# Response of Growing Rabbits to Feed Restriction and Some Additives on Performance, Carcass and Hepatic Gene Expression under Egyptian Summer Conditions

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## Abstract

This work aimed to study the response of growing female NZW rabbits to quantitative feed restriction (FR) at 60% of ad-libitum applied and the effect of adding either copper sulfate (CuSO<sub>4</sub>) and/or sodium bicarbonate (NaHCo<sub>3</sub>) to drinking water on performance and hepatic expression of apolipoprotein (apoA1), lipoprotein (cholesteryl ester transfer protein CETP, hepatic lipase HL) and insulin-like growth factor-I (IGF-1) during the summer period. Over the fattening period, rabbits fed AL during RES period (28-49 day of age) were significantly higher in most growth performance (GP) than RES-group, but all hepatic expression of genes tested decreased in feed restrictions group when compared to control on the other hand, adding either CuSO<sub>4</sub> or NaHCo<sub>3</sub> to drinking water slightly insignificant increase the expression of all the tested gens. When RES. rabbits fed freely after restriction (49-84 days of age). Logically, feed intake (FI) was significantly increased for AL group during FR period while, it was nearly similar, but FCE was more favorable for RES group throughout the whole period. Also, performance index (PI) was significantly higher for RES-groups. All values of carcass traits were significantly affected by FR. However, adding either CuSO<sub>4</sub> or NaHCo<sub>3</sub> to drinking water decreased GP during FR period. It can be concluded that FR had no significant effect on GP and slight decrease in gene expression for IGF-1 and lipoprotein gens, so it can applied FR to produce at slaughter age, rabbits with the same LBW of rabbits fed AL. Also, CuSO<sub>4</sub> or NaHCo<sub>3</sub> in drinking water is more practical to alleviate of heat load under the summer condition of Egypt.

Keywords: Growing rabbits, Feed restriction, Copper sulfate, Sodium bicarbonate, Performance, Carcass

#### 1. Introduction

Rabbit meat is an important source of protein for human because of its high quality and low fat. But during the summer months in Egypt can generate a state of stress and evoke a combination of behavioral, biochemical and physiological changes causing a reduction in rabbit performance. However, limiting the FI is widespread in animal breeding such as for adjusting the ration to the nutrient requirements or to manage the fattening and the

meat quality (Gidenne et al, 2009). Also, an intake restriction is frequency applied for the young rabbit female to avoid excessive fattening troubles (Rommers et al, 2004). However, feed restriction became systematic in much country as a preventive methods against post-weaning digestive disorders (Gidenne et al, 2003; Boisot et al, 2004 and Bergaoui et al. (2008). Boisot et al. (2003) demonstrated that the interest of a preventive restricted feeding to reduce the negative effect of this disorders on the growth performance of rabbits, they found that a feeding level of 60% was more efficient than 80% in Epizootic Rabbit Enteropathy (ERE) conditions. Moreover, restricted feeding induces compensatory growth by realimination and increases feed efficiency (Tumova et al, 2002, 2003 and Dalle Zotte et al., 2005) and body fat can be reduced (Washburn, 1990). However, many additives are recently added to rabbit feed or water as a way to help alleviate adverse effect during summer months and to enhance productive performance and immune response of rabbits. Copper sulfate ( $CuSO_4$ ) has been recognized as a feed additive for rabbits to improve growth rate and reduce entric disease (Lebas et al., 1986 and Liang et al., 1988). Different studies had been conducted to evaluate CuSO<sub>4</sub> as found by Bassuny (1991) who reported an improvement of daily gain, feed intake and feed conversion ratio for the copper supplement in NZW rabbits. Also, the addition of 100 ppm copper (as Cu o form) to the basal ration improved growth performance in the growing rabbits without accumulative effect of Cu on liver tissues and did not adversely influence and kidney functions under subtropical conditions (Abdel-Same and El-Masry, 1997). Adding of rabbits diet with copper could improve growth performance and reproductive efficiency for NZW rabbits (Maria et al., 2000; Moce et al., 2000 and Attia 2003). However, valuable studies have been conducted using sodium bicarbonate (NaHCo<sub>3</sub>) showing growth response when stimulated water and feed intakes and improved weight gain (Gorman, 1992; Abdel-Khalek, 1999 and Al-Shanti, 2003). Furthermore, Balnave and Oliva (1991) found that diets supplemented with NaHCo<sub>3</sub> in diets or drinking water produced a significant improvement in bird production response. Therefore, the objective of the present study was to investigate the effect of adding drinking water either with CuSO<sub>4</sub> or NaHCo<sub>3</sub> on performance of growing rabbits subjected to quantitative restriction feeding and hepatic gene expression apoA1, CETP, HL and IGF-1 during summer months in Egypt.

#### 2. Materials and Methods

#### 2.1 Animal Management

This experiment was carried out in animal house, National Research Centre, Egypt during summer season to investigate the response of growing rabbits to quantitative feed restriction which was 60% of its requirements and effect of adding copper sulfate (CuSO<sub>4</sub>) and/or sodium bicarbonate (NaHCo<sub>3</sub>) to drinking water on some productive and physiological traits. A total number of sixty female New Zealand White (NZW) weaned rabbits, 28 days old, with nearly equal homogenous in live body weight at the beginning of the experiment, were randomly identified and distributed to form five treatment groups of 12 each. Each group contained 3 replicates. Water was available ad-libitum. The rabbits were fattened until 84 days of age and received commercial pelleted diet, its composition is given in Table (2). The diet was formulated to cover the nutrient requirements of the growing rabbits according to National Research Center (1977). The experimental groups were classified as follow: Group1: fed ad libitum (AL) served as control. Group2: fed restricted diet (60%) + tap fresh water. Group3: fed as group 2 + 0.1(g/L) CuSO<sub>4</sub> in water. Group4: fed as group 2 + 10 (g/L) NaHCo<sub>3</sub> in water, while group5: fed as group 2 + 0.1(g/L) CuSO<sub>4</sub> + 10 (g/L) NaHCo<sub>3</sub> in water. Following the restriction period at 49 days of age, six of each group sacrificed for gene expression tests and the remaining rabbits were fed ad-libitum. All rabbits were housed in galvanized metal wire cages provided with feeders and automatic drinking system and kept under managerial and hygienic conditions. The experimental period was extended for 8 weeks. Live body weights (LBW) were controlled individually at 49 and 84 days of age. Feed intake (FI) was controlled when rabbits were recorded daily; also, water consumption was recorded at different periods for each group. While live body gain (LBG) and Feed conversion ratio (FCR) were calculated during the experimental periods. At the end of the experimental period, 4 representative rabbits of each treatment were weighted and slaughtered to complete bleeding, then all the parts of the body were weighed and carcasses were proportional to LBW of the rabbits.

## 2.2 RNA extraction

The liver of each group was promptly excised, and frozen in liquid nitrogen for extract total RNA form liver with Trizol reagent according to the manufacturer's procedures. The concentration and purity of RNA was measured at 260/280 nm using ultraviolet spectrophotometer. Equal amounts of RNA isolated from individual liver of each group were prepared for the semi-quantitative RT-PCR (Marone et al., 2000).

## 2.3 Semi-quantitative reverse transcription and PCR reaction

First strand cDNA synthesis was conducted using semi-quantitative RT-PCR ready to go you primer–First Strand Beaks kit (Ferments) according to the manufacturer's procedures., cDNAs were amplified using specific primers for rabbit apoA1, CETP, HL and IGF-1 table (1). The PCR reaction was carried out as described [Chen & Klebe, 1993] and quantified as described below.

## 2.4 Electrophoresis

Semi-quantitative RT-PCR products were subjected to electrophoresis on 1.5% agarose gel and visualized over a UV Trans-illuminator. The ethidium bromide-stained gel bands were scanned and the signal intensities were quantified by the computerized Gel-Pro (version 3.1 for window 3). The ratio between the levels of the target gene amplification product and the  $\beta$ -actin (internal control) was calculated to normalize for initial variation in sample concentration as a control for reaction efficiency (Raben, et al., 1996).

The proximate chemical analysis of feed was determined according to A.O.A.C. (1990) and the data were subjected to one way analysis of variance using SPSS program and significant differences were verified by Duncan's new multiple range test (Duncan, 1955).

# 3. Results and discussions

## 3.1 Feed restriction period

## 3.1.1 Live body weight

At the beginning of the trial, the rabbits of both groups fed ad-libitum or restricted feed ( $T_1$  vs. others) showed a similar LBW (Table 3) but at the end of the 60% restricted period, LBW of rabbits fed ad-libitum was significantly higher than the restricted ones (at 49 days old). When restricted rabbits were fed freely (after 49 days old) we observed restricted group showed a compensatory growth that induce a progressively decrease in the differences between both groups which resulted insignificant differences in LBW (at 84 days old). A compensatory growth following growth retardation resulted in insignificant differences in BW at the end of experiment (Szendro et al., 1989; Scholkaut and Lange, 1990 and Perrier, 1998).Adding CuSO4 (0.1g/Lwater), NaHCO3 (10g/Lwater) or mixed induced slightly improvement for LBW at 49 days of age ( $T_2$  vs.  $T_3$ ,  $T_4$  or  $T_5$ ). The same result was observed in final LBW (at 84 days of age).

## 3.1.2 Weight gain

During the restricted period, the values of TWG in restricted rabbits was lower than in full-feed rabbits, but after the next 4 weeks of restriction, the control group recorded the lowest value of TWG compared to others with significant differences. Maertens and Peeters (1988) reported high weight gain in the first two weeks after restriction. In rabbits during restriction period, we observed a significantly reduction in DWG for groups subjected to FR, but when restricted rabbits were fed freely after 49 days old we found a significant compensatory growth which increased with the feed restriction applied (Table3). In this connection, Perrier and Ouhayoun (1998); Perrier (1998). Dalle-Zotte et al.(2005) found that after a quantitative restriction, a compensatory growth was previously found in the young rabbit. Also, Gidenne et al.(2003) found with a 80% of FR from weaning to 49 days of age, found significant differences in DWGs for ad-libitum and restricted groups. During the FR period, the groups received CuSO<sub>4</sub> and/or NaHCo<sub>3</sub> recorded insignificant improvement in TWG and DWG for T2, but TWG and DWG during 49-84 days of age in which the improvement was not significantly affected. All groups were nearly similar.

## 3.1.3 Feed intake and feed conversion

In growing rabbits, FI and DFI (Tables 3&4) of restricted groups were significantly lower than the control ad-libitum group during the restricted period. While, at flushing period (49-84 day old) FI and DFI values were slightly higher for Res group compared to control. In this respect, Szendro et al (1989), Jerome et al. (1998) reported lower feed consumption in restricted rabbits compared to in rabbits fed ad-libitum. Regarding to the effect of adding either CuSO<sub>4</sub> or NaHCo<sub>3</sub> during the period 28-49 days old, feed intake was restricted. But after the restricted period (49-84), FI decreased with no significant as a result of CuSO<sub>4</sub> or NaHCo<sub>3</sub> addition (T<sub>2</sub> vs. T<sub>3</sub>, T<sub>4</sub> or T<sub>5</sub>).

Feed conversion efficiency was significantly better for AL-group than the RES-group, whereas, adding the tested additives ( $T_3$ ,  $T_4$  or  $T_5$ ) caused an improvement in the efficiency of FCR compared to  $T_2$  during the first 3 weeks of the trial. Starting from 49 day due to compensation growth, FCR was more favorable for restricted groups. During the whole period, no difference was observed between  $T_1$  and  $T_2$  but an improvement was detected due to

tested additives. Similar results were reported by Scholaut and Lange (1990) who reported that feed consumption and feed efficiency were not significantly affected by restriction treatments.

## 3.1.4 Growth performance index (PI)

Performance index values in Table (4) showed that PI in RES-rabbits during restricted period was significantly lower than those fed ad-libtum This may be due to the lower FI, BWG and the higher FC, whereas, no significant effect was noted among RES-groups, but during the whole period, the results were nearly similar between AL-group ( $T_1$ ) and RES-group ( $T_2$ ). However, the tested additives significantly improved PI %, this result due to the realimenation period, growth accelerated and compensatory growth achieved full-recovery, the next-5-weeks after restriction. However, we have to take into account that at environmental temperatures of 32°C, heat stress occurs, leading to production losses. El-Raffa (2004) showed that when temperature of 35°C and higher persist, the greatest losses from heat stress may result. Therefore, FR for rabbits must be considered as a stress condition and applied with other stressors occur (Bovera et al. 2008).

In respective to CuSO<sub>4</sub> addition, the results showed that adding CuSO<sub>4</sub> to drinking water of NZW growing rabbits is a suitable and effective for most of performance traits, which may be attributed to the promoting, ability of copper to growth (Liang et al, 1988 and Bassuny, 1991) and its protective effect against enteritis (Grobner et al., 1986). In addition, copper acts mainly by its antimicrobial action in the gut of animals and had a role in metabolism and absorption of iron and hemoglobin which are reflected on the BWG (Shurson et al. 1987). Also the mode of action of copper is by reducing the wall thickness of the ceacum of rabbits that facilities the uptake of nutrients and consequently improves daily and FBG,s (King, 1975 and Omole, 1977). Contrary to what expected, this effect was not associated with an improvement of the FC. Reversely, a clear compensatory growth was observed when rabbits were fed freely. On the other hand, the improvement with NaHCO<sub>3</sub> may be attributed to that KHCO<sub>3</sub> (as appetizer) supplement to rabbits diets may stimulate the appetite, increase fiber digestibility and improve feed efficiency (Abdel-Samee et al, 2003). Similar results were obtained by Marai et al. (1994) who found that 1.25 or 2.5% NaHCo<sub>3</sub> improved GPs, rectal temperature, respiration rate and blood components due to correcting acid -base balance disturbances, such under stress conditions. However, NaHCo<sub>3</sub> addition to drinking water of stressful rabbits had controversial suggestions, e.g. Teeter et al. (1985) found NaHCo<sub>3</sub> could increase respiratory alkaloids severity, while Haydon and West (1990) suggested that the improvement resulted from adding NaHCo<sub>3</sub> to heat –stressed rabbits probably due to increase blood buffering capacity.

## 3.1.5 Carcass traits

Data of the present study revealed that most values of carcass traits were significantly affected by FR. The results in Table (5) revealed that AL-group had no effect in dressing and hot carcass weight percentages compared to RES-group ( $T_1$  vs.  $T_2$ ), while adding either CuSO<sub>4</sub> or/and NaHCo<sub>3</sub> ( $T_3$ , $T_4$  or  $T_5$ ) in drinking water increased dressing and carcass %. These results were in accordance with the results of Ferreira and Carregal (1996), Tumova et al.(2003) and Boisot et al.(2004) who reported that restriction did not affect dressing % but in constant with the results of Combes et al.(2003). However, the internal organs were significantly affected with exception kidney and liver between restricted and ad-libitum fed rabbits. Similar results were reported by Perrier and Ouhayoun (1996) and Tumova et al(2003). Moreover, the tested additives had no clearly effects on internal organs. The effect of feed restriction on abdominal fat seems not to be clear and this result coincides with Combes et al.(2003) and Gidenne et al. (2009) who showed no clear effect of FR on perirenal fat proportion.

## 3.2 Gene expression Patterns

The gene transcripts (mRNAs) of the four genes apo A-I, CETP, hepatic lipase and IGF-1 were successfully detected in liver tissues within all groups (Fig. 1). The gene expression was normalized with the expression values of the  $\beta$ -Actin gene. The results revealed that apo A-I, mRNA expression in the liver tissues of the group fed with FR diet was significantly decrease (P <0.05) than the control group. These observations extend our previous studies that show that apolipoprotein gene expression is much more susceptible to regulation in the liver. The same results observed by Staels et al. (1992); Richards et al. (2003) which found apoA1 gene expression and hepatic apolipoprotein declined during restricted breeders chickens. The decrease in apoA1 mRNA steady-state levels is associated with a lowered transcription rate for the gene These results suggested that feed restriction affect in apolipoprotein production at a posttranscriptional or translational level. Meanwhile Mooradian et al. (2006) suggested that reduction in the expression of apo A-I may be due to a reduction in total dietary.

On the other hand, adding either  $CuSO_4$  and /or NaHCO<sub>3</sub> in drinking water insignificantly increased the hepatic expression of Hepatic apo A-I when compared to FR group and significantly decreased (P<0.05) when compared to control group. Similar results observed by Hoogeveen et al. (1995) which noticed that copper deficiency

increases hepatic apo A-I synthesis and secretion but does not alter hepatic total cellular apo A-I mRNA abundance. Thus, the enhanced hepatic apo A-I synthesis observed in copper-deficient cells may have resulted from alterations in posttranscriptional and translational processes.

The hepatic lipase gene expression insignificantly decreased by FR similar results observed by Wade et al.(2002) which noticed that decrease in body weight as a result decrease in hepatic lipase gene expressions. While adding either  $CuSO_4$  and /or NaHCO<sub>3</sub> in drinking water slightly increased the hepatic lipase expression and this increase insignificantly when compared to FR group and insignificantly decreased when compared to control group. The hepatic cholesteryl ester transfer protein (CETP) gene expression insignificantly decreased by FR similar results observed by Wood et al. (2006) which noticed that decrease in body weight as a result decrease in hepatic CETP gene expressions. While adding either  $CuSO_4$  and /or  $NaHCO_3$  in drinking water slightly increased the hepatic lipase expression. While adding either  $CuSO_4$  and /or  $NaHCO_3$  in drinking water slightly increased the hepatic lipase expression and this increase insignificantly when compared to FR group and insignificantly decreased when compared the hepatic lipase expression and this increase insignificantly when compared to FR group and insignificantly decreased when compared to control group.

Feed restriction lead to weight loss result in reliable and dramatic changes in lipoprotein metabolism characterized by decreased triglycerides and remodeling of LDL and HDL cholesterol to form larger particles (Volek & Feinman 2005; Wood et al., 2006). Since weight loss has similar effects, we surveyed various apolipoproteins and enzymes regulating triglyceride and lipoprotein metabolism including CETP and apolipoprotein A-I. Our study show an association of a polymorphism in CETP gene to weight loss. The finding suggests that the weight loss response to feed restriction may be mechanistically linked to the intravascular processing of lipoproteins.

In addition, expression of IGF-I was influenced in a similar manner by feed restriction, whereas Hepatic IGF-I expression significantly (p<0.05) decreased by feed restriction when compared by control similar results observed by Brameld et al. (2000) which reported that the Fr decreased the expression of IGF-I and refeeding increased the amount of IGF-I mRNA in hepatic fetal sheep after maternal. Kobayashi et al. (2002) decided that feed restriction decreased the hepatic expression in dairy cows nutrient restriction. Similar result noticed by Connor et al. (2010) in beef cattle. While adding either  $CuSO_4$  or/and  $NaHCo_3$  in drinking water insignificantly increased the hepatic expression of IGF-I than in FR group. On the other hand, adding either  $CuSO_4$  or/and  $NaHCo_3$  in drinking water significantly (p<0.05) decreased when compared to control group.

## 4. Conclusions

It can be concluded that under the condition of the study, feed restriction for rabbits have to be considered as a possible methods to reduce FI, quantitative RT-PCR can be a useful tool in studying genetic adaptations to acute dietary challenges. Using this technique, we found a very rapid change in hepatic gene expression also, FR improving growth performance and a better control of the quality of the carcasses. Moreover, adding either  $CuSO_4$  or/and NaHCO<sub>3</sub> in drinking water for growing rabbits is very useful, more practical for alleviation of heat load, reduced FC and improved most GP traits.

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| Primer name | Primer sequence (5 –3 )         | Annealing<br>temperature (°C) | PCR product<br>size (bp <u>)</u> |
|-------------|---------------------------------|-------------------------------|----------------------------------|
| ß-actin     | CGTGACATCAAAGAGAAGCTGTGC        | 64                            | 376                              |
|             | GCTCAGGAGGAGCAATGATCTTGAT       | 04                            |                                  |
| IGF         | AAGCCTACAAAGTCAGCTCG-           | 57                            | 114                              |
|             | GGTCTTGTTTCCTGCACTTC            | 51                            |                                  |
| СЕТР        | ATGGAATTCACACCATCTCCAACATCATGGC | 60                            | 445                              |
|             | TTCTGGCAGGAGATCTTGGGC           | 00                            |                                  |
| Hepatic     | ATGGAATTCTCCAGCCTGGCTGCCACTTC   | 60                            | 300                              |
| lipas       | TTGAAGGGGGACTGGGCTCG            | 00                            |                                  |
| apoA-1      | CGAGGAGGTCGAGGCCTAC             | 59                            | 360                              |
|             | TCCTCGGCCACAACCTTTAG            |                               | 500                              |

Table 1. Summary of the primers performed in PCR amplification

| INGREDIENTS                 | %     |  |  |  |  |
|-----------------------------|-------|--|--|--|--|
| Yellow corn                 | 32.0  |  |  |  |  |
| Wheat bran                  | 20.0  |  |  |  |  |
| Soybean meal (44%)          | 18.0  |  |  |  |  |
| Wheat straw                 | 12.0  |  |  |  |  |
| Alfalfa hay                 | 5.0   |  |  |  |  |
| Rice bran                   | 5.0   |  |  |  |  |
| Linseed straw               | 2.8   |  |  |  |  |
| Sunflower meal              | 2.5   |  |  |  |  |
| Lime stone                  | 2.0   |  |  |  |  |
| Sodium chloride             | 0.3   |  |  |  |  |
| Vitamins and minerapremix * | 0.3   |  |  |  |  |
| DL-Methionine               | 0.1   |  |  |  |  |
| Total                       | 100   |  |  |  |  |
| Calculated analysis:        |       |  |  |  |  |
| DE(Kal/Kg)                  | 2463  |  |  |  |  |
| CP %                        | 16.35 |  |  |  |  |
| CF %                        | 11.54 |  |  |  |  |

Table 2. Ingredients and chemical analysis of the experimental diet

\*Vitamins and minerals premix per kilogram contains: Vit. A,10.000 IU; Vit.D3, 900IU; Vit.E,50.0mg; Vit.K,2.0mg; Vit.B1,2.0mg; Vit.B 2,6.0mg; Vit.B6,2.0mg; Vit.B12,0.01mg; Biotin,0.2mg; Choline, 1200 mg; Niacine, 50mg; Zinc,70mg; Cu.,0.1mg; Mn.,8.5mg; Fe.,75.0mg; Folic acid,5mgand Pantothenic acid, 20.0mg. \*\* According to NRC (1977) for rabbits.

| ITEMS                                   | TREATMENTS  |                          |  |                          |                          |  |  |
|---|---|--------------------------|--|--------------------------|--------------------------|--|--|
|   | Control F. R.   |                          | F. R.+ CuSo <sub>4</sub>                         | F. R.+NaHCo <sub>3</sub> | F. R. + Mix              |  |  |
| Live body weight (g)                    |   |                          |  |                          |                          |  |  |
| 28 day of age                           | 1121±23.89  | 21±23.89 1127±22.48      |  | 1122±25.59               | 1117±23.79               |  |  |
| 49 day of age                           | 1622 <sup>a</sup> ±35.46  | 1372 <sup>b</sup> ±29.00 | 1437 <sup>b</sup> ±26.82                         | 1403 <sup>b</sup> ±21.98 | 1394 <sup>b</sup> ±21.15 |  |  |
| 84 day of age                           | 2422±49.38  | 2455±40.35               | 2478±30.17 2483±52.04                            |                          | 2466±47.87               |  |  |
| Body weight gain (g)                    |   |                          |  |                          |                          |  |  |
| 28 - 49 day of age                      | - 49 day of age 501 <sup>a</sup> ±23.30 245 <sup>b</sup>  |                          | 311 <sup>b</sup> ±33.89                          | 281 <sup>b</sup> ±27.96  | 277 <sup>b</sup> ±22.73  |  |  |
| 49 - 84 day of age                      | day of age 800 <sup>b</sup> ±40.82  |                          | 1041 <sup>a</sup> ±43.25 1080 <sup>a</sup> ±56.1 |                          | 1072 <sup>a</sup> ±54.08 |  |  |
| 49 - 84 day of age                      | 1301±54.40  | 1328±46.44               | 1352±35.82                                       | 1381±45.11               | 1349±50.15               |  |  |
| Daily weight gain (g / day)             |   |                          |  |                          |                          |  |  |
| 28 - 49 day of age                      | 8 - 49 day of age 23.86 <sup>a</sup> $\pm 1.11$ 1   |                          | $14.81^{b} \pm 1.61$                             | 13.39 <sup>b</sup> ±1.33 | $13.18^{b} \pm 1.08$     |  |  |
| 49 - 84 day of age $14.29^{b} \pm 0.73$ |   | 19.35 <sup>a</sup> ±0.87 | 18.59 <sup>a</sup> ±0.77                         | 19.29 <sup>a</sup> ±1.00 | 19.15 <sup>a</sup> ±0.97 |  |  |
| 49 - 84 day of age                      | 49 - 84 day of age 16.90±0.71 1   |                          | 17.56±0.47                                       | 17.68±0.59               | 17.52±0.65               |  |  |
| Total feed intake (g)                   |   |                          |  |                          |                          |  |  |
| 28 - 49 day of age                      | $28 - 49 \text{ day of age}  1785^{a} \pm 24.21  1365^{a} \pm 24.21  1365^$   |                          | 1365 <sup>b</sup> ±00.0                          | 1365 <sup>b</sup> ±00.0  | 1365 <sup>b</sup> ±00.0  |  |  |
| 49 - 84 day of age                      | 49 - 84 day of age 3529±236.75 39   |                          | 3785±106.36                                      | 3901±189.21              | 3701±180.38              |  |  |
| 49 - 84 day of age                      | 5314±253.47   | 5346±214.74              | 5150±106.36                                      | 5266±189.21              | 5066±180.38              |  |  |
| Daily feed intake (g/day)               |   |                          |  |                          |                          |  |  |
| 28 - 49 day of age                      | $28 - 49 \text{ day of age}  84.98^{a} \pm 1.15  65.00^{b} \pm 0.00  65.00^{b} \pm 0.00^{b} \pm 0.0$ |                          |  |                          | $65.00^{b} \pm 0.00$     |  |  |
| 49 - 84 day of age                      | ay of age 63.03±4.23 71.09±3.83   |                          | 67.58±1.58                                       | 69.66±3.38               | 66.09±3.22               |  |  |
| 49 - 84 day of age                      | - 84 day of age 69.01±3.29 69.43±2.79   |                          | 66.88±1.38                                       | 68.39±2.46               | 65.79±2.34               |  |  |

# Table 3. Performance of NZW rabbits supplemented with CuSo<sub>4</sub> and NaHCo<sub>3</sub> and subjected to feed restriction

a, b. Means with different superscript in the same row for the same item differ significantly (p <0.05).

| ITEMS                                   | TREATMENTS               |                           |                          |                           |                          |  |  |
|---|--------------------------|---------------------------|--------------------------|---------------------------|--------------------------|--|--|
|   | Control                  | F. R.                     | F. R.+ CuSo <sub>4</sub> | F. R.+NaHCo <sub>3</sub>  | F. R. + Mix              |  |  |
| Feed conversion ratio (g feed / g gain) |                          |                           |                          |                           |                          |  |  |
| 28 - 49 day of age                      | $3.61^{b}\pm 0.15$       | 6.40 <sup>a</sup> ±0.93   | $4.86^{ab}\pm 0.56$      | 5.24 <sup>ab</sup> ±0.51  | $5.30^{ab} \pm 0.58$     |  |  |
| 49 - 84 day of age                      | 4.40 <sup>a</sup> ±0.15  | $3.68^{b} \pm 0.15$       | $3.68^{b} \pm 0.18$      | 3.46 <sup>b</sup> ±0.15   | $3.46^{b}\pm 0.08$       |  |  |
| 49 - 84 day of age                      | 4.08 <sup>a</sup> ±0.6   | $4.02^{ab}\pm 0.05$       | $3.82^{bc} \pm 0.07$     | 3.87 <sup>c</sup> ±0.07   | $3.76^{\circ}\pm0.06$    |  |  |
| Performance index % ( PI)               |                          |                           |                          |                           |                          |  |  |
| 28 - 49 day of age                      | 45.81 <sup>a</sup> ±2.87 | 25.06 <sup>b</sup> ±3.52  | $33.16^{b} \pm 4.01$     | 29.08 <sup>b</sup> ±3.10  | $28.38^{b}\pm 2.50$      |  |  |
| 49 - 84 day of age                      | 55.39 <sup>b</sup> ±1.58 | 67.43 <sup>a</sup> ±2.57  | 68.64 <sup>a</sup> ±3.69 | 69.25 <sup>a</sup> ±3.28  | 71.65 <sup>a</sup> ±2.42 |  |  |
| 49 - 84 day of age                      | 59.46 <sup>a</sup> ±1.38 | 61.19 <sup>ab</sup> ±1.17 | 65.16 <sup>b</sup> ±1.88 | 64.37 <sup>ab</sup> ±1.90 | 65.76 <sup>b</sup> ±1.82 |  |  |

a, b, c. Means with different superscript in the same row for the same item differ significantly (p < 0.05).

|                      | TREATMENTS          |                    |                     |                      |                    |  |
|----------------------|---------------------|--------------------|---------------------|----------------------|--------------------|--|
| ITEMS                | Control             | F. R.              | F. R.               | F. R.                | F. R.              |  |
|                      |                     |                    | $+ CuSo_4$          | + NaHCo <sub>3</sub> | + Mix              |  |
| Live hade weight     | 2450                | 2400               | 2166                | 2416                 | 2250               |  |
| Live body weight     | $\pm 104.08$        | $\pm 76.38$        | $\pm 60.09$         | ±72.65               | ±160.72            |  |
| Dragging noroontogo  | 60.33 <sup>ab</sup> | 58.97 <sup>b</sup> | 61.57 <sup>a</sup>  | 61.67 <sup>a</sup>   | 62.77 <sup>a</sup> |  |
| Dressing percentage  | ±0.55               | ±0.81              | $\pm 0.88$          | ±0.84                | ±0.73              |  |
| Hat approach the 0/  | 50.84 <sup>b</sup>  | 50.23 <sup>b</sup> | 52.49 <sup>ab</sup> | 52.53 <sup>ab</sup>  | 53.86 <sup>a</sup> |  |
| Hot carcass weight % | ±0.57               | ±0.85              | ±0.90               | $\pm 0.88$           | ±0.50              |  |
| Shin maight 0/       | 16.53 <sup>a</sup>  | 14.50 <sup>b</sup> | 15.63 <sup>a</sup>  | 15.83 <sup>a</sup>   | 15.70 <sup>a</sup> |  |
| Skin weight %        | ±0.27               | ±0.35              | ±0.22               | ±0.33                | ±0.40              |  |
| Les maisht 0/        | 3.30 <sup>b</sup>   | 3.80 <sup>a</sup>  | 3.40 <sup>ab</sup>  | 3.40 <sup>ab</sup>   | 3.73 <sup>ab</sup> |  |
| Leg weight %         | ±0.15               | ±0.06              | ±0.15               | ±0.17                | ±0.12              |  |
| Used weight 0/       | 6.70 <sup>a</sup>   | 5.23 <sup>b</sup>  | 5.70 <sup>ab</sup>  | 5.60 <sup>ab</sup>   | 5.43 <sup>b</sup>  |  |
| neau weight %        | ±0.09               | ±0.09              | ±0.15               | ±0.21                | ±0.20              |  |
| Liver weight 0/      | 2.57                | 2.53               | 2.40                | 2.57                 | 2.56               |  |
| Liver weight %       | ±0.67               | ±0.07              | ±0.15               | ±0.12                | ±0.9               |  |
| Heart weight 0/      | 0.25 <sup>b</sup>   | 0.35 <sup>a</sup>  | 0.41 <sup>a</sup>   | 0.41 <sup>a</sup>    | 0.39 <sup>a</sup>  |  |
| Heart weight 76      | ±0.01               | ±0.03              | ±0.02               | ±0.01                | ±0.01              |  |
| Vidnova waight 0/    | 0.61 <sup>a</sup>   | 0.61 <sup>a</sup>  | 0.56 <sup>ab</sup>  | 0.56 <sup>ab</sup>   | 0.51 <sup>b</sup>  |  |
| Kiuneys weight %     | ±0.02               | ±0.02              | ±0.01               | ±0.02                | ±0.02              |  |
| Abdominal fat 0/     | 1.18 <sup>a</sup>   | 0.95 <sup>b</sup>  | 0.90 <sup>b</sup>   | 0.99 <sup>b</sup>    | 0.96 <sup>b</sup>  |  |
| Addominal lat %      | ±0.01               | ±0.01              | ±0.02               | ±0.05                | ±0.03              |  |

Table 5. Carcass traits of NZW rabbits supplemented with CuSo4 and NaHCo3 and subjected to feed restriction

a, b. Means with different superscript in the same row for the same item differ significantly (p < 0.05).



Figure 1. The mean of ratio between hepatic gene expression /  $\beta$ -actin after 49 day of feed restriction of rabbit where, (IGF-1) insulin-like growth factor-I, (CETP) cholesteryl ester transfer protein, (HL) hepatic lipase, (apoA1) apolipoprotein