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Effects of ephedrine and its combination with caffeine on body composition and blood attributes of fat-tailed Mehraban lambs

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Summary

The effect of ephedrine at 0 (E_0C_0, n = 10), 8 (E_8C_0, n = 10) and 10 ($E_{10}C_0$, n = 10) mg per kg metabolic body weight or mixture of ephedrine/caffeine, at doses of 8 mg ephedrine/80 mg caffeine (E_8C_{80}, n = 10), or 10 mg ephedrine/100 mg caffeine ($E_{10}C_{100}$, n = 7), per kg metabolic body weight on body composition of feedlot Mehraban ram lambs (8-month-old) was studied. The lambs were fed for 95 days with a fattening ration *ad lib*., and ephedrine and ephedrine/caffeine, dissolved in distilled water, were drenched daily. The control sheep (E_0C_0) were drenched with distilled water only. Ephedrine/caffeine mixture caused a significant decrease in weight and daily gain. Dressing percentage was not affected by treatment, but carcass depreciation (shrinkage) was significantly reduced in $E_{10}C_{100}$ treatment (1.3% vs. 2.0 in control lambs). The mixture significantly increased the crude protein (in dry matter) but decreased dry matter and fat contents of the carcass meat. Internal fat (absolute values and as a percentage of slaughter weight) was significantly higher in the control sheep as compared with other groups. Serum glucose concentration was significantly lower in the control than in other groups. Serum cholesterol levels increased in groups receiving the ephedrine (E_8C_0 and $E_{10}C_0$) compared with the control, but caffeine returned their values to the control levels. Total serum protein level increased slightly but significantly in E_8C_0 and $E_{10}C_{100}$ groups, and serum total lipid and triacylglycerol levels did not change significantly. The results showed that oral administration of ephedrine/caffeine altered the body composition of Mehraban fat-tailed rams, and that feeding of 10 mg ephedrine and 100 mg caffeine per kg metabolic body weight ($E_{10}C_{100}$) was most effective in changing the body composition.

Key words: Ephedrine, Caffeine, Beta-agonists, Mehraban lamb, Carcass composition

Introduction

Beta-adrenergic receptor (β-AR) agonists can alter the carcass characteristics of a number of meat producing animals (Hanrahan, 1987; Wellenreiter, 1991; Avendano-Reyes *et al*., 2006). Large responses have been observed in carcass characteristics with a net result of increased lean and less fat. Zamiri and Izadifard (1995) studied the effect of metaproterenol on carcass characteristics of 18-month-old rams of two Iranian fat-tailed breeds of sheep (Ghezel and Mehraban). Metaproterenol affected body composition in a manner which was dependent on breed, dose and period of treatment. Unlike most published results in thin-tailed sheep (Baker *et al*., 1984; Convey *et al*., 1987; Fennessy *et al*., 1990), carcass chemical protein as well as fat were increased by metaproterenol, but Zare Shahneh *et al*. (2001) reported that subcutaneous injection of metaproterenol (7 and 14 μg kg^{-1} $W^{0.75}$) significantly reduced the fat and increased the protein content of carcasses of 10-month-old Varamini fat-tailed ewes. Zamiri and Karimi (2005) showed that ephedrine increased the protein, and decreased the fat content of the meat in crossbred fat-tailed ewes; a daily oral dose of 8 mg kg^{-1} $W^{0.75}$, administered for 70 days, was effective in changing the carcass chemical composition.

Ephedrine is structurally similar to

epinephrine and norepinephrine and is a non-selective beta-adrenergic agonist that releases norepinephrine at nerve endings. It is resistant to monoamine oxidase and has a half-life of 3 to 6 h (Adams, 1995). One of the effects of beta-agonists is to increase the level of adenosine which is diffused into the interstitial compartments where it binds to A_1 receptors resulting in a decrease in cAMP levels thus reducing the efficiency of beta-agonists. Methylxanthines, such as caffeine, inhibit adenosine A_1 receptors, and thus can be synergistic with ephedrine. A dose-ratio of 1:10 between ephedrine and caffeine acted synergistically on thermogenesis and on body weight loss in humans on dietary restriction (Astrup *et al*., 1990). Oksbjerg and Sorensen (1995), using the same ratio, studied the effects of increasing dietary levels of ephedrine/caffeine mixture protein and lipid deposition in castrated male pigs. The mixture markedly improved the performance and body composition of finishing, castrated pigs. Based on these findings, it was hypothesized that a combination of ephedrine and caffeine would be more effective than ephedrine (Zamiri and Karimi, 2005) for the fat-tailed sheep as well. Therefore, this study was conducted to evaluate the effect of 8 and 10 mg ephedrine per kg metabolic body weight along with 80 and 100 mg caffeine on body composition of a fat-tailed sheep breed.

Materials and Methods

This experiment was carried out at the Animal Research Station, College of Agriculture, University of Shiraz, 15 km north of Shiraz, Iran. Fifty 8-month-old fat-tailed Mehraban ram lambs, with a mean body weight of 41.4 ± 6.1 kg, were allotted to five experimental groups (n = 10 lambs per group). The lambs were fed *ad lib.* with a ration consisting of (dry matter basis) alfalfa hay (65%), and barley (35%), containing 2.3 Mcal metabolizable energy and 123 g crude protein per kg. Salt licks and water were freely available.

The lambs were drenched daily with a solution containing 0 (E_0C_0), 8 (E_8C_0) and 10 ($E_{10}C_0$) mg ephedrine per kg metabolic body weights or a mixture of 8/80 (E_8C_{80}) and 10/100 ($E_{10}C_{100}$) mg ephedrine/caffeine per kg metabolic body weights for 95 days. Doses of drugs were adjusted at 15-day weighing intervals. The control sheep were drenched with an equivalent volume of water. Ephedrine tablets, each containing 25 mg ephedrine hydrochloride, were purchased from Daroupakhsh Pharmaceutical Company (Tehran, Iran) and caffeine was purchased from Boehringer Co. (Germany). Feed and water were removed for 12 h before slaughter. The lambs were weighed on the day of slaughter and a blood sample was taken from each lamb via jugular vein. Blood samples were centrifuged at 5000 rpm for 15 min, and the serum samples were kept at -30°C until analyzed for glucose (Teuscher and Richiterich, 1971), triacylglycerols (Schattler and Nussel, 1975) and cholesterol (Richmond, 1973) by using commercially available kits (Pars-Azmoon Co.). Total lipid concentration was measured according to Zollner and Kirsch (1962) and total protein level was measured by the biuret method (Burtis and Ashwood, 1994).

Upon slaughter, internal organs (heart, kidneys, liver, lungs, stomach and spleen) and internal fats (pericardial, perinephric, pelvic and gastrointestinal) were weighed. Hot and cold (after 24 h at 5°C) carcass weights were also determined. The fat-tail was removed, and the right side of the carcass was divided into conventional cuts including the leg, shoulder, back, breast, neck and abdominal wall (Farid, 1989). Physically separated fat, bone and lean meat of each of the cuts were weighed. Dissected fat (excluding the tail) and lean from the right side of the carcass were thoroughly minced, mixed and a sample was frozen at -30°C for analysis of dry matter (DM), crude protein, ether extract and ash (Official Methods of Analysis, 1975). Subcutaneous fat depth (SCFD) was measured with calipers between the 12th and 13th ribs; measurements were taken at four points and their mean was used as SCFD. The greatest width and depth of the cross section of *Longissimus dorsi* (LD) muscle at 12th rib were measured by calipers. LD cross-sectional area was traced on a paper, and its area was determined by using a planimeter. The means for the width, depth and LD area of the right and left cross-sections were used

in the analysis of variance. Three lambs from $E_{10}C_{100}$ group died due to pulmonary edema.

Data were analyzed by using the GLM procedure of SAS (1996). Slaughter and carcass weight were used as covariates and means were compared using the Duncan's multiple range test. Percentage data were transformed into arcsine √X before analysis, but actual percentages are reported in the paper.

Results

Slaughter weight was significantly lower in treatments containing ephedrine and caffeine mixture (Table 1). Ephedrine, alone or in combination with caffeine, significantly reduced the average daily gain, while dressing percentage was not affected by the treatments. Treatment $E_{10}C_{100}$ resulted in about 30% less shrinkage in the carcass.

Ephedrine/caffeine mixture significantly decreased the live weight percentages of the internal fat, including the perinephric, pericardial, gastrointestinal, and pelvic fats (Table 2). Meat composition was also affected by the mixture (Table 3). Dry matter and fat contents decreased but protein content increased by ephedrine/caffeine mixture, with no significant differences between the two levels used in the present experiment. Ephedrine did not significantly affect the meat composition. Table 4 shows the effect of treatments on blood serum attributes. Serum glucose concentration was

Table 1: Effects of ephedrine and ephedrine/caffeine mixture[1] (per kg metabolic weight per day) on daily gain, dressing percentage and carcass shrinkage in feedlot lambs (mean ± SEM)[2]

Measurement	E_0C_0	E_8C_0	$E_{10}C_0$	E_8C_{80}	$E_{10}C_{100}$
No. of lambs	10	10	10	10	7
Slaughter weight (kg)	57.1 ± 1.9^{a}	58.0 ± 2.2^{a}	59.4 ± 2.2^{a}	53.8 ± 2.6^{b}	53.3 ± 3.1^{b}
Daily gain (g)	206.3 ± 7.2^{a}	170.3 ± 8.7^{b}	177.2 ± 5.6^{b}	160.1 ± 12.8^{b}	173.7 ± 10.3^{b}
Dressing percentage	51.9 ± 0.8	51.9 ± 0.8	52.9 ± 0.5	52.6 ± 0.7	52.1 ± 0.8
Carcass shrinkage (%)	2.0 ± 0.2^{a}	2.3 ± 0.3^{a}	2.1 ± 0.2^{a}	1.8 ± 0.1^{ab}	1.3 ± 0.2^{b}

[1]E_0C_0 (control), E_8C_0 (8 mg ephedrine), $E_{10}C_0$ (10 mg ephedrine), E_8C_{80} (8 mg ephedrine plus 80 mg caffeine) and $E_{10}C_{100}$ (10 mg ephedrine plus 100 mg caffeine). [2]In each row, means with common superscript(s) are not significantly different (Duncan's test; P>0.05)

Table 2: Effects of ephedrine and ephedrine/caffeine mixture[1] (per kg metabolic weight per day) on internal fat (% of slaughter live weight) of feedlot lambs (mean ± SEM)[2]

Measurement	E_0C_0	E_8C_0	$E_{10}C_0$	E_8C_{80}	$E_{10}C_{100}$
Pericardial	0.16 ± 0.01^{a}	0.12 ± 0.01^{abc}	0.13 ± 0.01^{ab}	0.09 ± 0.01^{c}	0.10 ± 0.02^{bc}
Perinephric	0.47 ± 0.01^{a}	0.29 ± 0.05^{ab}	0.33 ± 0.04^{ab}	0.25 ± 0.06^{bc}	0.16 ± 0.04^{c}
Gastrointestinal	1.54 ± 0.19^{a}	1.28 ± 0.18^{a}	1.05 ± 0.19^{a}	0.92 ± 0.17^{b}	0.67 ± 0.12^{b}
Pelvic cavity	0.29 ± 0.06^{a}	0.20 ± 0.02^{ab}	0.16 ± 0.02^{bc}	0.13 ± 0.02^{dc}	0.08 ± 0.01^{d}
Total internal	2.45 ± 0.32^{a}	1.89 ± 0.24^{a}	2.07 ± 0.24^{a}	1.39 ± 0.23^{b}	0.99 ± 0.14^{b}

[1]E_0C_0 (control), E_8C_0 (8 mg ephedrine), $E_{10}C_0$ (10 mg ephedrine), E_8C_{80} (8 mg ephedrine plus 80 mg caffeine) and $E_{10}C_{100}$ (10 mg ephedrine plus 100 mg caffeine). [2]In each row, means with common superscript(s) are not significantly different (Duncan's test; P>0.05)

Table 3: Effects of ephedrine and ephedrine/caffeine mixture[1] (per kg metabolic weight per day) on chemical composition of carcass meat (muscle plus fat) of feedlot lambs (mean ± SEM)[2]

Measurement	E_0C_0	E_8C_0	$E_{10}C_0$	E_8C_{80}	$E_{10}C_{100}$
Protein (% in fresh meat)	16.5 ± 0.3	16.6 ± 0.3	17.1 ± 0.2	16.5 ± 0.3	16.9 ± 0.3
Protein (% in meat dry matter)	39.0 ± 1.8^{c}	40.9 ± 1.1^{c}	43.1 ± 1.6^{abc}	45.0 ± 1.4^{ab}	46.6 ± 2.4^{a}
Fat (% in fresh meat)	24.2 ± 1.5^{a}	22.4 ± 1.1^{a}	20.9 ± 1.4^{ab}	18.4 ± 1.1^{bc}	17.5 ± 1.5^{c}
Fat (% in meat dry matter)	56.0 ± 1.9^{a}	54.5 ± 1.2^{a}	52.0 ± 1.8^{ab}	49.5 ± 1.3^{bc}	47.1 ± 2.2^{c}
Meat dry matter (%)	42.7 ± 1.2^{a}	40.8 ± 1.2^{a}	39.9 ± 1.3^{ab}	36.0 ± 1.2^{b}	36.7 ± 1.6^{b}

[1]E_0C_0 (control), E_8C_0 (8 mg ephedrine), $E_{10}C_0$ (10 mg ephedrine), E_8C_{80} (8 mg ephedrine plus 80 mg caffeine) and $E_{10}C_{100}$ (10 mg ephedrine plus 100 mg caffeine). [2]In each row, means with common superscript(s) are not significantly different (Duncan's test; P>0.05)

Table 4: Effects of ephedrine and ephedrine/caffeine mixture[1] (per kg metabolic weight per day) on blood serum attributes of feedlot lambs (mean ± SEM)[2]

Measurement	E_0C_0	E_8C_0	$E_{10}C_0$	E_8C_{80}	$E_{10}C_{100}$
Glucose (mg/dl)	41.6 ± 2.3^{b}	60.1 ± 11.4^{a}	54.1 ± 3.9^{a}	49.9 ± 4.3^{ab}	62.0 ± 3.8^{a}
Cholesterol (mg/dl)	59.7 ± 9.7^{b}	99.3 ± 8.8^{a}	100.8 ± 14.3^{a}	70.7 ± 17.1^{ab}	50.0 ± 6.3^{b}
Triacylglycerols (mg/dl)	81.8 ± 25.7	63.0 ± 6.9	74.2 ± 15.8	51.2 ± 7.91	47.5 ± 6.7
Total proteins (g/dl)	3.9 ± 0.1^{c}	4.4 ± 0.2^{a}	4.1 ± 0.1^{abc}	4.0 ± 0.1^{c}	4.3 ± 0.1^{ab}
Total lipids (g/dl)	1.1 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1

[1] E_0C_0 (control), E_8C_0 (8 mg ephedrine), $E_{10}C_0$ (10 mg ephedrine), E_8C_{80} (8 mg ephedrine plus 80 mg caffeine) and $E_{10}C_{100}$ (10 mg ephedrine plus 100 mg caffeine). [2] In each row, means with common superscript(s) are not significantly different (Duncan's test; $P>0.05$)

lower in the control sheep as compared with the groups receiving either ephedrine or ephedrine and caffeine. Serum cholesterol concentration was significantly increased by ephedrine but was not affected when ephedrine was administered with caffeine. Total lipid and triacylglycerol concentrations were not significantly affected but serum protein level was higher at higher doses of ephedrine and caffeine (E_{10}/C_{100}). Total lean, trimmed fat, and bone (absolute and as a percentages of carcass weight) in major cuts (leg, shoulder and back), SCFD, the weight and proportion in the live weight of the tail, lungs, heart, kidneys, spleen and liver, and the depth, width and cross-sectional area of the LD muscle, were not significantly affected in this experiment.

Discussion

Studies on the effect of β-AR agonists on Iranian fat-tailed sheep are very few (Zamiri and Izadifard, 1995; Zare Shahneh *et al*., 2001; Zamiri and Karimi, 2005). The effects of β-AR agonists on carcass weight and dressing percentage are not consistent (Hamby *et al*., 1986; Kim *et al*., 1987; Forsberg *et al*., 1989; Fennessy *et al*., 1990; Shackelford *et al*., 1992; Zamiri and Ehsani, 1995; Zamiri and Izadifard, 1995; Zamiri and Karimi, 2005; Avendano-Reyes *et al*., 2006).

Although metaproterenol was effective in reducing the fat-tail weight and total dissected fat from the carcass of 18-month-old Ghezel and Mehraban rams (Zamiri and Izadifard, 1995), ephedrine (Zamiri and Karimi, 2005; and present data) or ephedrine/caffeine mixture did not have a significant effect on these measurements. In line with the data of Zamiri and Karimi (2005), ephedrine by itself did not have a significant effect on internal fat in the present experiment, but the percentage of the internal fat in live weights of lambs receiving ephedrine/caffeine mixtures was 40 to 60% less than the values for control animals. The effect of β-AR agonists on several fat depots in thin-tailed sheep have also been inconsistent (Baker *et al*., 1984; Kim *et al*., 1987; Fennessy *et al*., 1990; Shackelford *et al*., 1992), but it seems that caffeine is effective in reducing the internal fat depots.

Effects of β-AR agonists on the weights of internal organs have been studied in sheep (Fennessy *et al*., 1990; Zamiri and Izadifard, 1995; Zare Shahneh *et al*., 2001; Zamiri and Karimi, 2005) and other species (Jones *et al*., 1985; Kim *et al*., 1987; Forsberg *et al*., 1989; Moloney *et al*., 1990; Warriss *et al*., 1990; Zamiri and Ehsani, 1995). Neither in the study with crossbred ewes (Zamiri and Karimi, 2005) nor in this experiment, the SCFD was not significantly affected by ephedrine. Likewise, mixtures of caffeine and ephedrine did not significantly affect SCFD. Subcutaneous injection of metaproterenol decreased SCFD in Varamini ewes (Zare Shahneh *et al*., 2001) and Mehraban rams, but not in Ghezel rams (Zamiri and Izadifard, 1995). Studies with several BAA in thin-tailed sheep found decreases in SCFD (Baker *et al*., 1984; Kim *et al*., 1987; Fennessy *et al*., 1990). In contrast to the present data, cross-sectional area of the *Longissimus dorsi* muscle (LD area) was significantly increased by all doses of ephedrine (1 to 8 mg kg^{-1} $W^{0.75}$) in crossbred ewes (Zamiri and Karimi, 2005), and by metaproterenol in Varamini ewes (Zare Shahneh *et al*., 2001). However,

Zamiri and Izadifard (1995) did not find any significant effect of metaproterenol on this measurement in fat-tailed rams of Mehraban and Ghezel breeds. β-AR agonists also increased the LD area in thin-tailed sheep (Baker *et al*., 1984; Shackelford *et al*., 1992).

Although feeding ephedrine at 8 mg kg^{-1} $W^{0.75}$ for 70 days increased crude protein and decreased fat percentages of the carcass meat in crossbred ewes (Zamiri and Karimi, 2005), doses of 8 and 10 mg kg^{-1} $W^{0.75}$ for 95 days did not significantly affect these attributes in the present experiment. However, in sheep receiving ephedrine/ caffeine mixtures, the protein content (in dry matter) was increased between 15 to 20%, and the fat content was decreased between 12 to 16% over the control sheep. The mixtures also decreased the meat dry matter content by about 15% over the control group. Beta-agonists have generally decreased the fat content of the meat but their effects on protein content have been dependent on the species, type of beta-agonists and the duration of treatment (Baker *et al*., 1984; Thornton *et al*., 1985; Fennessy *et al*., 1990; Zamiri and Izadifard, 1995; Zare Shahneh *et al*., 2001).

Oksbjerg and Sorensen (1995) reported that a mixture of ephedrine and caffeine resulted in increased muscle protein deposition and decreased lipid accretion rates in castrated finishing pig. The increased protein deposition was attributed to ephedrine and both compounds contributed almost equally to the decrease in lipid deposition. Decreased lipid accretion was interrelated with reduction in food intake, and also to a lower efficiency of energy deposition caused by caffeine. In the present experiment, meat protein and fat contents and internal fat of the sheep receiving ephedrine were non-significantly different from the control sheep, but the values were significantly different from the control animals when caffeine was also administered. This indicates that caffeine could contribute to both reduction in fat and increase in protein deposition. It is interesting to see whether higher levels of ephedrine alone will exert a profound effect on fat and protein deposition in fat-tailed rams. It is noted that β-AR agonists increase protein deposition in skeletal muscles, but not in other tissues (Williams, 1987). Feed intake was not measured in our study, but ephedrine/caffeine mixture decreased feed intake and improved the feed:gain ratio in castrated male pigs (Oksbjerg and Sorensen, 1995). Decreased daily gain and weight of rams in the present experiment could be due to decreased feed intake which decreases fat deposition. Decreased weights of organs, not measured in the present work, may have contributed to overall weight loss. Higher blood glucose levels (10 to 20 mg/dl) in treated rams as compared with the control could have an anorexic effect on rams.

Although β-AR agonists have a considerable effect on carcass composition, there are species differences in the magnitude of their effects. There are several reasons for such differences: some species have been intensively selected for growth rate; a particular β-AR agonist may not be as effective in one species as in another; β-AR in target tissues may be rapidly inactivated, or a particular species may have a limited number of these receptors on its tissues, thus, reducing the response to β-AR agonists (Wellenreiter, 1991; Mersmann, 1998). Several β-AR have been identified in animal species, including α_1, α_2, β_1, β_2 and β_3. The β-receptor subtypes show differential desensitization; β_3 is generally less responsive than the other two subtypes (Marullo *et al*., 1995). The β-receptor subtype populations may change with the stage of differentiation of a cell or with the hormonal milieu provided to the cell. The proportion of these subtypes present on skeletal and adipose tissues in a particular species may suggest the response of the cell to a particular agonist (Mersmann, 1998).

The most frequently observed effect of the orally administered β-AR agonists is an increase in the muscle mass; this could be due to increased protein synthesis, decreased protein degradation or a combination of both (Mersmann, 1998). The fat reducing effect of β-AR agonists could be due to stimulation of adipocyte triacylglycerol degradation and inhibition of fatty acid and triacylglycerol synthesis, although the response of adipose tissue has not been as persistent as that of the skeletal muscles. Other mechanisms of

action of β-AR agonists include increased blood flow to certain regions of the body. Increased blood flow to muscles may provide more nutrients for protein synthesis. On the other hand, increased blood flow to adipose tissues might be envisioned to carry non-esterified fatty acids away from the tissue to enhance lipolysis. Modulation of circulating concentrations of hormones could be another mechanism.

Systemic effects of β-AR agonists have been found in some species. Acute and chronic increases in plasma non-esterified fatty acids were reported in steers (Eismann *et al*., 1988) and pigs (Mersmann, 1998) with clenbuterol and in lambs with cimaterol (Kim *et al*., 1987). There were no effect of treatments on serum triacylglycerol and total lipids in the present experiment. Serum glucose concentration was increased by ephedrine, but caffeine did not seem to modify this effect. Blood glucose decreased in pigs treated with salbutamol (Warriss *et al*., 1990), and chronically increased in steers with clenbuterol (Eismann *et al*., 1988). However, Ricks *et al*. (1984) did not find any changes in glucose concentration of steers fed clenbuterol. Ephedrine alone caused a substantial increase (about 70%) in serum cholesterol concentration, but caffeine prevented this hypercholesterolemic effect of ephedrine. Salbutamol also increased serum levels of lipase, triacylglycerols, cholesterol and glucose in guinea pigs (Zamiri and Ehsani, 1995). Any of these mechanisms, or more likely, some combinations of them could be operative in a given species administered a given β-AR agonists, at a particular age, with a specific genetic background, under a designated husbandry condition, and fed a particular diet.

Treatment with β-AR agonists results in tougher meat production, due to increases in the amount of connective tissue, decreased activity of proteolytic enzymes and formation of bonds between collagen molecules (Chesworth *et al*., 1998). We did not measure the toughness of meat in the present experiment, but Oksbjerg and Sorensen (1996) reported that ephedrine caused a change in the proportion of fiber types and increased the toughness of meat in the pig. Concern has been raised as to the tissue contamination by some compounds (Smith, 1998). Ephedrine and caffeine suppress appetite and stimulate energy expenditure in humans. Ephedrine has no effect on weight loss; yet with the addition of caffeine, lean body mass is increased and body fat is reduced. A moderate and careful intake of ephedrine and caffeine combined with a healthy diet and sufficient exercise has been shown to be beneficial for losing weight in humans (Dickson, 2006). Data indicate that combination of caffeine and ephedrine is effective in increasing protein and decreasing the fat deposition and increasing protein accretion of feedlot fat-tailed rams.

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