



## ***C/EBP $\alpha$* gene as a genetic marker for beef quality improvement**

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**ABSTRACT.** Intramuscular fat (IMF) or intramuscular triglycerides are interspersed throughout the skeletal muscles. The IMF, also called marbling, imparts meat with flavor and juiciness and is one of the core criteria for judging carcass value. The quantity of IMF is influenced entirely by genetics. Recently, understanding the underlying genetic bases of IMF has been a focus particularly in the beef industry. In this study, with the deep insights of ameliorating the beef quality by genetic means, the role of the CCAAT/enhancer binding protein alpha (*C/EBP $\alpha$* ) gene was investigated by over-expressing *C/EBP $\alpha$*  in bovine muscle stem cells (MSCs) to initiate the adipogenic program. Prior to this, bovine MSCs were isolated and induced to differentiate into adipocytes from cells that were exposed to dexamethasone isobutylmethylxanthine and indomethacin; the presence of insulin and fetal bovine serum was examined. Either ectopic expression of *C/EBP $\alpha$*  or treatment with dexamethasone and insulin induced the accumulation of fat droplets and the expression of adipogenic induction genes (*LPL*, *PPAR $\gamma$* , *C/EBP $\beta$* , and *C/EBP $\delta$* ). The expression levels of myoblast-related genes (*MyoD*, *Myf5*, and *Pax7*) were also measured to assess the accuracy of the

differentiation process. This study provides evidence that the *C/EBPα* gene is essential for cattle adipose tissue growth and development. Hence, this finding can contribute to improving beef carcass quality.

**Key words:** *C/EBPα* gene; Bovine muscle stem cells; Over-expression; Adipocyte differentiation

## INTRODUCTION

A variety of pluripotent and preadipocyte cell lines that have been developed from mouse embryonic tissue are useful for analysis of the adipocyte differentiation program (Cornelius et al., 1994). Two widely studied models, 3T3-L1 and 3T3-F442A, are already committed to the adipocyte lineage and are thus considered preadipocyte cell lines (Green and Kehinde, 1974, 1975). Analyses of the differentiation program in these cell lines have shown that CCAAT/enhancer binding proteins (*C/EBPs*) and PPAR $\gamma$  transcription factors work sequentially and cooperatively to stimulate the genetic events that result in differentiation. Research from a number of investigators has demonstrated that the expression of *C/EBPα* is both necessary and sufficient for the differentiation of 3T3-L1 preadipocytes to adipocytes. To show that the expression of *C/EBPα* is necessary for preadipocyte differentiation, Lin and Lane (1992) blocked its expression through the introduction of antisense RNA into 3T3-L1 preadipocytes. In the absence of *C/EBPα*, adipose-specific genes were not expressed and triacylglycerol accumulation was not detected. In addition, the conditional expression of *C/EBPα* in stably transfected clones of 3T3-L1 preadipocytes was sufficient to bring about differentiation as measured by the cytoplasmic accumulation of lipid and the expression of *422/aP2*, *GLUT4*, and endogenous *C/EBPα* (Lin and Lane, 1994; MacDougald and Lane, 1995). Skeletal muscle contains various cell types and can give rise to both muscle-derived satellite cells and adipose tissue-derived adipocytes, both of which are important to animal agriculture. Recently, it has become apparent that muscle stem cells (MSCs) exhibit more plasticity than previously thought because they can be differentiated into cells with adipocyte features (Grimaldi et al., 1997; Singh et al., 2007). Based on the key role that the *C/EBPα* gene plays in the differentiation of 3T3-L1 cell lines and in regulating gene expression, as well as considering the importance of intramuscular fat (IMF) in food-producing animals, it is critical to study the *in vitro* regulation of cattle adipose tissue by the *C/EBPα* gene. In this study, we addressed this issue by isolating bovine MSCs and quantifying lipid droplet accumulation, as well as the expression of adipogenic markers after insulin treatment and *C/EBPα* up-regulation.

## MATERIAL AND METHODS

### Isolation and cultivation of bovine MSCs

Bovine MSCs were cultivated using muscle samples collected from the hind leg of a Qinchuan cow's newborn calf. Samples were immediately soaked in 75% alcohol for 30 s and then washed 3 times with sterile phosphate-buffered saline (PBS). The following steps were carried out under a laminar sterile hood: samples were washed 3 times with PBS supplemented with penicillin and streptomycin antibiotics and then cut into a piece of meat approximately 1 mm<sup>3</sup>. In order to induce digestion, samples were soaked in collagenase type IV-containing

medium (2 mg/mL) and subsequently placed in an incubator at 37°C for 3 h. The tissue pellet was pipetted every 30 min for faster and complete tissue digestion. Two volumes of normal growth medium (DMEM) were added to the pellet, and the obtained solution was centrifuged at 1000 *g* for 5 min. The supernatant was discarded, and the cell pellet was resuspended in medium containing 20% fetal bovine serum (FBS) and 10% horse serum (HS). Approximately 400 selected cells were then transferred to a new Petri dish. This first generation of cells was denoted as PP1. After 1 h of incubation (37°C, 0.5% CO<sub>2</sub>), the suspension of non-adherent cells was transferred to a new Petri dish and denoted as PP2. Likewise, PP2 cells were treated as mentioned above, and the suspension of non-adherent PP2 cells was transferred to a new Petri dish and denoted as PP3 cells. After 24 h of incubation, the suspension of non-adherent PP3 cells was centrifuged at 1000 *g* for 5 min. The supernatant was discarded, and the pellet was resuspended in medium containing 20% FBS and 10% HS and subsequently transferred to a new Petri dish. This new generation of cells was denoted as PP4 (Figure 1a).

After 48 h of incubation, the same treatment strategy that was applied to the PP3 cells was applied to the PP4 cells. The new generation of cells that was obtained was denoted as PP5 (Figure 1a). After 72 h of incubation, the PP5 cells generally reached over 80% confluence and were either stored in liquid nitrogen or sub-cultivated (Figure 1b).

### **Construction of bovine MSC growth curve**

The prime aim of this study was to gain insights into the growth status of bovine MSCs. Cells were cultivated on a 24-well culture plate (approximately 1 x 10<sup>4</sup> cells/well). Every 24 h, the cells of 3 wells were digested by trypsin enzyme (0.25%) and counted with a hemocytometer. This was done for 6 continuous days to allow us to construct a bovine MSC growth curve (data not shown).

### **Identification of bovine MSCs**

#### ***Morphological identification***

Both bovine preadipocytes and bovine fibroblasts were used as control cells to observe the morphological difference of isolated MSCs.

#### ***Identification by differentiation induction***

A differentiation medium (DMEM + 2% HS) was used to assess the ability of the isolated MSCs to form a myotube.

### **Adipogenic differentiation of bovine MSCs by insulin**

For this experiment, 4 treatment groups were designed. In each group, either insulin-free serum-containing medium or serum-containing medium with insulin was used (Table 1). After every 3 days, a half volume of culture medium was renewed. One 6-well plate was used per treatment. Cells were collected every 3 days to conduct RNA extraction and real-time polymerase chain reaction (PCR). The whole experiment lasted 15 days. On the last day of the experiment, cells of the remaining 2 wells were used for RNA extraction and oil red staining.

**Table 1.** Insulin test design.

	Group I	Group II	Group III	Group IV
Culture medium	DMEM + 20% FBS + 10% HS	DMEM + 20% FBS + 10% HS + 1 $\mu$ M dexamethasone + 10 $\mu$ g/mL insulin + 0.5 mM IBMX + 200 $\mu$ M indomethacin	DMEM + 10% FBS	DMEM + 10% FBS + 1 $\mu$ M dexamethasone + 10 $\mu$ g/mL insulin + 0.5 mM IBMX + 200 $\mu$ M indomethacin

FBS = fetal bovine serum; HS = horse serum; IBMX = isobutylmethylxanthine.

### RNA extraction, reverse transcriptase-PCR, and real time-PCR

All cellular RNA was extracted using the total RNA kit I protocol from Omega (USA). First-strand cDNA synthesis was performed by reverse transcription using total RNA as a template and PrimeScript RT Master Mix protocol from Takara (Japan). Real-time PCR was performed in a 20- $\mu$ L volume composed of 50 ng cDNA, 0.4  $\mu$ M of each primer, 1X SYBR Premix Ex Taq<sup>TM</sup>II, and 1X ROX reference dye. PCR cycles were as follows: 1 repetition of 95°C for 30 s, 40 repetitions of 95°C for 5 s and 60°C for 34 s, and 1 repetition of 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s.

### Oil red O staining

To prepare the oil red O working solution, 0.5 g oil red powder was dissolved in 100 mL isopropanol and filtered using a qualitative filter paper. Then, this solution was diluted with distilled water in a ratio of 3:2. Once again, the overall dye was filtered. Before staining, culture medium was removed from cells, and cells were washed 3 times with 2 mL 1X PBS and fixed for 30 min by 4% paraformaldehyde. Paraformaldehyde was discarded from each well, and cells were rinsed with 1 mL 1X PBS per well. Oil red O working solution (1 mL) was added to each well, covering the entire monolayer. Following 20-30 min of incubation, cells were rinsed with 1X PBS and observed under the microscope.

### Adipogenic differentiation of bovine MSCs via C/EBP $\alpha$ gene over-expression

#### Generation of adenovirus

To generate the pAd-Track-CMV-GFP virus, a cDNA encoding C/EBP $\alpha$  that was cleaved from the PMD-19T-simple vector was inserted into the shuttle vector pAd-Track-CMV (kindly provided by Professor Luo Jun, Northwest A&F University, China) using *Bgl*III and *Xho*I restriction endonucleases and a DNA ligase. Following cleavage by *Pme*I, the resultant plasmid was linearized and subsequently transferred to the BJ5183 bacterial strain (kindly provided by Professor Luo Jun). Kanamycin-resistant colonies were selected and were subjected to restriction endonuclease analyses for confirmation. Following cleavage with *Pac*I, confirmed recombinant adenovirus plasmids were transferred to the HEK 293A cell line. The viral propagation, purification, and plaque assay were performed using established adenoviral protocols (Luo et al., 2007).

#### Cell cultivation and viral infection

The cells used for this experiment were obtained from the third sub-cultivation of

the bovine MSCs that we previously isolated. Three groups of treatments were designed in the experiment that lasted 7 days. The first group was the control group, where nothing was added to the cells. The second group, denoted as the negative adenovirus group, had a *C/EBP $\alpha$*  gene-free recombinant adenovirus (2 mL) added to it. The third group had a recombinant adenovirus containing the *C/EBP $\alpha$*  gene (2 mL) added to it. Viral supernatants were removed 10 h after addition, and cells were supplemented with a normal myoblast growth medium, which was renewed every 24 h. Total RNA was extracted from each group on days 1, 3, 5, and 7 for further real-time PCR analysis, and the oil red O test was performed according to the protocol described previously.

### Statistical analysis

*GAPDH* was used as an internal control gene for the real-time PCR analysis. The expression levels of all targeted genes were analyzed using the  $2^{-\Delta\Delta C_t}$  formula, where the  $C_t$  value represents the number of the cycle at which the fluorescence intensity of each reaction intersects with the threshold. The formulas are described as follows:

$$\Delta C_t = C_{t\text{mean}}(\text{target gene}) - C_{t\text{mean}}(\text{GAPDH}) \quad (1)$$

$$\Delta\Delta C_t = C_{t\text{mean}}(\text{sample}) - C_{t\text{mean}}(\text{max sample}) \quad (2)$$

$$\text{Relative quantity} = 2^{-\Delta\Delta C_t} \quad (3)$$

Once the efficiency of both reactions reached 100%, the expression ratio between samples was equal to the relative quantity (Logan et al., 2009). All results are reported as means  $\pm$  standard error of mean using SPSS 17.0. P values less than 0.05 and P values less than 0.01 were considered to be statistically significant or statistically very significant, respectively.

## RESULTS

### Isolation and cultivation of bovine MSCs

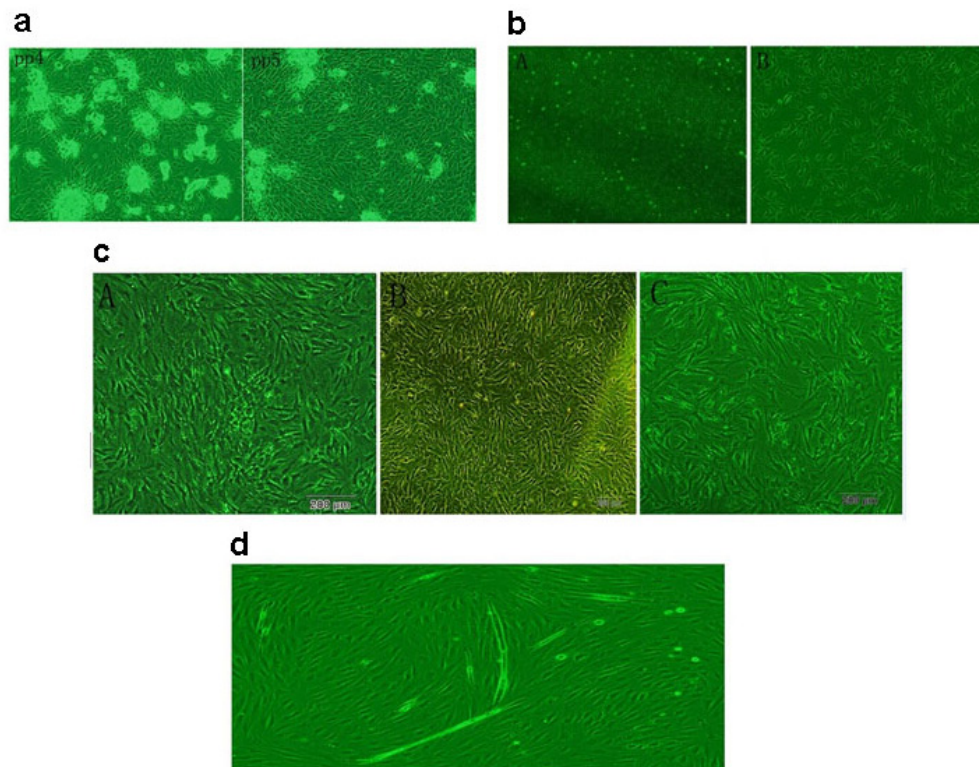
In this experiment, we successfully isolated and cultivated bovine MSCs (Figure 1a). Overall, we noticed that MSC adherence is relatively slow (beginning 12 h after incubation). MSCs were fusiform or fusiform-like. With increased incubation time, the density of the cells increased and the fusiform shape became more obvious. Cells were regularly arranged in parallel and tended to grow rapidly. Overall, the confluence of cells reached 90% after 3 days. We normally began the sub-cultivation at this time. Cells began to adhere 1 h after sub-cultivation, and adherence was completed after 24 h. Healthy sub-cultivated cells were fusiform, grew well, and showed a good refraction (Figure 1b).

### Construction of an MSC growth curve

The constructed cell growth curve had an ‘‘S’’ shape. Overall, cells had approximately 1 day of latent or slow growth; this could be denoted as the cellular adaptation stage. After-



wards, cells entered the exponential growth phase. The maximum number of cells was reached on the 4th day. We observed that between the 4th and the 5th day, cells showed constant growth. This was because cells reached the maximum density, and cell growth was likely inhibited by in-between cell contact. The number of cells began to decrease on the 5th day because of cell senescence and apoptosis (data not shown).



**Figure 1.** a. Isolated MSC magnification time (100X). b. Sub-cultivated MSC magnification time A (40X) and B (100X). c. Stem cell morphological comparison (A - MSC; B - preadipocyte, C - fibroblast). d. Myotube formation.

### Morphological identification of bovine MSCs

Figure 1c shows the results of the morphological comparison of bovine MSCs with both bovine preadipocytes and bovine fibroblasts. All 3 types of cells were fusiform, but we observed clearly that MSCs were slightly oval, fibroblasts were slightly elongated, and the width of preadipocytes lay between these two (Figure 1c).

### Identification by differentiation induction

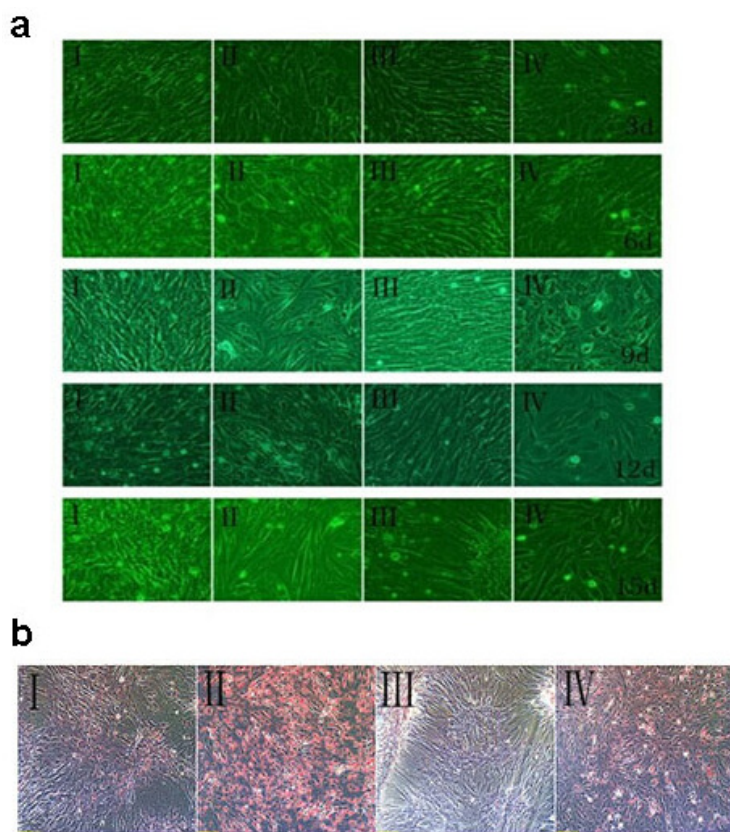
Differentiation medium (DMEM + 2% HS) was used to induce MSC differentiation into myotubes. The result was obvious from day 3 of differentiation. The number of differentiated MSCs increased with time. The myotubes displayed in Figure 1d were observed on day

7. The occurrence of myotubes is evidence that we successfully isolated and cultivated bovine MSCs (Figure 1d).

### Adipogenic differentiation of MSCs using insulin

#### *Effect of insulin and different media on bovine MSC morphology*

MSC morphological changes were observed under an inverted microscope during the experimental time. Differences within the group were observable beginning on day 9. Group I cells were shuttle-like, group III cells were elongated, group II cells were oval, and group IV cells were almost the same but seemed to be slightly elongated. As with cell proliferation, group I and group III cell numbers increased gradually, and this could be seen clearly on day 9. However, group I cells seemed to proliferate more rapidly than group III cells. Group II and group IV cells were treated with isobutylmethylxanthine (IBMX) that inhibited their proliferation and promoted their differentiation. With time, a lipid droplet-like substance appeared in the cells of group II and group IV (Figure 2a).



**Figure 2. a.** Differentiation of MSC into adipocyte under insulin and dexamethaxone control. **b.** Oil red O-stained cells.

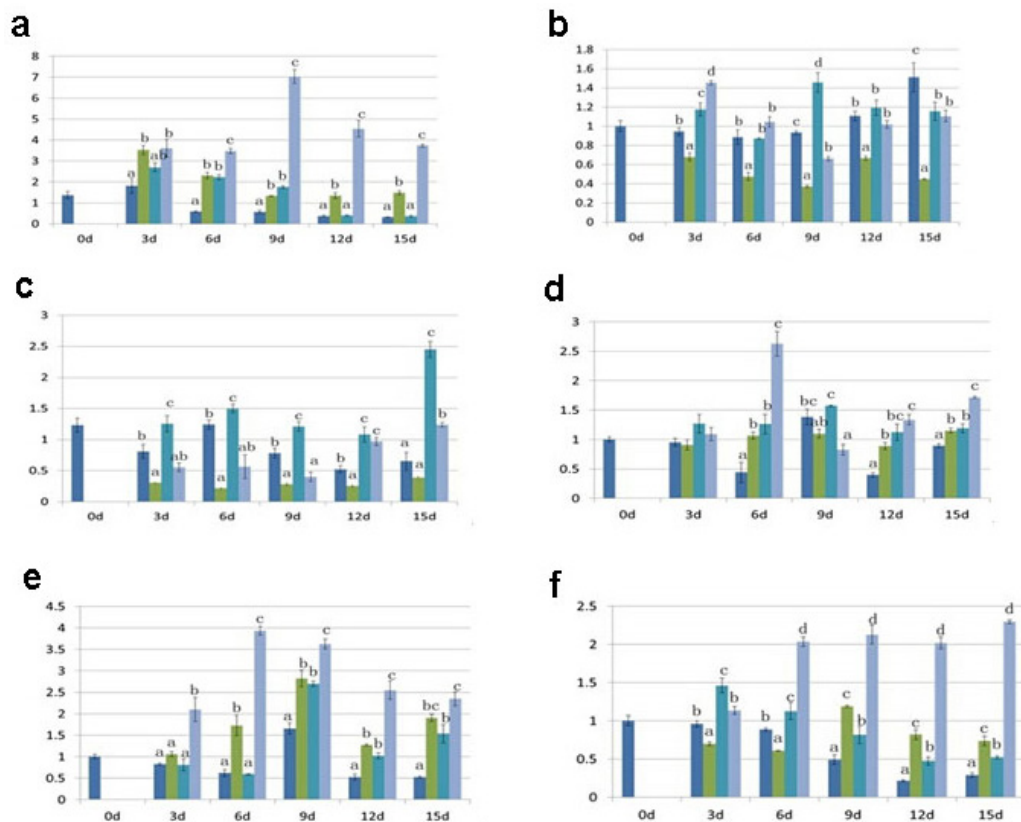
### Oil red O-staining test

The oil red O-staining test was performed on day 15, which showed that there was a higher accumulation of lipid droplets in group II and group IV than in group I and group III. In addition, lipid droplet accumulation was more important in group I than in group III and in group II than in group IV (Figure 2).

### Expression profile of myoblast- and adipocyte-related genes

#### *MyoD*

By comparison, *MyoD* gene expression was significantly higher in groups II and IV than groups I and III ( $P < 0.05$ ). Group III expression of *MyoD* was significantly higher than that of group I on day 6 and day 9 ( $P < 0.05$ ). In group IV, the expression of *MyoD* was significantly higher than that of group II on days 6, 9, 12, and 15 (Figure 3a).



**Figure 3.** a. *MyoD* mRNA expression. b. *Myf5* mRNA expression. c. *C/EBPα* mRNA expression. d. *C/EBPβ* mRNA expression. e. *C/EBPδ* mRNA expression. f. *PPARγ* mRNA expression. Group I (dark blue); group II (green); group III (light blue); group IV (purple).



### ***Myf5***

*Myf5* gene expression was significantly lower in group II than in group I from day 3 to day 15 and significantly lower in group IV than in group III on day 6 only ( $P < 0.05$ ). Group III expression of *Myf5* was significantly higher than that of group I on day 3 and day 9 and lower on day 15 ( $P < 0.05$ ). Group IV expression of *Myf5* was significantly higher than that of group II from day 3 to day 15 (Figure 3b).

### ***C/EBP $\alpha$***

Concerning *C/EBP $\alpha$*  gene expression, the recorded values were significantly higher in groups I and III than in groups II and IV from day 3 to day 9 ( $P < 0.05$ ). On day 12, only group I's *C/EBP $\alpha$*  gene expression was significantly higher than that of group II, and on day 15, only group III's *C/EBP $\alpha$*  gene expression was significantly higher than that of group IV. The expression of the *C/EBP $\alpha$*  gene in group III was significantly higher than that in group I ( $P < 0.05$ ). The expression of *C/EBP $\alpha$*  in group IV was significantly higher than that in group II on days 12 and 15 ( $P < 0.05$ ; Figure 3c).

### ***C/EBP $\beta$***

*C/EBP $\beta$*  gene expression was significantly higher in group II than in group I on days 6, 12, and 15 and significantly higher in group IV than in group III on days 6 and 15 ( $P < 0.05$ ). Group III's *C/EBP $\beta$*  gene expression was significantly higher than that of group I on days 6, 12, and 15. Likewise, group IV's *C/EBP $\beta$*  gene expression was significantly higher than that of group II on days 6, 12, and 15 (Figure 3d).

### ***C/EBP $\delta$***

The recorded values of *C/EBP $\delta$*  gene expression were significantly higher in groups II and IV than in groups I and III ( $P < 0.05$ ), except on day 3, when *C/EBP $\delta$*  gene expression was not significantly different between groups I and group II ( $P > 0.05$ ). On days 9, 12, and 15, *C/EBP $\delta$*  gene expression in group III was significantly higher than that in group I. From days 3 to 15, *C/EBP $\delta$*  gene expression in group IV was significantly higher than that in group II (Figure 3e).

### ***PPAR $\gamma$***

The recorded values of *PPAR $\gamma$*  gene expression were significantly higher in groups II and IV than in groups I and III on days 9, 12, and 15, and they were higher in group IV than in group II on day 6 ( $P < 0.05$ ). From day 3 to day 15, *PPAR $\gamma$*  gene expression was significantly higher in group III than in group I. Likewise, *PPAR $\gamma$*  gene expression was significantly higher in group IV than in group II from day 3 to day 15 (Figure 3f).

## **Adipogenic differentiation of MSCs via *C/EBP $\alpha$* gene over-expression**

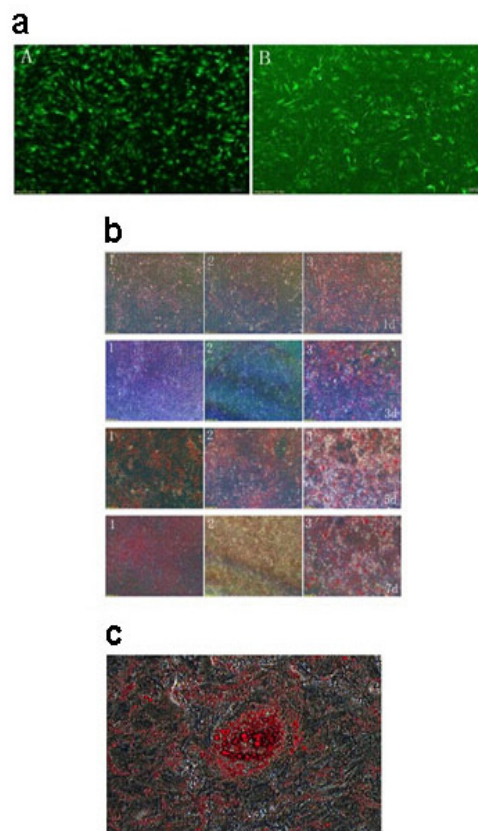
### ***Viral transfection efficiency***

One day prior to the transduction, approximately  $1 \times 10^5$  cells/well were seeded into

7 wells of a 24-well plate so that their confluence at the time of transduction was about 50-70%. The next day, virus particles were thawed on ice and added to the wells according to Table 2. No virus particles were added to one of the wells, which served as the control. Cells were incubated for 48 h at 37°C under standard cell culture conditions. Cell pictures were acquired by fluorescence microscopy after 24 and 48 h, and the rate of transduced cells per well for each multiplicity of infection (MOI) and at each time point was determined. As a result, MOI 40 appeared to be the lowest MOI at which enough cells showed transgene expression without cytotoxic side effects (Figure 4a). Therefore, MOI 40 was used for further experimentation.

**Table 2.** Amounts of adenovirus required to determine the multiplicity of infection (MOI).

Size	Amount of recombinant adenovirus ( $\mu$ L)						
	No. of cells	MOI 10	MOI 40	MOI 100	MOI 200	MOI 500	MOI 1000
24 wells	100,000	0.1	0.4	1	2	5	10



**Figure 4.** a. Adenovirus transfection efficiency (A, Ad-NC recombinant adenovirus-infected cells; B, Ad-C/EBP $\alpha$  recombinant adenovirus-infected cells). b. Oil red O-stained cell (group I = control; group II = Ad-NC recombinant adenovirus-infected cells; group III = Ad-C/EBP $\alpha$  recombinant adenovirus-infected cells). c. Oil red O-stained Ad-C/EBP $\alpha$  recombinant adenovirus-infected cells on day 7.

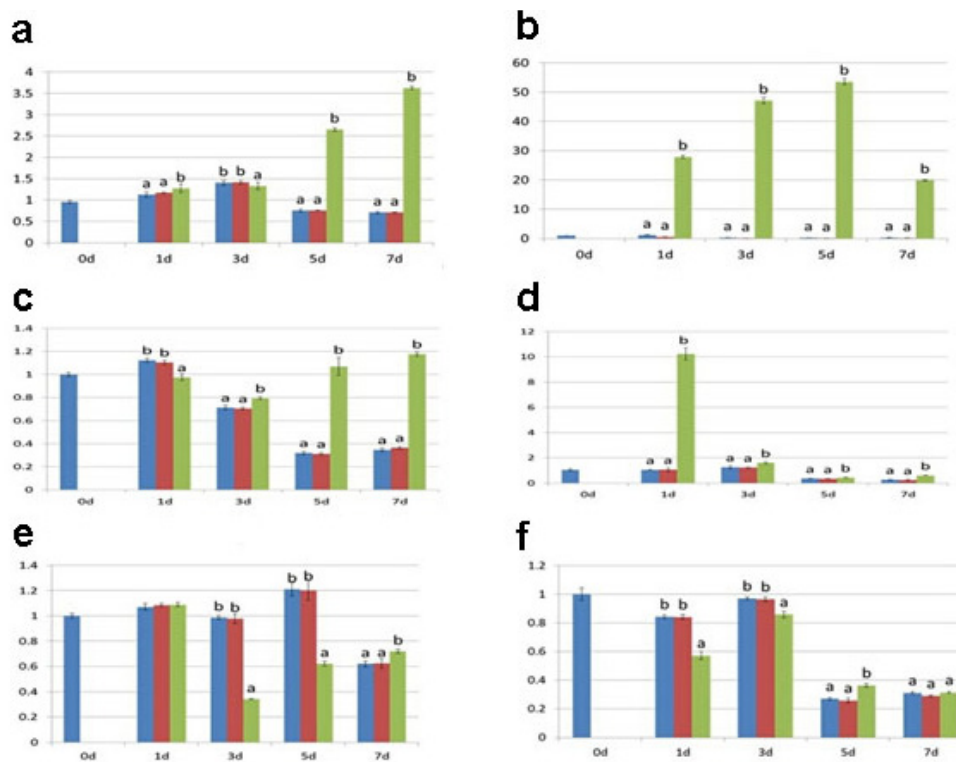
### Oil red O-staining test

Figure 4b displayed the results of the oil red O-staining test performed on days 1, 3, 5, and 7. On day 1, there was already an obvious difference in coloration between group I, group II and group III, where a better coloration was observed in group III. Overall, with an increased number of days, the coloration increased in all groups. The coloration in groups I and II was similar, and the coloration in group III was always higher (Figure 4b). On day 7, large lipid droplets were already formed in group III (Figure 4c).

### Relevant gene expression profiles

#### *C/EBP $\alpha$*

As we might predict, group III (presence of recombinant adenovirus containing the *C/EBP $\alpha$*  gene) showed a significant difference in endogenous *C/EBP $\alpha$*  gene expression from day 1 to day 7 compared to group I and group II ( $P < 0.05$ ). *C/EBP $\alpha$*  gene expression of group I and group II was not significantly different ( $P > 0.05$ ). With the increase in the number of days, group III's *C/EBP $\alpha$*  gene expression gradually increased. The highest expression was obtained on day 5 and was followed by a decline on day 7 (Figure 5a).



**Figure 5.** a. *C/EBP $\alpha$*  mRNA expression. b. *LPL* mRNA expression. c. *PPAR $\gamma$*  mRNA expression. d. *MyoD* mRNA expression. e. *Myf5* mRNA expression. f. *Pax7* mRNA expression. Group I (blue); group II (red); group III (green).

### **LPL**

*LPL* gene expression was significantly higher in group III than in groups I and II, except on day 3 ( $P < 0.05$ ) (Figure 5b). No significant difference in expression was observed between group I and group II ( $P > 0.05$ ).

### **PPAR $\gamma$**

On day 1, *PPAR $\gamma$*  gene expression was lower in group III than in groups I and II; however, it was significantly higher in group III than in groups I and II from day 3 to day 7 ( $P < 0.05$ ) (Figure 5c). No significant difference in *PPAR $\gamma$*  gene expression was observed between group I and group II ( $P > 0.05$ ).

### **MyoD**

Group III *MyoD* gene expression was significantly higher than that of group I and group II from day 1 to day 7 ( $P < 0.05$ ). No significant difference was observed between group I and group II ( $P > 0.05$ ; Figure 5d).

### **Myf5**

On both days 3 and 5, the expression of the *Myf5* gene in group III was significantly lower than that in groups I and I, but it was higher on day 7 ( $P < 0.05$ ). Overall, there was no significant difference between groups I and II from day 1 to day 7; likewise, the same pattern was observed amongst the 3 groups on day 1 ( $P > 0.05$ ; Figure 5e).

### **Pax7**

On days 1 and 3, expression of the *Pax7* gene in group III was significantly lower than that in groups I and II, but it was higher on day 5 ( $P < 0.05$ ). From days 1 to 7, there was no significant difference in *Pax7* gene expression between groups I and II. Likewise, the same pattern was observed amongst the 3 groups on day 7 (Figure 5f).

## **DISCUSSION**

C/EBPs are a family of transcription factors with a highly conserved basic leucine zipper domain as the main family characteristic; 6 isoforms have been identified to date (Williams et al., 1991; Lekstrom-Himes and Xanthopoulos, 1998). Currently, the function of C/EBPs has been investigated by various approaches. A number of researchers have reported a pivotal role played by this family in the control of cellular proliferation, differentiation, metabolism, and numerous other responses, particularly in adipocytes (Ramji and Foka, 2002; Rosen, 2005). As demonstrated in *in vitro* and *in vivo* studies, isoforms of C/EBPs can activate fat metabolism and adipocyte differentiation-related genes by interacting with their promoters (Cao et al., 1991; Ramji and Foka, 2002). The coordinate activation of C/EBPs and adipocyte markers provides correlative evidence for the hypothesis that the induction of C/EBP $\beta$  and C/EBP $\delta$  increases the expression of C/EBP $\alpha$ , which, in turn, activates the expression of adi-

pocyte genes and thus stimulates the differentiation process (MacDougald and Lane, 1995). A number of studies have provided evidence that *C/EBP $\alpha$*  promotes adipogenesis in 3T3-L1 cells (Freitag and Geddes, 1992; Lin and Lane, 1992; Wu et al., 1995). However, its role in the skeletal muscle tissue is not yet reported. Skeletal MSCs from food-producing animals captivated the attention of agricultural life scientists seeking to develop a better understanding of the molecular regulation of lean tissue and IMF development. Here, we investigated the possible adipogenesis regulation role of the *C/EBP $\alpha$*  gene in bovine MSCs. MSCs were properly isolated from a newborn calf's muscle tissue and then confirmed by morphological comparison and myotube differentiation induction. The activation of adipogenesis in isolated MSCs by exposure to the adipogenic inducers dexamethasone, insulin, IBMX, indomethacin, and FBS was marked by a major change in cell morphology, lipid deposition, and adipogenic-relevant gene expression stimulation. The highest fat deposition was obtained in group II, which contained the highest amount of FBS. *C/EBP $\beta$* , *C/EBP $\delta$* , and *PPAR $\gamma$*  gene expression levels were significantly higher in the insulin-treated group, which is consistent with early observations and predicted ongoing adipogenesis activity. However, *C/EBP $\alpha$*  gene expression was unexpectedly and significantly lower in the same group. The specific reasons and mechanisms in this case could be the subject of further study. *MyoD* gene expression was higher in the insulin-treated group; in contrast, *Myf5* gene expression was lower in the same group. This result is consistent with the current thinking that *Myf5* specifically defines the majority of quiescent satellite cells, whereas *MyoD* expression increased after cell activation (Beauchamp et al., 2000; Marchildon et al., 2012). With the ectopic expression of the *C/EBP $\alpha$*  gene, a significant increase was noted in endogenous *C/EBP $\alpha$*  gene expression in the group that received adenovirus with the *C/EBP $\alpha$*  gene until day 7. The decline in *C/EBP $\alpha$*  gene expression that was observed on day 7 coincided with a higher deposition of lipids and might be the result of gene expression auto-regulation. The higher expression of the *LPL* and *PPAR $\gamma$*  genes with the adenovirus expressing the *C/EBP $\alpha$*  gene, where lipid deposition was higher, correlates with previous findings. Although *MyoD* gene expression was significantly higher in this adenovirus containing the *C/EBP $\alpha$*  gene group, a progressive loss of expression was observed with the passing of the day. *Myf5* and *Pax7* showed lower expression at an earlier stage, but their expression was higher at the later stage. This instability of expression might be because activated satellite cell markers change with the degree of activation (Crameri et al., 2005).

## CONCLUSION

In summary, in this experiment we have successfully established an *in vitro* system of bovine MSC cultivation. Cell adipogenic differentiation was demonstrated via dexamethasone and insulin treatment and by up-regulating the *C/EBP $\alpha$*  gene. Our findings showed that the *C/EBP $\alpha$*  gene is important for lipid metabolism in bovine skeletal muscle tissue. This is essential for developing technologies and strategies to improve IMF and consumer acceptance and satisfaction of muscle foods.

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