



# Feeding a High-Concentrate Diet Reduces Milk Production is Associated with Endogenous Growth Hormone in Lactating Goats

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## ABSTRACT

The aim of this work was to evaluate the effects of endogenous GH-IGF-1 axis on lactating goats fed with high-concentrate diets. Ten lactating goats were used and randomly divided into two groups, in a 2×2 Latin square experiment design with different forage to concentrate rations of 40:60 (the control group) and 60:40 (the high-concentrate group), respectively. During the experiment, milk samples were collected to assay the content of milk compositions; plasma samples were collected to measure the content of IGF-1 and GH. The liver and mammary gland samples were collected to observe the expression of IGF-1R and GH. The results showed that compared with the control group, the content of plasma GH and IGF-1 were decreased in the high-concentrate group, and the percentage of lactose and milk yield were also decreased. Meantime, the mRNA expression of IGF-1R in mammary gland was down-regulated in the high-concentrate group. Our results indicated that feeding with high-concentrate diets for 9 weeks would decrease the contents of endogenous GH and IGF-1, and eventually lead to the decreased milk yield.

## Article Information

Received 16 February 2023

Revised 09 March 2023

Accepted 27 March 2023

Available online 18 May 2023

(early access)

Published 12 April 2024

## Authors' Contribution

PSY performed the experiment, drafted the manuscript and analysed the data. LL and YLW contributed to experimental design and manuscript revision. YSZ conceived the idea, designed the experiment and finalized the manuscript. All authors read and approved the final manuscript.

## Key words

High-concentrate diets, Endogenous GH, Galactopoietics effect, Milk yield, Sannen goats

## INTRODUCTION

The growth hormone (GH) plays a crucial role in ruminant mammary development, onset of lactation, and maintenance of milk secretion (Akers, 2006). GH was synthesized and secreted by the acidophilic cell of pituitary anterior lobe, and regulated by growth hormone releasing hormone and growth hormone release inhibiting hormone of the hypothalamus (Tucker, 2000). The primary function of GH is to regulate somatic growth, but many of its growth-promoting effects are indirectly mediated by stimulation of liver to produce insulin-like growth factor-1 (IGF-1) (McCoard *et al.*, 2016). *In vivo*, GH and IGF-1 form the GH-IGF-1 axis to produce important action to regulate cell growth, differentiation, maintenance of normal cellular function and metabolism (Berryman *et al.*, 2008).

Several studies have found that GH-IGF-1 pathway is related to lactation. It has been shown that in lactating ruminant, high levels of GH profit glucose and fat into the mammary gland, and could significantly improve milk yield. The content of plasma GH has an overall increasing trend with the increased proportion of dietary roughage. The increased proportion of roughage in total mixed ration diets can not only promote the synthesis and secretion of GH, but also contribute to the process of anabolic metabolism that improves milk yield (Min *et al.*, 2005).

At present, concentrate supplementation is used to compensate nutritional deficiencies in the forage supply to improve animal performance such as milk production (Tarazon-Herrera *et al.*, 2000). However, long-time feeding with high-concentrate not only induces the decrease of milk quality and milk yield, but also causes metabolic disorders and increases the risk of various diseases (Ma *et al.*, 2022). How to find a balance between milk yield and body health is the main focus of dairy production. The GH-IGF-1 pathway participates in lactating and is directly related to milk yield, but its change and effect on milk production under high-concentrate feeding are unclear. In this study, we assessed the level of endogenous GH and IGF-1 in lactating goats with different forage to concentrate ratio diets and analyzed the expression level

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0030-9923/2024/0003-1225 \$ 9.00/0



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of related receptor genes to reveal the effect of endogenous GH-IGF-1 axis on the decreased of milk yield caused by feeding with high-concentrate diet.

## MATERIALS AND METHODS

### *Experimental animal and experimental design*

The trial was conducted in a standard animal house at Nanjing Agricultural University (Nanjing, China). Ten health multiparous dairy Sannen goats, with an average body weight of 45±7 Kg (mean ± SD), were used at mid-lactating with an average milk yield of approximately 0.5 Kg.

After 14 days adaptation period for experiment environment, goats were divided into two experimental groups in a 2×2 Latin square experiment design, which were balanced for body weight, parity and milk yield. The experimental design was a completely randomized, with four replicates per treatment. One group received diets with low concentrate (40% of dry matter) as the control group, and another group received high concentrate diets (60% of dry matter) as the high-concentrate group. Ingredients and nutrient compositions of experimental diets were presented in Table I. The content of total energy and total protein were equivalent between the two experimental diets. Goats were housed in individual stalls and free access to fresh water throughout the experimental time. Goats were fed twice daily (feeding time were from 07:00 to 08:00 h and from 19:00 to 20:00 h) and milked twice daily (the time were consistent with feeding time) for statistical daily milk yield. The health condition was checked throughout the study period and no cases of clinical mastitis were recorded.

### *Sample collections*

Daily milk yield of each goat was recorded by means of graduated measuring cylinders attached to individual milking units. Fifty milliliter of milk was sampled from individual goats, consisting of proportional volumes of morning (from 07:00 to 08:00 h) and evening (from 19:00 to 20:00 h). Samples were collected in 60 mL sterile plastic containers with potassium dichomate and stored at the 4°C until milk composition analysis conducted by the commercial company of Nanjing Weigang dairy industry Co., Ltd.

We collected blood samples in the 2<sup>nd</sup>, 5<sup>th</sup> and 8<sup>th</sup> week. Samples were taken at the same hour (from 07:00 to 08:00 h) each time. At the 9<sup>th</sup> week, blood was sampled at feeding before, after feeding 15, 30, 45 and 60 min. Blood samples were obtained by puncturing of the jugular vein and stored at EDTA-containing vacuum tubes. Blood samples were centrifuged (2,300 g×15 min at 4°C), and

plasma was removed and stored at -20°C until assayed.

Goats were executed at the end of experiment, the liver and mammary gland tissue samples were collected immediately, and immediately frozen in liquid nitrogen. All samples were stored at -80°C until assayed.

**Table I. Ingredient and nutrient composition of experimental diets.**

Ingredients (%)	Forge to concentrate ratio	
	4:6	6:4
<i>Leymus chinensis</i>	40.00	26.70
<i>Medicago sativa</i> Hay	20.00	13.30
Corn	22.99	23.24
Bran	0	20.77
Soybean meal	15.00	13.66
Powder	0.65	1.43
DCP	0.46	0
Premix <sup>a</sup>	0.50	0.50
Salt	0.40	0.40
Total	100	100
<b>Nutrient levels</b>		
Net energy(MJ/kg)	5.63	5.83
Digestible crude protein (%)	9.90	10.00
Neutral detergent fiber (%)	36.64	34.55
Acid detergent fiber (%)	24.74	20.35
Calcium (%)	0.80	0.90
Phosphorus (%)	0.33	0.38

a, Provided per kg of premix: Vitamin A 6 000U, Vitamin D 2 500U, Vitamin E 80ng, Cu 6.25mg, Fe 62.5mg, Ze 62.5mg, Mn 50mg, I 0.125mg, Co 0.125 mg, Mo 0.125 mg.

### *Analyses*

IGF-1 concentration in the jugular plasma samples were determined by enzyme linked immunosorbent assay using commercial kits (Goat Insulin Like Growth Factor 1(IGF-1) ELISA Kit, Beijing Rigobio Science Development Co.,Ltd. Beijing, China). GH concentration were determined by radioimmunoassay using commercial kits (Iodine [<sup>125</sup>I] Growth Hormone Radioimmunoassay Kit, Beijing North Institute of Biotechnology, Beijing, China).

Total RNA was extracted from the liver and mammary gland tissue samples using the Trizol reagent (Takara, Dalian, China) according to the manufacturer's instructions. The purity of the extracted RNA was verified by measuring the ratio of absorbencies at 260nm and 280nm.

**Table II.** The primer sequences of the target genes.

Gene	Genbank accession number	Primers sequence (5'-3')	Orientation	Product size(bp)
<i>B-actin</i>	JN033788	CCGTCATTAGTGCCTCAGTTC	Forward	175
		ATCCTCACCCAGTCTTCGTC	Reverse	
<i>GH</i>	M82912.1	TCCAGCCTCTGTTTCA	Forward	94
		CCACTGCCAAGGTCAA	Reverse	
<i>IGF-1R</i>	JN200823.1	GCTCA ACCCA GGGAA CTACA C	Forward	161
		CCACT ATCAA CAGAA CCGCA AT	Reverse	

The RNA was stored at  $-80^{\circ}\text{C}$  until use. Total RNA was transcribed into cDNA by using standard techniques of the Superscript first strand synthesis system (Shengxin Bio, Nanjing, China). The relative expression quantification of IGF-1R and GH mRNA in liver and mammary gland were observed by the Real-time PCR method. The genes sequence were obtained from Genbank and designed with the software of Primer 5 according to the principle of primer design (the primer sequences of the target genes were reported in Table II). All primers were synthesized by Beijing Dingguo Changsheng Biotechnology Co., Ltd (Beijing, China). The SYBR Green Supermix (Bio-Rad, 10023434, USA) was used to quantify the relative abundance of target mRNA. The detection was performed on a Real-Time PCR Detection System (Bio-RAD Q5 Applied Biosystems Inc., Foster City, USA).  $\beta$ -actin was used as a reference gene. The threshold cycle (CT) for the test samples was subtracted from the CT for the control sample to obtain the change(delta) in CT ( $\Delta\text{CT}$ ). In order to calculate differences in the expression level of the target gene, the  $\Delta\Delta\text{CT}$  method for relative quantification was used according to the manufacturer's manual.

#### Statistical analyses

All data were analyzed statistically by using SPSS 16.0 with independent-samples T-test. Results were expressed as the average  $\pm$  standard error (mean $\pm$ SE). The significance level was declared at  $P < 0.05$ .

## RESULTS

#### Milk yield and milk compositions

In the experiment, we analyzed the change of weekly milk yield between the control group and the high-concentrate group. As shown in Figure 1, the average milk yield of goats with high-concentrate diet was lower than the control group during the 9 weeks trial time. However, there was no significant differences between the two groups.

We also collected milk samples in the 2<sup>nd</sup> and 5<sup>th</sup> week and sent to the commercial company of Nanjing Weigang dairy industry Co., Ltd to analyze the milk

compositions. According to the results (Fig. 2), compared to the control group, the percentage of milk lactose in the high-concentrate group were lower.

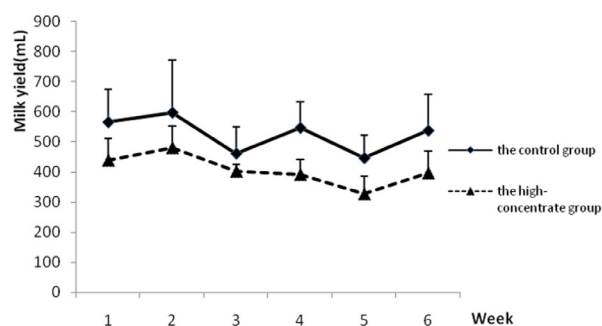


Fig. 1. Effect of different forage to concentrate ratios on weekly milk yield in goats.

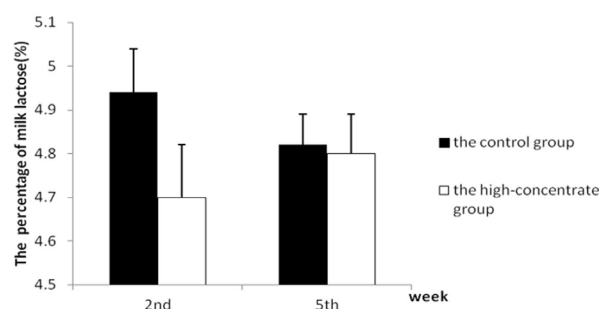


Fig. 2. Effect of different forage to concentrate ratios on lactose content of milk.

#### Plasma IGF-1 and GH.

As shown in Figure 3A, the content of plasma IGF-1 in the high-concentrate group were significantly lower than the control group in samples collected from week 2 ( $P < 0.05$ ) and week 5 ( $P < 0.01$ ).

As shown in Figure 3B, the plasma GH levels in the 9<sup>th</sup> week samples showed decrease after feeding in both groups. And the contents of plasma GH in the high-concentrate group were lower than the control group, with no significant differences.

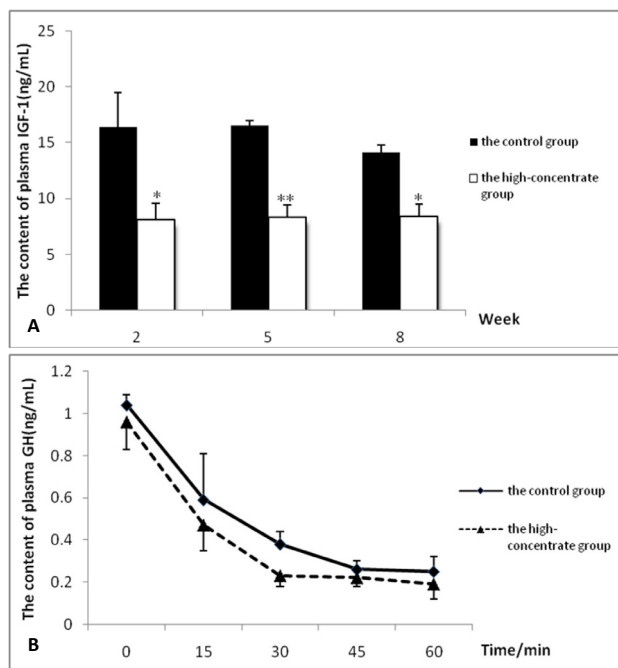


Fig. 3. Effect of high concentrate diet on plasma IGF-1R and GH in goats.

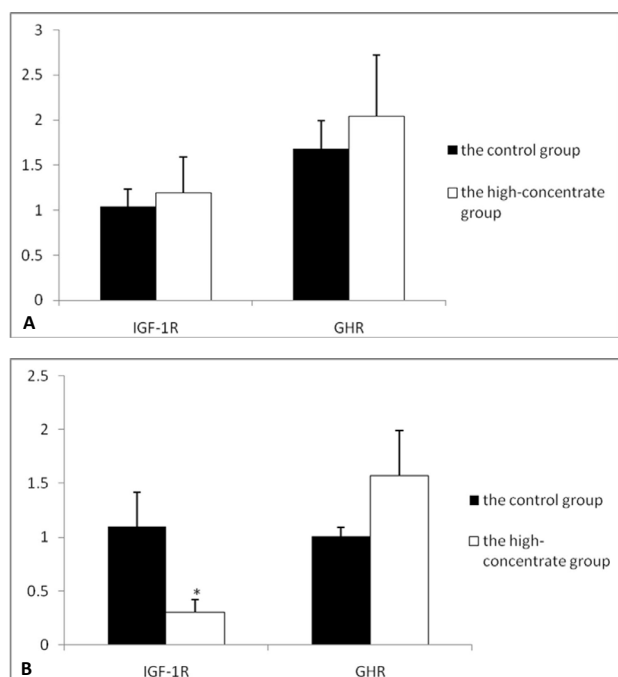


Fig. 4. Effect of high concentrate diet on mRNA expression of IGF-1R and GHR in liver (A) and mammary gland (B) of goats.

#### *mRNA expression of IGF-1R and GH in liver and mammary gland tissues*

The results (Fig. 4A) showed that the mRNA expression of IGF-1R and GH in the liver of the high-concentrate group were higher than the control group, but there had no significant different ( $P>0.05$ ).

As shown in Figure 4B, the mRNA expression of IGF-1R of mammary gland in the high-concentrate group was significantly lower than the control group ( $P<0.05$ ), while the mRNA expression of GH was higher than the control group.

## DISCUSSION

GH is one of the most important hormones which participate in ruminant mammogenesis, onset and maintenance of milk secretion. A large number of studies indicated that GH has a galactopoietic effect (Tucker, 2000). With respect to animal agriculture, administration of exogenous somatotropin (ST) is a biotechnology that increases the food output (meat or milk) per unit of feed resource input (Ferreira *et al.*, 2021). Pocius and Herbein (1986) demonstrated that milk yield increased up to 40% with no adverse effects in the treated cows which were injected with bovine growth hormone lasting 10-12 weeks (Pocius and Herbein, 1986). In 1985, the first longer term study reported that there was sufficient recombinant bst for 188 days of treatment, and impressive increases in milk yield and productive efficiency were observed (Bauman *et al.*, 1985). Milk yield response to bst treatment has been observed in cows of all parities, but the magnitude of the increase in milk yield varies according to the stage of lactation. Typical milk yield responses are increase by 10-15% (4-6kg/day), although even greater increases occur when the management and care of the animal are excellent (Tucker, 2000). In our experiment, we observed that the content of plasma GH in the high-concentrate group (the forage to concentrate ratio was 40:60) was lower than the control group (the forage to concentrate ratio was 60:40). Scilicet the release of endogenous GH in the control group was more than the high-concentrate group. Considering the changes of milk lactose and milk yield, this finding was justified, because prior studies had demonstrated that milk yield would increase when the content of GH increased (Pocius and Herbein, 1986), indicating the endogenous GH has the same galactopoietic effect as exogenous GH.

The effect of increasing milk yield by adding exogenous GH has been reported by numerous studies, but the underlying mechanism is not fully understood. Most studies indicated that the galactopoietic effect of GH is indirectly mediated by the endocrine-, autocrine-, or paracrine-acting of IGF-1 (Belli *et al.*, 2018). Cohick *et al.*

(1989) demonstrated circulating concentrations of IGF-1 began to increase about 6-12 h after the bst injection and reached to a maximum concentration in approximately 48h (Cohick *et al.*, 1989). Likewise, IGF-1 also stimulates DNA synthesis in mammary tissue cultures and thus may play a role in maintaining cell number during long-term ST treatment. Prosser *et al* found that infusion of IGF-1 into the pudenda artery of lactating goats for 6 h could increase milk production by about 30%. So IGF-1 plays a direct effect on promoting lactating and maintaining lactating. In our experiment, though detecting the content of plasma IGF-1, we found that compared to the control group, the content of plasma IGF-1 in the high-concentrate group was significantly lower. The result matched with the changes of plasma GH. Therefore, we thought that the increase of milk yield in control group was related to the higher level of endogenous GH, which promotes the release of IGF-1.

The somatomedin hypothesis suggested that GH exerts its effects by stimulating IGF-1 release from the liver, which in turn circulates to the target tissues (Isaksson *et al.*, 2001; Kleinberg *et al.*, 2011). The liver is the primary source for IGF-1 secretion in to the blood, and IGF-1 is transported to local tissues to exert its effects (Nguyen *et al.*, 2013). A reasonable explanation is that IGF-1 binds to the IGF-1 receptor (IGF-1R) in mammary gland, which increases the activity and the proliferation of mammary epithelial cell, and ultimately promotes lactating (Nguyen *et al.*, 2013). In our experiment, we found that the mRNA expression of IGF-1R in mammary gland was down-regulated in the high-concentrate group. The result was consistent to the results of milk yield, plasma GH and IGF-1. These results illustrated that the galactopoietics effect of endogenous GH was exerted by up-regulation of the IGF-1 and IGF-1R.

Lactose synthesis and milk yield have been found to be positively correlated with glucose uptake in the mammary gland of ruminants. It indicates that glucose uptake of mammary gland may be an important determining factor for the rate of milk synthesis (Shi *et al.*, 2022). According to Baumrucker, uptake of glucose and other nutrients in the mammary gland is determined by the supplement to the gland and the rate of transmembrane transport into the cell, however, supply is determined by the arterial nutrient concentration and mammary blood flow (Baumrucker, 1985). Bequette *et al.* (2001) has shown that milk yield and mammary blood flow are stimulated in goats when infused with IGF-1 from close-arterially into one mammary gland. Heo *et al.* (2017) found that IGF-1 increased both casein synthesis and glucose transport in the culture of mammary explants from mid-pregnant mice. A plausible mechanism may be that IGF-1 could promote glucose uptake in mammary gland by increasing mammary blood flow and

glucose transport, and subsequently improves milk yield. In our study, we also found that the percentage of milk lactose in the high-concentrate group was lower than in the control group. We speculated that the galactopoietics effect of endogenous GH was related to the high level of lactose, which caused by IGF-1 promoting glucose uptake in the mammary gland.

We found that in the high-concentrate group, the mRNA expression of IGF-1R in the liver was higher than in the control group. But the result was contrary to the mRNA levels in the mammary gland. It is possible that the decreased endogenous GH caused by feeding with high-concentrate diet leads to the decrease of IGF-1, which generates in liver tissue. In mammary gland, IGF-1 binding to IGF-1R exerts its effects of promoting lactating and glucose uptake in the mammary gland. Therefore, glucose uptake in the mammary gland was attenuated when the content of IGF-1 decreased, and the milk yield was decreased. The reason for the induction of the mRNA expression of IGF-1R in the liver of high-concentrate group could be related to desensitizing protection, which caused by excess IGF-1 generated in liver. The detailed mechanism needs further study.

## CONCLUSIONS

Numerous studies have found that milk quality and milk yield were decreased by long-term feeding with high-concentrate diets. Our study has demonstrated that in the high-concentrate diets feeding conditions, the content of endogenous GH was decreased, and then the content of IGF-1 was decreased possibly via the GH-IGF-1 axis. The mRNA expression of IGF-1R in mammary gland was down-regulated, leading to the weak action of glucose uptake in the mammary gland. It leads to the decreased lactose content and decreased milk yield.

## ACKNOWLEDGMENTS

We thank our colleagues from the Laboratory of Animal Physiology and Biochemistry for their assistance and comments that greatly improved the manuscript.

### Funding

Financial support for this work was provided by Priority Academic Program Development of Jiangsu Higher Education and National Basic Research Program of China (Project No. 2011CB100802). This work was supported by the Hebei Youth Natural Science Foundation (C2020108002), Provincial Science and Technology Project of Hebei Province (22326616D), Scientific Research Project of Hebei Higher Education Institutions

(BJK2024001), Xingtai Innovation Capability Plan Project (2022zz104).

#### IRB approval

The protocol for this study was reviewed and approved under project number 2011CB100802. The slaughter and sampling procedures strictly followed the 'Guidelines on Ethical Treatment of Experimental Animals' (2006) no. 398 created by the Ministry of Science and Technology in China as well as 'Regulation regarding the Management and Treatment of Experimental Animals' (2008) no. 45 from the Jiangsu Provincial People's Government.

#### Ethical approval

The Institutional Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, People's Republic of China) approved all of the procedures (surgical procedures and care of goats).

#### Statement of conflict of interest

The authors have declared no conflict of interest.

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