Astragalus Membranaceus, Seed of Cowheb Alters Milk Protein Yields and Lactoferrin, Casein Gene Expression in Cow Mammary Epithelial Cells

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Key words : MEC ,Astragalus membranaceus ,seed of cowheb ,lactoferrin ,casein

Introduction Several studies (Zhou ,2005 ,Wu . ,2004) have examined the effects of Chinese herbal medicines on milk output and quality of lactating cows *in vivo* .But there were few studies reported about their undergoing mechanism *in vivo* and using *in vitro* system to determine if medical herbs could alter milk yields and milk proteins synthesis by altering cell number and/or milk production per cell .So the present research focuses on the direct cellular level effect of aqueous extracts of *Astragalus membranaceus* (AM) ,*seed of cowheb* (DSC) and the commixture of DSC and AM(DSC+AM) on the cow mammary epithelial cells (MEC) cultured *in vitro* .The purpose of this study was to examine the alteration of α-casein (α-CN) ,β-casein (β-CN) and lactoferrin (LF) gene expression and milk protein secretion in response to aqueous extracts of herbs modification in cow MEC in culture .

Materials and methods The MEC line used in this study was established in the laboratory .After preincubation in 6-well paltes for 24 hours ,the cells were washed with PBS and then grown in the control medium and aqueous extracts of 3 herbs treatment media (AM ,20mg/ml ; DSC ,10mg/ml ; DSC+ AM ,10mg/ml+20mg/ml) for 48 hours ,then cells were collected and rinsed twice in PBS .Lamemmli lysis buffer was added ,followed by vortexing and sonication ,the protein concentration of cell lysates were determined by a coomasie brilliant blue staining assay .Total RNA was isolated using Tri Reagent according to the manufacturer's recommendations ,primer pairs were newly designed using published bovine nucleic acid sequences ,α-CN ,β-CN and LF gene expression were quantified by real-time PCR .Data were expressed as means ± *SD* ,statistical analysis was performed using one-way ANOVA .

Results The herbs of DSC ,AM and the commixture of DSC and AM had a multiplicative effect on the ability of the MEC to synthesize milk proteins .The amounts of milk proteins were quantified ,the milk proteins production of the control group ,DSC , AM and DSC+AM were 0 .57±0 .04 ,0 .63±0 .02 ,0 .59±0 .02 ,0 .64±0 .03 μg/ml/ well ,respectively .Compared with the control ,AM ,DSC groups ,the level of lactoferrin mRNA was much higher in the DSC+AM group .The α-casein gene expression level in the DSC group was much higher than that in the control ,AM ,DSC+AM groups .The level of β-casein mRNA were much higher in the DSC+AM ,DSC groups than that in the control ,AM groups .

Table 1 *The effect of herbs on milk proteins production (MPP) and LF ,β-CN ,α-CN mRNA of MEC .*

Group	n	Dose(mg/ml)	MPP(μg/ml/ well)	LF($2^{-\Delta\Delta CT}$)	β-CN($2^{-\Delta\Delta CT}$)	α-CN($2^{-\Delta\Delta CT}$)
Control	6	/	0 .57±0 .07^{bcB}	0 .68±0 .08^{b}	1 .29±0 .09^{b}	0 .85±0 .01^{Bc}
DSC	6	10	0 .63±0 .04^{abAB}	0 .71±0 .04^{b}	1 .46±0 .07^{a}	0 .97±0 .01^{Aa}
AM	6	20	0 .59±0 .12^{bcAB}	0 .69±0 .13^{b}	1 .38±0 .04^{ab}	0 .83±0 .05^{Bc}
DSC+AM	6	10+20	0 .64±0 .08^{aA}	0 .85±0 .07^{a}	1 .48±0 .13^{a}	0 .93±0 .08^{Ab}

Note : Differing superscripts (A ,B) indicate most significant differences ($P<0$ .01) ,and (a ,b) indicate significant differences ($P<0$ .05)

Conclusions *Seed of cowheb* (DSC) ,*Astragalus membranaceus* (AM) and the commixture DSC and AM could obviously enhance the ability of cow mammary epithelial cells to secrete milk proteins in culture .Moreover ,the commixture of DSC in 10mg/ml concentration and AM in 20mg/ml had the best role of getting the biggest milk protein yields ,and the highest levels of β-casein mRNA and lactoferrin mRNA expression .The DSC in 10mg/ml concentration group had the highest level of α-casein mRNA expression .

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