Asian-Aust. J. Anim. Sci.
Vol. 22, No. 8 : 1180 - 1185
August 2009

www.ajas.info

Effect of Dietary Energy Level on Nutrient Utilization, Insulin-like Growth Factor-I and Insulin-like Growth Factor Binding Protein-3 in Plasma, Liver and *Longissimus dorsi* Muscle in Growing-finishing Pigs Using Soybean Oil as an Energy Source*

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ABSTRACT : Two experiments were carried out to study the effects of dietary energy level on nutrient digestion, nitrogen (N) utilization, growth performance, insulin-like growth factor-I (IGF-I), and insulin-like growth factor binding protein-3 (IGFBP-3) in plasma, liver and *longissimus dorsi* muscle in growing-finishing pigs. In experiment 1 (Exp 1), 15 castrated male pigs (Duroc×Landrace ×Large White) (Body weight, BW, 55.6±1.8 kg) were divided into three groups and fed rations containing 13.33, 14.87 and 17.35 MJ digestible energy (DE)/kg as treatments I, II and III, respectively, using soybean oil as an energy source. The experiment lasted 8 days and faecal and urinary samples were collected during the last 3 days. The results showed that the digestibility of dry matter (DM), energy and N was increased from treatments I to III ($p<0.01$). N-retention and N-retention rate were not influenced by dietary DE level ($p>0.05$). In experiment 2 (Exp 2), 36 female pigs (Duroc×Landrace×Large White) (BW 41.5±3.8 kg) were divided into three groups. The pigs were fed with the same three rations used in Exp 1 for 60 days. At the end of Exp 2, eight pigs were selected from each group for blood sampling and 4 pigs for slaughter trial. The results indicated that average daily feed intake (ADFI) and N-intake were significantly decreased ($p<0.01$), and DE intake ($p<0.01$) and average daily gain (ADG) ($p<0.05$) were increased. IGF-I and IGFBP-3 in plasma were increased ($p<0.05$). No significant differences in IGF-I and IGFBP-3 in liver and *longissimus dorsi* muscle were found between different treatments. It was concluded that higher dietary DE level improved nutrient digestibility, ADG and feed/gain ratio when soybean oil was used as an energy source in the ration of growing-finishing pigs. No significant differences were found in N-retention and IGF-I and IGFBP-3 in liver and *longissimus dorsi* muscle between different treatments. (**Key Words :** Dietary Energy, IGF-I, IGFBP-3, Pigs, Soybean Oil)

INTRODUCTION

Dietary energy is a key nutritional factor which influences growth and fattening of animals. Many studies have examined the effect of dietary energy level on protein and fat deposition of weaned piglets and young pigs. Van Lunen and Cole (1998) reported that pigs from 9.1 to 25.4 kg BW fed with high DE rations had higher ADG, N-retention rate and lipid deposition rate than pigs fed on low DE rations. In addition, many researchers have investigated the effect of dietary energy density on growth performance and carcass characteristics (Smith et al., 1999; Liu et al., 2007), and physical and/or chemical composition of the total body at slaughter (Quiniou and Noblet, 1997) in pigs. It is well established that increased energy supply can improve growth performance.

The GH/IGF axis is important in postnatal growth in most mammals. IGF-I is predominantly regulated by growth hormone (GH) while IGFBP-3 plays an important role in regulating IGF-I actions (Lee et al., 2000). The IGF-I and IGFBPs in plasma are sensitive to changes of nutritional status in humans and animals (Thissen et al., 1994). Some studies have indicated that fasting and restriction of protein and energy supply decreased

* Part of National Basic Research Program of China (2004CB117502).

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Received January 8, 2009; Accepted March 10, 2009

circulating IGF-I in pigs (Hathaway et al., 1999; Guay and Trottier, 2006) and cats (Maxwell et al., 1999). It was also reported that fasting decreases serum IGFBP-3 in pigs (White et al., 1991). Bee et al. (2002) reported that dietary energy level influenced growth performance, GH, insulin and IGF-I in pigs.

Soybean oil is commonly used as an energy source in animal rations since it has a high energy density. When soybean oil is used as an energy source in the ration of growing-finishing pigs, the effect of increased soybean oil on growth performance and hormone metabolism has not been studied in detail. The objective of the present experiments was to study the effects of dietary energy level on nutrient digestion, growth performance, and IGF-I, IGFBP-3, GH in plasma and IGF-I, IGFBP-3 in tissue of growing-finishing pigs using soybean oil as an energy source in the ration.

MATERIALS AND METHODS

Animals, rations and feeding

Exp 1. Nutrient digestion and N utilization : Fifteen castrated male pigs (Duroc×Landrace×Large White) with average BW of 55.6±1.8 kg were used. The pigs were randomly divided into three groups, each comprising 5 pigs. Three rations with an energy content of 13.33, 14.87 and 17.35 MJ DE/kg ration were formulated and fed to the pigs as experimental treatments I, II and III (Table 1), respectively. Except for dietary DE, other nutrients were adequate for growing-finishing pigs (NRC, 1998) and were similar among the experimental diets. The experiment lasted 8 days. The pigs were housed in individual pens with plastic floors. The ambient temperature was kept at 20-25°C during the experiment. The pigs were fed with 2,000 g of ration daily, in two equal meals at 08:00 and 16:00 h, and had free access to drinking water via low-pressure drinking nipples (Ruan et al., 2007; Yang et al., 2007; Yin et al., 2008). During the last three days of the experiment, total faeces and urine were collected and sampled for later determination and analysis.

Exp 2. Feeding and slaughter trial : Thirty-six female growing pigs (Duroc×Landrace×Large White) with BW of 41.5±3.8 kg were used. The pigs were randomly divided into three groups, each comprising 12 pigs. All the pigs were fed with the ration containing 14.87 MJ DE/kg for 10 days as an adaptation period. Then the three groups of pigs were fed with the three rations used in Exp 1 for 60 days as an experimental period. The pigs were fed *ad libitum*. The feeding and management of the pigs was similar to Exp 1.

The pigs were weighed and recorded at the beginning and the end of the feeding trial after a 12 h fasting period for the calculation of ADG. Feed intake was recorded daily during the 60 d period for the calculation of ADFI and feed/gain ratio. At the end of the feeding trial, 10 ml blood samples were taken from the jugular vein of 8 pigs of each group into vacuettes containing heparin (Greiner Bio-one Vacuette GmbH, Austria). The blood samples were centrifuged at 4,000×g for 15 min at room temperature. The plasma was collected and immediately frozen at -20°C for later analysis (Ruan et al., 2007).

Four pigs from each group were selected and fasted for 24 h before slaughter. The carcass was weighed and divided into two equal sides. The left side of the carcass was dissected into fat, muscle and bone and respective weights recorded. Samples of *longissimus dorsi* muscle from the right side of the carcass and liver were obtained, wrapped in aluminum foil, frozen in liquid nitrogen and immediately kept at -70°C for later analysis.

Table 1. Composition and nutrient level of the experimental rations

	Treatments		
	I (13.33 MJ/kg)	II (14.87 MJ/kg)	III (17.35 MJ/kg)
Ingredients (%, as fed basis)			
Corn	57.70	64.98	59.15
Soybean meal	22.85	24.35	27.35
Wheat bran	10.20	4.50	0.00
Soybean oil	0.00	3.17	10.50
Rice bran	6.25	0.00	0.00
Limestone	0.70	0.70	0.70
$CaHPO_4$	1.00	1.00	1.00
Sodium chloride	0.30	0.30	0.30
1% Premix[1]	1.00	1.00	1.00
Nutrient level (as fed basis)[2]			
DM (%)	87.20	87.20	88.10
DE (MJ/kg)	13.33	14.87	17.35
Crude protein (%)	16.20	16.20	16.30
Crude fiber (%)	4.10	2.68	2.34
Crude fat (%)	3.11	7.21	13.04
Calcium (%)	0.64	0.64	0.64
Total phosphorus (%)	0.58	0.56	0.51
Lysine (%)	0.80	0.78	0.81
Methionine and cysteine (%)	0.58	0.58	0.58
Tryptophan (%)	0.22	0.22	0.23
Threonine (%)	0.68	0.67	0.69

[1] Providing per kg diet: vitamin A, 3,086 IU; vitamin D_3, 386 IU; vitamin E, 15.4 IU; vitamin K, 2.3 mg; vitamin B_{12}, 15.4 μg; riboflavin, 3.9 mg; pantothenic acid, 15.4 mg; niacin, 23 mg; choline chloride, 500 mg; Cu, 10 mg; Fe, 100 mg; Zn, 100 mg; Mn, 10 mg; I, 1 mg; Se, 0.30 mg.

[2] DM, crude protein, crude fiber, crude fat, calcium and total phosphorus were determined values. DE was calculated value based on the digestible energy of feed obtained from the digestion trial. Amino acids were obtained from Zhang (2000).

Determinations and analysis

The gross energy of feeds, faeces and urine were determined using an oxygen bomb calorimeter (WZR-1,

Table 2. Effect of dietary energy on nutrient digestion and N metabolism in growing-finishing pigs (Exp 1)

Items	Treatments			p-value
	I (13.33 MJ/kg)	II (14.87 MJ/kg)	III (17.35 MJ/kg)	
Body weight (kg)	55.2±1.2	56.8±0.7	57.8±1.2	0.277
DM digestibility (%)	59.82±1.83[c]	65.21±1.62[b]	70.55±1.87[a]	<0.001
Energy digestibility (%)	78.95±2.47[c]	85.53±2.09[b]	91.20±2.37[a]	<0.001
N-intake (g/d)	51.84±0.00	51.84±0.00	52.16±0.00	-
Faecal N excretion (g/d)	9.11±1.00[a]	7.72±0.78[a]	4.74±0.67[b]	0.008
Urinary N excretion (g/d)	10.40±0.44	8.92±1.17	10.17±1.95	0.709
N-digestibility (%)	82.44±4.30[b]	85.11±3.37[b]	90.91±2.86[a]	0.008
N-retention (g/d)	32.34±1.11	35.20±1.02	37.93±1.03	0.149
N-retention rate (%)	62.38±2.15	67.90±1.97	71.42±4.65	0.169

[a, b, c] Means in the same row with different superscripts differ significantly ($p<0.05$), n = 5.

Changsha Instrument Company, China). The total N of feeds, faeces and urine were determined by the Kjeldahl method.

The IGF-I in plasma was extracted with acid-ethanol cryoprecipitation and analyzed using specific radioimmunoassay kits consisting of anti-hIGF-I, ^{125}I-IGF-I and hIGF-I (Diagnostic System Laboratories, USA). The IGFBP-3 in plasma was analyzed using specific radioimmunoassay kits consisting of anti-anti-hIGFBP-3, ^{125}I-IGFBP-3 and hIGFBP-3 (Diagnostic System Laboratories, USA). The samples of liver and *longissimus dorsi* muscle were boiled in saline (1 ml) for 3 min, then homogenized with 1 N glacial acetic acid (0.5 ml) and neutralized with 1 N sodium hydroxide solution (0.5 ml) as pretreatment. Then the IGF-I and IGFBP-3 of the tissue samples were analyzed. GH in plasma was analyzed using specific radioimmunoassay kits (Sino-UK Institute of Biological Technology, Beijing, China).

The total protein in homogenized tissue (liver and *longissimus dorsi* muscle) was determined using colorimetric methods with a Technicon RA2100 automatic biochemistry analyzer using reagent kits (Jiancheng Bioengineering Institute, Nanjing, China).

Calculation and statistical analysis

Nutrient digestibility (%)
= (Nutrient intake (g/d)-Faecal nutrient (g/d))
/Nutrient intake (g/d)×100

N-retention in Exp 1 (g/d)
= N-intake (g/d)-Faecal N (g/d)- Urinary N (g/d)

N-retention rate in Exp 1 (%)
= N-retention (g/d)/N-intake (g/d)×100

N-retention in Exp 2 (g/d)
= N-intake in Exp 2 (g/d)
×N-retention rate in Exp 1 (g/d)

The dressing percentage and lean, fat and bone percentages were calculated as following.

Dressing percentage (%)
= Carcass weight (kg)/Liveweight (kg)×100

Carcass fat percentage (%)
= Fat (kg)
/(Fat (kg)+Lean (kg)+Bone (kg)+Skin (kg))×100

Carcass lean percentage (%)
= Lean (kg)
/(Fat (kg)+Lean (kg)+Bone (kg)+Skin (kg))×100

Carcass bone percentage (%)
= Bone (kg)/(Fat (kg)+Lean (kg)+Bone(kg)
+Skin (kg))×100

The experimental data were expressed as Mean±SE. ANOVA in SPSS 10.0 (Chicago, IL, USA) was used for the comparison of differences in parameters between treatments.

RESULTS

Exp 1 : Nutrient digestion and nitrogen utilization

The results are shown in Table 2. There was no difference in N-intake between different treatments. Faecal N excretion was decreased from treatments I to III ($p<0.01$). The digestibility of DM, energy and N was significantly increased from treatments I to III ($p<0.01$). No differences in urinary N-excretion, N-retention and N-retention rate were found between different treatments ($p>0.05$).

Exp 2 : Feeding and slaughter trial

Growth performance and carcass characteristics : The results are shown in Table 3. The results indicated that ADFI ($p<0.01$), N-intake ($p<0.01$) and feed/gain ratio ($p<0.01$) were decreased and daily DE intake ($p<0.01$) and

Table 3. Effect of dietary energy on growth performance and carcass yield in growing-finishing pigs (Exp 2)

Items	Treatments			p-value
	I (13.33 MJ/kg)	II (14.87 MJ/kg)	III (17.35 MJ/kg)	
Initial body weight (kg)	41.0±1.4	41.7±1.0	41.9±1.0	0.873
Final body weight (kg)	88.4±2.6	90.6±1.4	94.5±1.5	0.119
Feed intake (as fed basis, g/d)	2,661±91[a]	2,400±47[b]	2,307±58[b]	0.004
DE intake (MJ/d)	35.48±1.22[b]	35.68±0.70[b]	40.04±1.00[a]	0.008
N-intake (g/d)	69.0±2.4[a]	62.2±1.2[b]	60.2±1.5[b]	0.005
Average daily gain (ADG, g/d)	789±26[b]	815±22[ab]	878±22[a]	0.049
Feed/gain ratio	3.38±0.07[a]	2.96±0.06[b]	2.64±0.06[c]	<0.001
N-retention (g/d)*	43.0±1.5	42.2±0.8	43.0±0.7	0.872
Dressing percentage (%)	73.68±1.89	75.09±0.90	74.42±0.65	0.729
Lean (%)	60.66±0.89	60.47±1.16	59.32±0.35	0.451
Fat (%)	20.45±0.54	21.14±1.60	23.07±0.87	0.230
Bone (%)	11.47±0.90	11.33±0.74	10.52±0.50	0.588

[a, b, c] Means in the same row with different superscripts differ significantly ($p<0.05$), n = 12.

* N-retention in Exp 2 = N-retention rate in Exp 1×N-intake in Exp 2.

ADG ($p<0.05$) were increased with the dietary energy level. No significant differences were found in N-retention of the whole animal body, dressing percentage, and lean, fat and bone percentages between different treatments ($p>0.05$).

IGF-I, IGFBP-3 and GH in plasma : The results are shown in Table 4. The results indicated that IGF-I, IGFBP-3 in plasma were significantly increased with dietary energy level ($p<0.05$). However, there was no difference in GH in plasma between different treatments ($p>0.05$).

IGF-I and IGFBP-3 in liver and longissimus dorsi muscle : The results are shown in Table 5. The results indicated that IGF-I in liver tended to increase with dietary energy level, but the difference between different treatments did not reach a significant level ($p = 0.082$). No differences were found in IGFBP-3 in liver and IGF-I and IGFBP-3 in *longissimus dorsi* muscle between different treatments ($p>0.05$).

DISCUSSION

Effect of dietary energy level on nutrient digestion and nitrogen utilization (Exp 1)

Wheat bran, rice bran and soybean oil were used in the rations to adjust the dietary energy level in the present experiment. With the increased percentage of soybean oil, the percentages of wheat bran and rice bran were decreased from treatments I to III, and crude fiber content in rations was decreased from 4.10% to 2.68% and 2.34% from treatments I to III. This resulted in the increased digestibility of DM, energy and N and the decreased faecal

Table 4. Effect of dietary energy on plasma hormones in growing-finishing pigs (Exp 2)

Items	Treatments			p-value
	I (13.33 MJ/kg)	II (14.87 MJ/kg)	III (17.35 MJ/kg)	
IGF-I (ng/ml)	175.79±6.67[b]	183.98±18.46[b]	206.66±4.49[a]	0.011
IGFBP-3 (ng/ml)	40.83±3.66[b]	41.96±3.46[b]	53.60±3.18[a]	0.036
GH (ng/ml)	5.09±0.20	5.41±0.20	5.56±0.31	0.379

[a, b, c] Means in the same row with different superscripts differ significantly ($p<0.05$), n = 8.

Table 5. Effect of dietary energy on IGF-I and IGFBP-3 in liver and *longissimus dorsi* muscle in growing-finishing pigs (Exp 2)

Items	Treatments			p-value
	I (13.33 MJ/kg)	II (14.87 MJ/kg)	III (17.35 MJ/kg)	
Liver				
IGF-I (μg/g)*	0.99±0.12	1.73±0.14	1.80±0.38	0.082
IGFBP-3 (μg/g)	0.82±0.10	0.74±0.17	1.09±0.12	0.217
Longissimus dorsi muscle				
IGF-I (μg/g)	1.23±0.26	1.23±0.55	0.86±0.19	0.717
IGFBP-3 (μg/g)	0.51±0.08	0.51±0.20	0.25±0.09	0.319

* μg/g refers to μg per gram of tissue total protein, n = 4.

N from treatments I to III. The N-retention and N-retention rate tended to increase, however, the differences between different treatments did not reach statistical significance.

Effect of dietary energy level on growth performance of pigs (Exp 2)

Different ingredients and energy level of rations might influence palatability and feed intake. Indeed, it was found that ADFI significantly decreased with dietary energy level. However, the daily DE intake was significantly increased from treatments I to III. The reason for this could be that the pigs were fed *ad libitum* and might not have been able to effectively adjust DE intake to obtain a suitable amount of dietary energy when the dietary energy level was increased. It was found that the feed/gain ratio was significantly decreased with dietary DE level, therefore resulting in significant increase of ADG. The results were in agreement with James et al. (2002), who found that the growth performance of pigs was significantly improved by supplementation of fat in rations. Zhang et al. (2008) reported that lowering dietary energy content evidently reduced lean percentage and compensated to increase back fat deposition in later finishing pigs, whereas no significant differences in N-retention, dressing percentage and percentages of fat, lean and bone were found between different treatments in the present experiment, indicating that dietary DE level did not influence these indices when soybean oil was used as an energy source although ADG was increased with dietary DE level.

Effect of dietary energy level on IGF-I, IGFBP-3 and GH in plasma (Exp 2)

Nutritional status plays an important role in the regulation of circulating levels of IGF-I, IGFBP-3 and GH. It was found in Exp 2 that IGF-I and IGFBP-3 in plasma were significantly increased with dietary energy level from treatments I to III. The results were in agreement with both Vestergaard et al. (2003), who reported that high dietary DE and protein levels increased free and total IGF-I and IGFBP-3 of prepubertal heifers, and Thissen et al. (1994) who found that energy and/or protein deprivation in humans decreased IGF-I and IGFBP-3 in serum. The increase of ADG from treatments I to III in Exp 2 could be explained by the increase of IGF-I and IGFBP-3 in plasma.

In both humans and pigs, GH is required for maintenance of the circulating level of IGF-I (Simmen et al., 1998). Many studies have indicated that growth retardation induced by undernutrition was usually followed by an increase of GH in plasma in pigs, cattle and humans (Vance et al., 1992). In the present experiment, GH in plasma was not affected by dietary energy level. The reason for this could be that in the present experiment the nutrient intake of all the treatments met the nutrient requirements of the pigs. Thomas et al. (1996) reported that both soybean oil and animal tallow diets increased serum concentrations of GH in beef heifers relative to the control diet. The difference between the results of the present experiment and that of Thomas et al. (1996) could have resulted from different animal species and nutrition level.

Effect of dietary energy level on IGF-I and IGFBP-3 in liver and *longissimus dorsi* muscle (Exp 2)

IGF-I and IGFBP-3 have been identified to be expressed in hepatocytes and endothelial cells in pigs, respectively (Kim et al., 2008). Both energy and certain individual amino acids appear to control GH-stimulated IGF-I expression in cultured pig hepatocytes (Brameld et al., 1999). Some studies indicated that energy restriction reduced expression of IGF-I mRNA in liver and other tissues of heifers (Vandehaar et al., 1995). Weller et al. (1994) showed in pigs that the manipulation of dietary energy supply results in a positive correlation between growth rate and liver IGF-I, but not between growth and IGF-I in muscle. Pell et al. (1993) also reported in sheep that decreased growth correlated with decreased IGF-I mRNA expression in the liver, but not in skeletal muscle. It was found in the present experiment that the IGF-I in liver tended to increase with dietary energy level ($p = 0.082$), although it did not reach a significant level, while IGFBP-3 in liver and IGF-I and IGFBP-3 in *longissimus dorsi* muscle were not influenced by dietary energy level, which was in agreement with the results mentioned above. Soybean oil was used in the present experiment to increase dietary energy concentration. Different energy sources may have different effects on IGF-I gene expression. Further research is needed to compare the effects of different energy sources on IGF-I expression in liver and different tissues.

CONCLUSIONS

Supplementation of 10.5% soybean oil in the ration (as fed basis) of growing-finishing pigs improved the digestibility of DM, energy and N, and IGF-I and IGFBP-3 in plasma but had no significant effects on N-retention and IGF-I, IGFBP-3 in tissues. It was concluded that growth performance and hormone concentrations in plasma were affected by the increased dietary energy level, when soybean oil was used as an energy source in the ration of growing-finishing pigs.

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