programs or the administration of exogenous effectors. Interestingly, a CYP26 homolog has been characterized in chicks [42]. It's tempting to speculate that it may be possible to limit carcass adiposity through formulating rations containing CYP enzyme inhibitors thus increasing the impact of dietary vitamin A upon adiposity. Furthermore, CYP enzymes may prove to be useful markers that can be exploited to select for leaner birds through.

In conclusion, this study demonstrates for the first time that feeding RA to growing broilers can enrich hepatic concentrations of RA and reduce carcass adiposity. These results suggest that the further study of RA supplementation in broiler diets is warranted as manipulation of vitamin a status may represent a novel strategy for limiting adiposity in growing broilers.

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