Benha University
Faculty of Veterinary Medicine
Department of Animal Hygiene,
Behavior and Management

Behavior and performance of veal calves in relation to group housing

PhD Thesis Presented
By
ESSAM MAHMOUD MEIZAR ABDEL-FATTAH

B.V.Sc. Faculty of Veterinary Medicine, Benha University, 2005

M.V.Sc. Animal, Poultry Behavior and Management, 2008

Under Supervision of

Prof. Dr. Mohamed Moursy Ramadan Karousa

Professor of Animal & Poultry Behavior and Management and Vice Dean for community affairs and enviromental development, Faculty of Vet. Med., Benha University.

Prof. Dr. Gaafar Mohmoud El-Gendi

Professor of Poultry Management , Animal Production Department, Faculty of Agri., Benha University.

Prof. Dr. Susan Eicher

Professor of Animals physiology, USDA-ARS Livestock Behavior Research Unit, Purdue University. USA

Dr. Essam Ali Mahmoud

Assistant Professor of Animal & Poultry Behavior and Management, Faculty of Vet. Med., Benha University.

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ABSTRACT

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A study was conducted to investigate the effect of group size on behavior, health, growth, welfare and innate immunity of veal calves during the finishing period. The study was carried out in spring and summer of 2012 at one commercial veal farm. Holstein-Friesian bull calves (n = 168; 44 ± 3 d of age) were assigned randomly to 1 of 3 treatments of group housing with 2, 4, or 8 calves/pen. The pens used for housing were 3 × 1.20 m (2 calves/pen), 3 × 2.40 m (4 calves/pen), and 3 × 4.80 m (8 calves/pen), supplying a total pen space allowance of 1.82 m^2 /calf, regardless of pen size. Behavior was recorded from video data throughout the day from 0700 to 1900 h, during a single day each month for 5 mo using scan sampling every 5 min within 30-min observation sessions. On d 0, 1, 5, 14, 42, and 70 after grouping, continuous focal sampling around feeding time (30-min intervals before, during, and after feeding) focused on oral and aggressive behavior. Plasma cortisol, blood hemoglobin concentrations and differential leukocyte counts were determined and mRNA expression of interleukine-1β (IL-1β), IL-1 receptor antagonist (IL-1Ra), tumor necrosis factor (TNF)- α, toll-like receptor 4 (TLR4) and tachykinin 1 (TAC1) was determined using real-time RT-PCR in calves blood leukocytes. Health was evaluated monthly. Calves housed in large groups (4 or 8 calves/pen) showed more ($P \leq 0.001$) conspecific contact, walking, and standing, and less ($P < 0.001$) manipulation of objects, self-licking, and lying when compared to calves housed in small groups (2 calves/pen). Group size had no effect on play

behavior (P = 0.11) throughout the experiment. During feeding times group size had no ($P \geq 0.07$) effect on any behavioral patterns except for duration of conspecific contact ($P< 0.01$). Aggression at feeding time was not ($P > 0.23$) affected by treatment. Group size treatments were similar for hip height change (P = 0.41) and heart girth change (P = 0.18) over the duration of the experiment; however, both hip height and heart girth increased (P = 0.001) with calf age. During month 1, calves in groups of 8 or 4 coughed more than calves in groups of 2, whereas calves in groups of 8 coughed more than calves in groups of 4 or 2 in month 2. Furthermore, during month 4, calves in groups of 8 had less nasal discharge than calves in groups of 2 or 4 (treatment × month, P = 0.02). Ocular discharge, ears, and fecal scores did not differ ($P \geq 0.05$) among treatments. Neither plasma cortisol nor blood hemoglobin were not ($P \geq$ 0.37) affected by group size. Calves housed in groups of 8 tended to have greater neutrophil percentage (P = 0.09), neutrophil to lymphocyte ratio (P = 0.06), and had lower lymphocyte percentage (P = 0.06) than those housed in groups of 4 or 2. On the 1st month after grouping, veal calves housed in groups of 8 had greater expression of IL-1β mRNA and tended to have greater TAC1 mRNA expression than calves housed in groups of 4 and 2. In conclusion, the number of veal calves in a group when given the same space did not affect production and physiological indicators of welfare but had a transient effect on health. There is immunological evidence of stress from group housing of calves in groups of 8 when compared to those kept in groups of 4 or 2. Therefore, these data suggest that housing of veal calves in larger groups during the 5-months fattening period may lead to greater incidence of respiratory disease.

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List of abbreviation

Abbreviation	Definition
ACTH	Adrenocorticotropic hormone
ADG	Average Daily Gain
BW	Body Weight
cDNA	Complementary DNA
CP	Crude Protein
CT	Cycle Threshold
D	Day
DNA	Deoxy-nucleic acid
H	Hour
Hb	Hemoglobin
IL-1β	Interleukin 1 Beta
Min	Minute
Mo	Month
mRNA	Messenger RNA
OD	Optical Density
PBS	Phosphate Buffer Saline
qRT-PCR	Quantitative Reverse transcription Polymerase Chain Reaction
RNA	Ribonucleic acid
SE	Standard Error
TAC1	Tachykinin1 or Substance P
TLR4	Toll-Like Receptor4
TNF-α	Tumor Necrosis Factor-Alpha
WBCs	White Blood Cells
Wk	Week
18S rRNA	18S ribosomal RNA

INTRODUCTION

INTRODUCTION

The optimal environment for housing newborn and growing dairy calves should provide thermal, physical, psychological, and behavioral comfort **(Stull and Reynolds, 2008).** A majority of veal farms utilize individual stalls or pens, which is believed to be beneficial because of easy observation of each calf, effective management and handling of waste materials, limited cross contamination with pathogens among calves, and possible prevention of cross-sucking. Housing in single pens or hutches prevents calves from having physical contact with conspecifics. individual isolation, where calves are prevented from physical as well as visual contact with other calves, will influence their social development and affect their behavior thereafter **(Bøe and Færevik, 2003). The American Veal Association (2007)** called upon all U.S. veal producers to transition to group-housing by December 31, 2017 to address animal well-being pressures. From a welfare perspective, group housing is preferable to individual housing, because it enables calves to run and play **(Jensen et al., 1998; Babu et al., 2004)**, and have full social interactions with other calves **(Svensson and Liberg, 2006).** However, calves housed in groups may develop cross-sucking behavior **(de Passille, 2001; Jensen, 2003; Jensen and Budde, 2006)**, experience chronic social stress **(Veissier et al., 1998),** have higher risks of infection **(Svensson and Liberg, 2006),** and experience greater displacements from the feed trough **(Færevik et al., 2007).**

Grouping calves is an issue common to the dairy heifer, dairy beef, and veal industries. Group housing of veal calves may represent a source of chronic stress for the calf **(Hulbert and Ballou, 2012).** Immune status of animals under chronic stress can be compromised thereby reducing

disease resistance and welfare, and may even lead to death **(Muir and Woolf, 2001).**

Very little is known about how grouping of veal calves will influence innate and adaptive immune response. Traditionally, assessment of physiological stress has been estimated by measuring levels of adrenal hormones. However, there are associated drawbacks, such as the potential for rapid changes in hormone levels as a response to short-term acute stressors. Real-time PCR has been developed in recent years for the quantitation of several genes, and this method provides a simple and the rapid analysis for quantitation of cytokine expression associated with stress in cattle **(Satoru et al., 2003).** A marker of chronic pain that influences immunity will be useful in evaluating housing to improve animal welfare. Presently, no study has assessed the physiological leukocyte markers of stress that we propose to measure on a well-controlled studies of group housing for calves in North America. Peripheral blood leukocytes provide a broader picture of inflammatory responses during exposure to stressors. We will use mRNA to monitor the genomic transcription in leukocytes under the effect of group housing.

Objectives of study

Overall, information regarding group size and veal calf well-being is scarce and many questions not answered yet for this practice; therefore, the objectives of the present study were1) to explain the effect of group size on behavior, health, growth, and welfare of veal calves, 2) to investigate the effect of group size on immune system, and 3) to evaluate leukocyte gene expression of major pro- and anti-inflammatory cytokines mRNA of veal calves housed in different group size during the fattening period.

Hypothesis of study

We hypothesized that calves housed in larger groups will display more aggression, reduced growth performance, will have impaired immune function, greater incidence of diseases, altered expression of cytokines mRNA (IL-1β, IL-1Ra and TNF-α), toll-like receptor 4 (TLR4), and neurotransmitter associated with pain (TAC1) than those housed in smaller group.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I- Effect of group size on behavior and performance of veal calves

1.1 Grouping of young calves

Concern about animal welfare in U.S.A and worldwide is growing for both consumers and producers. Increasingly consumers are concerned about production practices that compromise the welfare of production animals, restrict normal behaviors, or create undue stress on the animals. Producers have similar concerns, and also understand that good animal welfare can enhance production efficiency and competitiveness. Several questions are not answered yet for this practice and under specific circumstances. It is known that the cattle are highly gregarious animals and housing them in groups rather than individually can improve their welfare **(Bouissou et al., 2001).** Group living is associated with both costs and benefits for the individual animal. Benefits of group life are discovering food sources more easily and this may explain higher feed intake in social groups of calves **(Warnic et al., 1977; Chua et al., 2002; Babu et al., 2004; Phillips, 2004).** Group living animals show less fear when group mates are present, and thus they can spend more time on vital activities such as resting and grooming **(Hopewell et al., 2005).** Studies showed that social grooming helps to maintain healthy fur on body parts that the individual cannot reach and the behavior itself has a calming effect on the animals by reducing the level of social tension which has been reported on cattle **(Sato, 1984; Sato et al., 1991).** In young animals, such as calves, group living also includes the opportunity to express play behavior, which stimulates motor and social skills that are crucial for

normal behavioral development **(Jensen et al., 1998; Babu et al., 2004).** The costs of group living can be measured in terms of competition for valuable resources such as food, water and attractive resting places **(Krause and Ruxton, 2002).** These costs can be minimized in different ways, for example by giving the animals the access to enough space and feeding places. Increased aggression connected with limited resources is also an important cost that must be taken into account. Providing a good environment and resources that fulfill everyone's individual needs can therefore reduce aggression in a group. Another cost of group housing systems is the increased susceptibility to disease transmission and subsequently increasing the relative risk for morbidity **(Losinger and Heinrichs, 1997; Svensson et al., 2000).** Therefore, a suitable animal housing method should be beneficial to both the well-being and performance of the animal. European Union **(Council Directive 97/2/EEC)** set requirements that calves reared individually should have social interaction with other calves, and calves older than 8 weeks is now compulsory to group-housed in order to satisfy calves' motivation for social contact **(Færevik et al., 2007).** In 2007, The American Veal Association's board of directors unanimously approved a policy that the veal industry fully transition to group housing by the end of 2017. Progress toward this goal has been substantial. In 2012, an American Veal Association (AVA) survey found that 70 percent of veal calves raised by AVA members would be group housed by the end of the year **(Smith, 2007)**. In general, keeping dairy calves in groups may provide a number of advantages to both producers and their calves. For calves, rearing calves in groups allows for early social interactions that are important in the development of normal social behavior **(Chua et al., 2002),** provides greater access to space, which, together with social contact, facilitates the expression of normal behavior **(Jensen et al.,**

1997). For producer, group housing is less labor intensive and therefore, less expensive compared to a single housing system **(de Passillé et al., 2004).** Group rearing can also reduce the labor of cleaning pens and feeding **(Kung et al., 1997).** On the other hand, an increase in disease transmission, stress of competition, and loss of individual observation could result in more morbidity and mortality, and lead to higher economic losses and arguably reduced animal welfare **(Cobb, 2012).**

In conclusion, group housing is important for young calves. Calves are motivated to seek the company of other calves and individual housing reduces the opportunities for social interaction. Behavioral problems claimed to be associated with group housing, that is, increased aggression, competition, and cross-sucking can be solved by appropriate management **(Rushen et al., 2008).**

1.2 Effect of group size on behavior

1.2.1 Ingestive behavior

The young ruminant naturally learns to take solid feed by allelomimicry, using its mother as a model, if available, or pen-mates. Calves housed in individual pens with solid sides have little opportunity for allelomimicry, and solid feed consumption may be delayed as a result. Grass intakes and time spent eating grass were greater for grouped calves than for individual calves **(Phillips, 2004).** In the experiment of **Færevik et al. (2007)**, the calves kept on 2.25m^2 total pen area for all group sizes (4, 8, and 16 calves/pen) and they found that group size had no effect on total feeding time. However, calves housed in larger groups (8 and 16 calves/pen) spent more time feeding with another calf (feed social) than calves housed in groups of four. While, **Jensen and Budde (2006)** showed that calves housed in groups of 6 were sucking milk faster than calves housed in groups of 2 (2.10 ± 0.13 vs. 3.03 ± 0.17 min/30-min

observation). They suggested that competition for milk was higher among calves in groups compared with calves in pairs which lead to higher rate of milk ingestion. A higher rate of ingestion, which may be taken as indicative of social constraint **(Nielsen, 1999)** has also been found in group-housed compared with individually housed calves when the milk was offered in a trough **(Babu et al., 2004).** Also, among calves fed from a computer-controlled milk feeder (in which the calves could not steal milk from each other but competed for access to the feeder), the rate of ingestion was higher in groups of 24 compared with groups of 12; the increased level of competition resulted in higher rate of milk ingestion and lower duration of time spent ingesting milk, however, did not affect the amount of milk ingested or the daily BW gain **(Jensen, 2004).** Furthermore, **De Paula et al. (2010)** found that paired calves spent more time at feeder, visited the feeder more often, and started ingesting concentrate more rapidly than did individually housed calves. In the experiment by **Richard et al. (1988),** milk replacer and water intakes of group-reared calves tended to be higher than that of individually fed calves but concentrate intake was not different. **Babu et al. (2004)** observed that group housed calves spent less time for milk sucking during morning and evening feeding with an average of 30.10 compared to 41.97 in individually reared ones. Quicker milk sucking time throughout the periods of observation in group housed calves was indicative of faster learning due to socialization, and also a sense of competitiveness than the individually reared calves.

1.2.2 rumination behavior

In the study of **Babu et al. (2004)** when calves offered milk replacer in bucket, they found that group housed calves spent more time ruminating while lying and observed ruminating early than individually

housed calves, and lying rumination showed an increasing trend with the age of calf. Also, **Phillips, (2004)** found that ruminating time was increased by offering grass to grouped calves compared with individual calves (333 vs. 248 min/d). Also for the effect of day period **Veissier et al. (1998)** mentioned that veal calves spent about 19.97% of the daytime doing chewing and rumination, and most of chewing occurred between milk meals. The time spent chewing and eating solid foods increased with age.

1.2.3 Abnormal oral behaviors

The veal calf is a young animal fed only milk or milk replacer and is usually slaughtered before 5 months. Calves are typically separated from their dams and artificially milk-fed for several weeks which do not satisfy the calf's motivation to suckle **(de Passillé, 2001).** Non-nutritive oral behaviors such as excessive sucking on body parts of other calves (cross-sucking) and on pen equipment such as walls or bars can be common in calves kept in groups **(Lidfors, 1993; de Passillé, 2001).** These activities are less frequent when veal calves receive some solid food. Also social deprivation is one of the factors which can cause abnormal oral behavior **(Veissier et al., 1997).**

Færevik et al. (2007) mentioned that group size had no effect on the occurrence of cross-sucking, and they found that calves in larger groups (16 calves/pen) performed more positive social interaction than smaller groups (4 and 8 calves/pen). Likewise, increasing group size could result in more social stimulation, as well as more disturbances. Also, **Jensen and Budde (2006)** found that group size (2 and 6 calves per pen) had no effect on cross-sucking and licking fixtures during the 30 min immediately after milk feeding. However, more calves in groups of 6 spent time on social grooming than groups of 2. However, **Veissier et al.**

(1998) observed that calves housed together in groups of 4 calves per pen spent more time on cross-sucking than calves in individual stalls.

In study of **Bokker and Koene (2001)** veal calves were assigned to three different housing systems: (1) individual housing (IH); group housing (GH) where calves kept in groups of 5-7 individuals after 8 weeks of age, and Peter's farm (PF) where calves kept in groups of 40-80 individuals from the start of fattening period with automatic feeding station. Over the whole fattening period calves in PF spent less time performing abnormal oral behavior than calves in IH and GH. In PF, calves lived in a social context from start of fattening period and most calf-directed behavior consisted of manipulating prepuce whereas in IH and GH it mainly consisted of manipulating other parts of the calves. In GH, most oral behavior in 30-min after milk feeding was consisted mainly of manipulating objects and in PF mainly of prepuce sucking while in IH, oral behavior was divided equally in manipulating calf and object.

Chua et al. (2002) found no differences between pair- and individually housed calves in amount of time spent in contact with pen. Moreover, **Babu et al. (2004)** found that the time involved in licking of inanimate objects and sucking of prepuce was significantly ($P < 0.01$) higher in group than individual housing both during 1 h before as well as after milk feeding. An increase in mutual sucking of mouth after milk feeding is presumably, because of stimulated temporary persistence of sucking behavior due to contact with milk. In conclusion, **Lidfors (1993)** reported that most of the mutual licking in group housing was directed towards mouth and ears (35-38%), scrotum (15%) and the rest towards prepuce, throat and other body parts. Whereas, Margerison **et al. (2003)** observed that most of the cross-sucking directed at the inguinal region

(78%), then the ear (8%), the mouth (6%), the throat (3%), the navel (2%), and other areas (4%).

1.2.4. Social interaction

As group housing lead to more social interaction among the calves, group housing has been developed as an animal friendly alternative for individual housing **(Veissier et al., 1998).** A study done by **Jensen et al. (1997)** shows that group rearing of calves makes it easier for them to develop a social behavior. Also they confirmed the importance of social interactions between calves in different ages. If calves are kept in groups it makes it possible for them to develop social bonds between each other.

1.2.5. Self-grooming

Veissier and Le Neindre (1994) described self-grooming as a comfort activity and concluded that self-grooming was less frequent in groups than in large stalls; calves in groups were also observed to be less clean than those in individual stalls. In contrast, **(Warnick et al., 1977; Dantzer et al., 1983; Bohus et al., 1987; Mason, 1993)** mentioned that behavioral indicators of stress may include excessive self- grooming, increased proportion of time spent resting or standing, and exhibition of stereotypic behavior.

Jensen and Budde (2006) found that group size had no effect on time spent self-grooming at milk feeding between pair-housed and group-housed calves**. Chua et al. (2002)** reported that there was no significant difference between treatments (individual vs. pair housing) for the amount of time spent in self-grooming. In contrast, **Bokker and Koene (2001)** mentioned that over whole fattening period of veal calves, self-grooming was significantly less in Peter's farm than in individual and group housing. **Mattiello et al. (2002)** found that the frequency of self-

grooming was higher around feeding times, and it decreased far from the milk meals. In contrast with results of **Veissier et al. (1997)** who found that veal calves spent around 5% of their time in this behavior far from the milk meals.

1.2.6. Aggression

Another issue associated with group housing is competition for milk. Calves in groups sometimes compete with pen mates. In one experiment using a simple teat-feeding system, they found that group-housed calves can displace one another from the milk teat many times each day if there are not enough teats **(von Keyserlingk et al., 2004).** However, giving each calf access to its own teat greatly reduced these displacements. This improved access to teats resulted in longer feeding times and increased milk intakes. **Kondo et al., (1989)** found that no correlation between group size and agonistic behavior in established groups of calves, but for heifers/cows there was a significant increase in agonistic interaction with group size. They also found that the occurrence of agnostic interaction were reduced as the space allowance per calf increased, which may indicate that sufficient space is of greater importance than group size in order to reduce the occurrence of agnostic behavior in calves.

Viesser et al., (1994) concluded that group housed calves showed less antagonistic behavior towards other calves compared to calves that previously had been housed in individual pens. When the calves were mixed at 14 weeks of age more antagonistic behavior were seen among calves that had been kept in single pens compared to group housed calves.

Jensen (2004) found that there was a higher level of competition for access to the feeder in groups with 24 calves and one computer-controlled milk feeder than in groups with 12 calves. When

competition for access to feed increases, the animals may adapt to this by feeding at a faster rate than preferred, feeding less than preferred, and by feeding at less preferred times of day.

Jensen and Budde (2006) observed the calves continuously for 30 min around milk feeding, they found that group size had no effect on percentage of calves bunting another at milk feeding throughout 6 weeks experimental period. The results suggested that the competition for milk was greater among calves in groups of 6 compared to calves in pairs.

Færevik et al. (2007) concluded that an increase in group size resulted in higher activity, fewer displacements from the feed barrier, more time spent resting and feeding in close proximity to other calves, and more positive social interactions between familiar calves. This suggests that the more tolerant social system that is reported in larger groups of other species also applies to young calves.

Telezhenko et al. (2012) found that number of displacements at the feed bunk was not affected by group size (6 vs. 12 calves/group). In addition to, **Færevik et al. (2007)** mentioned that the mean number of displacement from the feed barrier was higher in groups of 4 than in larger groups (groups of 8 and 16), but the difference was only significant on the first day after regrouping. Low level of aggression and rapid establishment of new social bound imply that regrouping of calves is acceptable from an animal welfare point of view.

1.2.7. Play behavior

Under natural or semi-natural conditions, play in calves is typically seen in a social context. Play behavior was categorized as locomotor play, social play, object play, or ground play. Locomotors play includes vigorous jumping, kicking, and running, often interrupted by fast stops

and turns in a new direction. Locomotor play is typically performed by several calves at the same time. Social play involves two or more individuals facing each other, pushing and bunting each other. Another type of social play is playful mounting. Finally, play behavior in calves also includes bunting and pushing objects, as well as ground play, where the calf rubs its neck and head against the ground while kneeling down **(Jensen and Kyhn, 2000).**

Compared to housing in small individual pens, group housing gives the calves' an opportunity to perform normal social behavior as well as play behavior **(Jensen et al., 1998; Babu et al., 2004)**. For these reasons, group housing is considered to improve calf welfare. Play is performed as a form of social contact, either in form of locomotion play or as social play, often in form of play fighting **(Vitale et al., 1986)**. The locomotion play often involves a group of calves and is carried out without physical contact. The locomotion play involves jumping, kicking and running. Social play on the other hand involves physical contact between two or several calves in form of non-reproductive mounting, pushing and bunting of each other. Play fights is often initiated by approach and rotation of the head

Sufficient space is essential for the expression of play behavior in domestic calves. Play is generally regarded as a positive indicator of health and well-being, and play may have several developmental benefits for the animal **(Spinka et al., 2001)**. An increase in the available space above minimum EU requirements which are 1.5 m^2 per calf increases the occurrence as well as the quality of locomotory play in calves **(Jensen and Kyhn, 2000; Jensen et al., 1998)**, and play is more likely to occur when calves are kept in groups **(Jensen et al., 1998)**.

Færevik et al. (2007) found that group size had no effect on the occurrence of play behavior. Social activities such as grooming and play behavior appeared to remain unchanged across group sizes.

1.2.8. Postures

In general, calves should rest adequately in either group or individual housing, provided that calf has sufficient space to adopt the important resting postures. Calves spent approximately 70% of their time lying down. Individually housed calves tended to spend slightly more time lying than paired calves, in part because the pair-housed calves spent more time standing inactive **(Chua et al., 2002)**. On the other hand, **DeWilt (1985)** suggested that a shorter lying time for calves housed in groups compared to being individually housed could be explained by the greater variety of stimuli, for examples, increasing group size result in more social stimulation, as well as more disturbances. While, **Wilson et al. (1999)** found that there were no consistent differences in postures or behaviors (e.g., lying, standing, chewing, tongue playing, grooming, and investigative activities) among calves in different housing designs or widths. Calves spent approximately 71 and 31% in lying and standing positions, with no reference for the right or left side while recumbent.

Færevik et al. (2007) found that time spent lying was greater to calves housed in groups of 4 and 8 than calves housed in groups of 16 and concluded that time spent lying decreased with increasing group size.

1.2.9. Locomotion

Group housing provides improved access to space that appears to be an important determinant for the expression of normal and naturally occurring locomotion **(Jensen et al., 1997)**. Confinement housing reduces expression of highly active movement in calves and results in impaired

locomotion. In study of **Chua et al. (2002)**, calves were recorded as moving for only 1% of the day, with pair-housed calves moving more than individually housed calves. Increased locomotion by the paired calves likely relates in part to the increased space available. Space allowance appears to be an important determinant for expression of normal and naturally occurring locomotion. Confinement housing reduces the expression of highly active movement in calves and results in impaired locomotion characterized by stumbling and falling in open field tests.

Telezhenko et al. (2012) found that group size had no effect on percentage of movement of cows and mentioned that pen size may be more important than density or group size and group-housed animals are able to share the space in the pen, such that even with the same number of animals per square meter and larger pens will provide more free space in which animals can move.

1.2.10. Inactivity

Jensen and Budde (2006) found that group size (4 vs. 6 calves/pen) had no effect on time spent inactive (standing or lying still) at milk feeding. However, **Færevik et al. (2007)** found that calves housed in groups of 16 were generally more active and spent less time standing inactive than calves housed in groups of 4 or 8. More incidences of calves standing inactive in the smaller groups could be a result of displaced animals waiting for access to the feed barrier. The increased activity of the calves in large groups cannot be explained by more social interactions (i.e. social grooming, social play, mounting or displacements). Therefore he suggests that the increased activity in larger groups could be explained by the fact that these calves are exposed to more social stimulation than

calves in smaller groups and that they move around more to avoid social conflicts.

Veissier et al. (1998) mentioned that between the milk meals, nibbling and eating remained at low levels, while chewing and inactivity occurred. Likewise, the time of inactivity decreased with age. It seems that the activity level of the calves varied according to a rhythm synchronized by milk distributions, rather than activities.

In study of **Bokker and Koene (2001)**, they found that activity was strongly affected by the two feeding times; activity was higher during feeding. While In study of **Chua et al. (2002)**, found that individually housed calves tended to spend slightly more time lying than paired calves, in part because the pair-housed calves spent more time standing inactive.

1.3 Effect of group size on performance (including growth, health, physiology and immunity)

1.3.1 Body weight and gain

In some experiments, there have been no differences in the average daily gain between group and individually reared calves **(Warnick et al., 1977; Nocek and Braund, 1986)**, but in some experiments the growth has been higher in individual pens than in groups **(Maatje et al., 1993)**. In contrast, **Xiccato et al. (2002)** found that the calves reared in groups showed higher final live weight than calves reared individually.in addition to **Andrighetto et al. (1999)** found that veal calves health, average daily gain and feed efficiency were similar between individual and group pen housing. However, group pen calves had higher ADG (1387 vs. 1317 g/d; $P< 0.1$) and better feed efficiency than individual housing in last 72 days of trail. Also, **Chua et al. (2002)**

found that pair-housed calves remained healthy and gained weight rapidly before and after weaning than individually housed calves. Indeed, he found no difference in weight gains between treatment groups except when calves were being weaned.

In regards to effect of group size, **Gottardo et al. (2005)** concluded that increasing from 3 to 7 the number of veal calves housed per pen doesn't impair their growth and slaughter performance as well as their meat color when space allowance is the same.

Svensson and Liberg (2006) found that calves housed in groups of 12-18 individuals had a reduced growth rate compared to calves that were held in groups of 6-9 individuals. The calves in large groups had approximately 40 g lower growth rate per day than calves in small groups. The calves in this study received a limited ration of whole milk or milk replacer and all the calves had free access to roughage and pelleted calf feed or rolled grain.

Færevik et al. (2007) mentioned that there was no significant effect of group size on average daily gain (group size 4: 707.5± 73.3; group size 8: 788.4± 58.2; group size 16: 867.8± 49.7 g/day, $P = 0.263$).

1.3.2 Body measurements

Shore and Roy (2011) reported that there was no difference in hip height between groups of dairy calves over 10 wk. trail (individual = 98.2 ± 1.3 cm; paired = 98.0 ± 2.4 cm; group of 6 = 97.2 ± 3.2 cm; group of 11 = 98.4 ± 3.1 cm), and also concluded that the number of calves in a group when given the same space did not affect growth parameters.

1.4. Effect of group size on health

Although individual housing is preferred method for reducing disease transmission between young calves, the research that has

examined health of calves housed individually or in groups has produced conflicting results **(Rushen et al., 2008)**. Diarrhea and respiratory illness are the most common diseases in young calves, and risk of the infection is higher in groups, because the calves are in close contact with each other **(Hepola, 2003).**

Webster et al. (1985) noted that the incidence of respiratory disease in calves was higher in group housed than individually housed (31% vs. 0%). However, other studies of veal calves reared in modern group housing **(Andrighetto et al., 1999; Xiccato et al., 2002)** reported very good health status and similar or improved growth rates compared to individual housing. Also, **Kung et al. (1997)** found fewer health problems on one farm when calves were kept in relatively large groups (12-15 calves) compared to calves kept in individual pens.

In a study of 122 dairy farm in Sweden **(Svensson et al., 2003)**; the young calves were kept individually, in small groups (3-8 calves fed milk manually) or large groups (6-30 calves fed with an automatic milk feeder). The incidence of diarrhea did not differ markedly among the types of housing. The incidence of respiratory disorders was twice as high in calves in large groups compared to calves in small groups or individual pens. In both cases of disease there was no difference between calves in small groups and those in individual pens. The authors suggested that the main disadvantage of the large groups was due to variation in calf age and the health effects reported in this study may not due to the group size per se.

In epidemiological surveys of pre-weaned U.S. and Swedish farms showed that calves kept in groups of more than 7 or 8 had higher morbidity and mortality and they concluded that housing young dairy calves in small groups is viable in terms of calf health **(Losinger and**

Heinrichs, 1997; Svensson et al., 2000). Most of researchers agree that calf immunity and the design and management of the housing systems, such as its cleanliness and ventilation, likely affect disease susceptibility more than group housing per se.

Svensson and liberg (2006) found that calves housed in pens for 12-18 calves had a higher incidence of respiratory illness than calves housed in groups of 6-9 calves. They detected no differences between calves kept in the small-sized versus the large-sized groups in terms of risk of diarrhea. They concluded that housing calves in groups of fewer than 10 calves is preferable from a health and growth perspective. They demonstrated that reducing group size is associated with a reduced risk of respiratory illness.

1.5. Effect of group size on physiological condition of veal calves

Grouping of veal calves may represent a source of chronic stress for the calf **(Veissier et al., 1998).** Chronic stressors are a major welfare issue in food producing animals. Social interaction is common stressors of livestock in the modern intensive farm animal industry. Immune status of animals under chronic pain can be compromised thereby reducing disease resistance and welfare, and may even lead to death **(Muir and Woolf, 2001).** Acute pain has a protective role, enabling healing and tissue repair. However, chronic pain has little or no protective value.Both induce biochemical and phenotypic changes in the CNS and immunity, but those associated with chronic pain result in systemic alterations that threaten allostasis and potentiate other illness or even death. Traditional measures of pain include behavior and stress responses, but neither seems to be sufficiently sensitive for chronic pain. Physiological indicators (e.g., cortisol levels) have been used in several domestic animals, including cattle **(Friend et al., 1987; Veissier et al., 1998; Munksgaard**

et al., 1999), to detect states of chronic activation of the hypothalamo-pituitary-adrenocortical (HPA) axis due to external stressors **(Terlouw et al., 1997).** Chronic stress can also lead to immune depression, which can be evidenced by an alteration of hematological measures, especially by an increase of the neutrophil/lymphocyte (N: L) ratio. This ratio has also been used as a welfare indicator in several studies on veal calves **(Friend et al., 1987; McFarlane et al., 1988; Stull and McDonough, 1994).**

5.1.1. Plasma cortisol

It is known that cortisol, a glucocorticoid of adrenocortical secretions, is released in response to stress or injury as a mean to maintain a state of physiological balance called homeostasis. Veal calves raised under commercial conditions are exposed to many stressors. The hypothalamic–pituitary–adrenal (HPA) axis is activated as part of the stress response and acts to maintain homeostasis. Neural information is coordinated into physiological responses by the release of hormones of the HPA axis, in a cascade effect, regulating the production of basal and stress-induced secretion of glucocorticoid hormones, which mediates many of the physiological effects of stress. In this axis, the response to stressors is initiated at the level of the hypothalamus. Neural activation stimulates the release of corticotrophin releasing hormone (CRH) from the hypothalamus, which is transported to the anterior pituitary by the hypophyseal portal vessels. At the pituitary, CRH stimulates adrenocorticotrophin (ACTH) secretion. In stressed animals increased circulating concentrations of ACTH causes the release of glucocorticoids, predominantly cortisol from the adrenal cortex **(Minton, 1994).** Calves reared in individual pens have higher cortisol responses to adrenocorticotrophic hormone (ACTH) than do calves reared in groups, and this is considered to be the result of chronic stress **(Dantzer et**

al.1983; Friend et al. 1985). According to **Dellmeier et al. (1985)**, this stress occurs because of the higher motivation of calves to interact with other calves. Corticosteroids which are involved in stress responses affect metabolism by increasing gluconeogenesis at the cost of protein synthesis **(Mormède 1995).** This is supported by the finding in several studies that calves reared alone grow more slowly than calves housed in groups **(Warnick et al., 1977; Veissier et al., 1994).**

Veissier et al. (1998) observed no effect of group housing on circulating ACTH level in response to a corticotrophin releasing factor challenges or on cortisol level during dexamethasone and ACTH challenges. The increased plasma cortisol level with advancing age may be attributed to changes because calves are growing; however, researchers **(Stull and McMartin, 1992; Wilson et al., 1999)** have noted that cortisol concentrations increased over time during the production cycle of special-fed Holstein veal calves.

Raussi et al. (2003) noted that calves housed in groups of 4 and 15 calves experienced higher basal cortisol concentrations, but when challenged with ACTH, individually-housed calves experienced a greater cortisol response than group housed calves.

5.1.2 Differential blood cell count

Traditionally, assessment of physiological stress has been estimated by measuring levels of adrenal hormones. However, there are associated drawbacks, such as the potential for rapid changes in hormone levels as a response to short-term acute stressors. Thus, an alternative method of assessing physical stress that has potential to reflect a more chronic state for the animal is investigation of relative white blood cell counts. Physiological stress causes a concurrent rise in neutrophil (N) number, and drop in lymphocyte (L) number, and thus a composite

measure that is often used to assess the stress response is the N:L ratio **(Davis et al., 2008).** Stress or administration of glucocorticoids in humans and laboratory animals generally leads to increased concentrations of neutrophil, and decreased concentrations of lymphocytes, eosinophil's and monocytes. Increased neutrophils and decreased lymphocytes are the most commonly used indicators of stress **(Siegel, 1987).** An increase in circulating neutrophil counts as a response to glucocorticoids is thought to occur because neutrophils are less able to migrate to the site of infection, and have increased longevity in blood **(Kulberg et al., 2002; Burton et al., 2005).**

Stull and McMartin (1992) concluded that N:L ratios greater than the range of 0.35 to 1.10 between 2 wk. of age and market weight (16wk) were indicative of undue stress in special-fed veal calves.

Friend et al. (1985) also reported higher basal cortisol levels, thyroid hormone levels, and N: L ratios in tethered or individually housed calves than in group-housed calves. However, **Friend et al. (1987)** found no differences in N: L ratio in response to different housing treatments.

Wilson et al. (1999) found that the mean blood leukocyte differentials at 4 wk of age in Holstein Veal calves were as follows: 40.2% segmented neutrophils, 0.32% banded neutrophils, 55.1% lymphocytes, 3.91% monocytes, 0.20% esinophils, 0.0% basophils, and a N:L ratio of 0.73. means at the end of the feeding period (18 wk) were as follows: 30.5% segmented neutrophils, 0.10% banded neutrophils, 64.1% lymphocytes, 4.2% monocytes1.1% esinophils, 0.13% basophils, and a N:L ratio of 0.48.

Mattiello et al. (2002) and Cozzi et al. (2002) mentioned that the decline in the N: L ratio from the beginning to the end of the fattening period, which they observed in all treatments, could be reflects the

physiological decrease of neutrophils and increase of lymphocytes which normally observed in calves during the 1st year of life. Also, they confirmed that eosinophil and monocyte values are less important for indicating changes in response to stressful situation in calves.

5.1.3 Blood hemoglobin concentration

Provision of veal calves with iron deficient diet in order to produce pale-colored meat inhibits the ability of body to produce red blood cells and results in reduced hemoglobin concentrations and eventually iron-deficiency anemia. **Andrighetto et al. (1999)** found that blood hemoglobin concentration was greater in group housed than individually housed calves (10.9 vs. 7.7 g/100 ml; $P < 0.01$). The restriction of movement forced by the individual crates could have caused the lower hemoglobin and PCV levels. However, **Gottardo et al. (2005)** concluded that hemoglobin concentration was not affected by increasing the number of calves from 3 to 7 per box when the space allowance per calf was the same (1.8 m^2/head). Many studies indicated that impaired performance and an increased disease susceptibility in calves whose blood hemoglobin concentration is below 4.5 mmol/l (European Union legal minimum concentration). **Agriculture Canada (1988)** concluded that blood hemoglobin concentrations of 6.5 g/dL or less are unacceptable since well-being of the veal calf is not ensured. **Mattiello et al. (2002)** noted that the rapid decrease in hematocrit values, together with a reduction in hemoglobin is common in veal calves at the end of fattening period due to their peculiar feeding plan.

II-Acute phase cytokines, TAC1, and Toll-like receptor4 mRNA expression association with housing in veal calves

Because of increasing awareness of pain or discomfort that production animals may experience, a more complete understanding of

the experience and biomarkers of pain is critical. Not only is there acute pain associated with some management procedures, but chronic pain may result from injuries or housing conditions. To better evaluate those housing conditions, biomarkers indicative of chronic pain are needed. Since group housing could be a source of chronic stress for the calf, and immune status of animals under chronic pain can be compromised thereby reducing disease resistance and welfare **(Muir and Woolf, 2001).** Presently, no study has assessed the physiological leukocyte markers of stress that we propose to measure on a well-controlled studies of group housing for calves. Peripheral leukocytes provide a mirror of inflammatory response during exposure to stressors; we will use mRNA to monitor the gene expression in peripheral leukocytes. Stress is known to influence both adaptive and innate immune response in both young and adult animals **(Nonnecke et al., 2009).** Innate immunity refers to nonspecific defense mechanisms that serve as the first line of defense against pathogen, injury, or stress and occurs very quickly. The phagocytic cells are the major players of innate immunity but also serve as the connection between innate and adaptive immunity. Adaptive immunity refers to an antigen-specific immune response that develops over time and is more complex than the innate responses. Macrophages and dendritic cells are specialized cells that initiate adaptive immune responses by presenting antigen to lymphocytes to initiate a cell-mediated or humoral response. Activation of lymphocytes leads to secretion of different types of cytokines. Quantitation of several of cytokine genes expression associated with stress in cattle provides a simple method and rapid diagnosis of stress **(Satoru et al., 2003).**

Recognition of antigen by immune cells initiating signaling pathways leading to release of pro-inflammatory cytokines such as IL-1β,

TNF-α and expression of receptors such as Toll-like receptors (TLR). Interleukine-1β secreted from phagocytes, fibroblasts, and T and B lymphocytes which trigger fever, stimulate T cell proliferation, and elicit the release of histamine at the site of inflammation **(Takashi and Kodama, 1994).** The pro-inflammatory effect of IL-1β can be inhibited by IL-1 receptor antagonist (IL-1Ra). IL-1Ra is produced by immune complex- or IL-4-stimulkated macrophages and by TNF-stimulated neutrophil. IL-1Ra inhibits IL-1 action by competing with IL-1 for binding to the IL-1 receptor **(Dinarello, 1991).** Tumor necrosis factors (TNF-α) are common cytokines that secreted from activated macrophages/monocytes, mast cells, and some T and natural killer cells. TNF-α, and IL-1 share several pro-inflammatory properties. Blood TNF-α concentration is frequently elevated during acute and chronic inflammation associated with body's response to infection and is considered a marker for immune activation **(Nonnecke et al., 2009).** Toll-like receptors are part of the IL-1 family. Their function is to distinguish antigens and to initiate an appropriate immune response. To date, 10 TLRs have been identified. TLR-4 is responsible for gram negative lipopolysaccharide (LPS) recognition and cell-signaling. Toll-like receptor 4 leads to increased interferon (IFN)-β, tumor necrosis factor (TNF)-α, interleukin (IL)-1b, IL-6, and IL-13 **(Eicher et al., 2004).** TLR-4 antagonist has been shown to have therapeutic promise for inflammatory arthritis **(Abdollahi-Roodsaz et al., 2007)** and alleviated neuropathic pain for a chronic restriction injury. **Christianson et al. (2011)** have demonstrated an association between TLR4 mediated nociception during inflammation phase and establishment of a chronic pain state.

Physiological studies have been performed to identify neuro-peptides present in the spinal cord in relation to neuropathic pain. Certain peptides, such as substance P, vasoactive intestinal peptide (VIP), cholecystokinin (CCK), and neurotensin, are associated with chronic pain mechanisms, and their concentrations have been measured for assessment of pain in farm animal. It was recently shown by time-of-flight mass spectrometry in a cuff-implanted sciatic nerve model that substance P and neurotensin are up-regulated in animals experiencing neuropathic pain. Detection and identification of peptides related to neuropathic pain may suggest new therapeutic strategies for the treatment of this condition **(Coetzee et al., 2008).** Substance P (SP) is a member of tachykinin (TAC) family neuropeptides which are small molecules secreted from the peripheral terminals of sensory nerve fibers and act as neurotransmitter or hormone. It mediates pain perception, regulates wound healing and tumor cell proliferation, and has a potential to induce angiogenesis. **O'Driscoll et al (2009)** found that keeping cows on rubber floor for prolonged time increase the expression Peripheral leukocytes genes associated with discomfort (TAC1) and lameness (MMP-13). In the study of **Coetzee et al., (2008)** they found that SP concentration in castrated calves was significantly higher than the concentration in control beef calves which strongly suggest that SP associated with pain and can be used as a valuable tool for assessment of pain in farm animals.

Assessment of animal well-being requires procedures that can be stressful for animals. So, measuring the expression of genes associated with stress could be a good approach for assessment of stress in calves.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was conducted at a Strauss Veal Feeds Inc. (North Manchester, IN, USA) finisher barn, which provided facilities, calves, and feed. The study was carried out over 5 months, from March 21 to July 21, 2012. All experimental procedures were in compliance with the **Guide for the Care and Use of Agricultural Animals in Research and Teaching (2010)**, and approved by Purdue University Animal Care and Use committee (Protocol no. 1112000434).

Experimental Animals and Housing

Holstein-Friesian bull calves (n = 168) from Strauss Veal Feeds Inc. starter barns were housed in individual pens until they were moved to the experimental pens in the finishing barn for this experiment. On arrival to the finishing barn, bull calves (44 ± 3 d of age) were allotted randomly in pairs to 84 pens that were in two rooms of the same barn. Experimental pens (3 m length × 1.2 m width × 1.21 m height) consisted of stainless steel partitions that enabled visual and tactile contact between calves (**Fig. 1, 2, and 3)**. After 1 wk of acclimatization, calves were assigned randomly to 1 of 3 group-housing treatments (2, 4, or 8 calves/pen). Those assigned into 2 calves per pen remained as they were, whereas those assigned to 4 or 8 calves per pen had the metal partitions between neighboring pens removed to form the larger groups.Twelve replicated pens (6/barn) of each treatment were formed; however; 1 pen of 8 calves was removed from the trial because of a congenital deformity of one calf. Pens used for housing were 3 × 1.2 m (2 calves/pen), 3 × 2.4 m (4 calves/pen), and 3 × 4.8 m (8 calves/pen), but the total pen area allowance for each calf was kept constant at 1.82 m^2 /calf for all group sizes **(Prevedello et al., 2012**). Initial BW of calves were 65.3 ± 3.7, 66.5 ± 3.7, and 67.5 ± 3.7 kg for calves in groups of 2, 4, and 8 calves,

respectively. The barn was naturally ventilated, utilizing automated curtain controllers and a chimney vent. The barn was new and had not been occupied previously. When calves arrived, the temperature was set to 12.7°C and lowered twice weekly by approximately 0.3°C each time over 5 wk until the temperature reached 10°C. Experimental pens were equipped with radiant floor heating to control the temperature of slatted concrete floors without additional bedding**.** No additional cooling was required during the time of the experiment. Natural lighting was used and the barn included transparent insulated curtains and a transparent ridge cap to maximize the natural lighting exposure. A feeding trough at the front of each pen was divided with a solid metal partition and provided a separated eating area for each calf (38.1 × 30.5 × 20.3 cm)

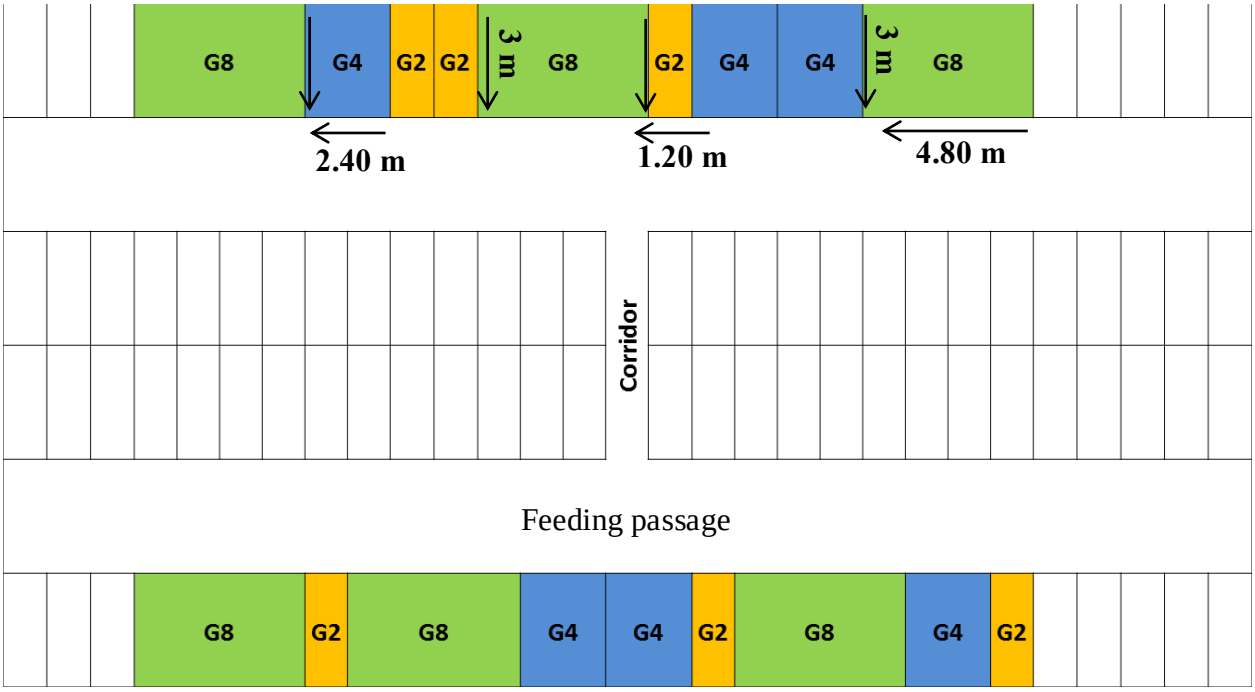


Diagram. 1. Layout of experimental barn; each barn contains 4 rows, each raw contains 64 pens. The only outside rows utilized in the study, we had six replicate in each barn with total twelve replicate for each treatment in the 2 barns used in this study.

Fig. 2. Group housing of veal calves (2 or 4 calves per pen)

Fig. 3. Group housing of veal calves (8 calves per pen)

Animal Management and Feeding

All experimental calves were individually identified with ear tags, and received a prophylactic treatment of oxytetracycline and lincomycine spectinomycine (LS-50; Zoetis, Madison, NJ), which was added to milk for 2 consecutive days after arrival to the finishing barn. Calves were fed their allowance of milk replacer **(Table 1)** and solid feed twice daily at 12-h intervals (5 am and 5 pm). They were offered a combination of dry and liquid milk replacer (Agri-Best Balancer 51/12 and LiquiKalf veal feed; Strauss Veal Feeds Inc., North Manchester, IN) which were blended together at ratio of 15% dry to 85% liquid milk replacer (16% crude protein and 18% crude fat), referred to as milk. At 100 days of age a small portion of 7/60 Fat is also introduced in milk. Milk was delivered to the calves by a hose and metered into an individualized trough which provided a separate feeding area for each calf in the pen (**Fig.4**). Additional iron was added in the milk replacer depending on blood tests (Vita-Iron, Strauss Veal Feeds Inc. North Manchester, IN). Once the milk was fed, calves were allowed 15 min to eat the dry-grain mixture (12% crude protein and 4% fiber) in the same trough before water was added to the trough. After each feeding all mix tanks, hose, feeding trough and walkways were cleaned with Bac-Drop water solution (EcoLab, Inc.). In the finishing barn, the calves received Iron dextran (0, 5, 10 cc based on blood test) for prevention of anemia and received Bar Vac vaccine (Boehringer Ingelheim Vetmedica, Inc. Missouri, USA) against clostridial diseases. During the experiment, calf health was monitored daily by trained, experienced personnel and appropriate medical treatment was provided when needed. All treatments (days of treatment, type of treatment, and dosage per calf) were recorded.

Table 1. Calculated analysis and ingredients of commercial calf milk replacer (Agri-best balancer 51/12) fed during the experimental period	
Calculated analyses	
Crude Protein, min	51.00%
Crude fat, min	21.00%
Crude fiber	0.15%
Calcium, min	1.25%
Calcium, max	1.50%
Phosphorus, min	0.90%
Vitamin A, min	100,000 IU/LB
Vitamin D3, min	10,000 IU/LB
Vitamin E, min	200 IU/LB
Ingredients	
Isolated wheat protein, Dried Whey, Dried Whey Protein Concentrate, Dried Whey Product, Animal and Vegetable Fat, Dried Skimmed Milk, Lecithin, Dried Milk Protein, Dicalcium Phosphate, Calcium Carbonate, Magnesium Sulfate, 1-Lysine, dl-Methionine, Vitamin A Acetate, Vitamin D3 Supplement, Vitamin E Supplement, Copper Proteinate, Manganese Proteinate, Zinc Proteinate, Iron Proteinate, Selenium Yeast, Dried Brewer Yeast, Vitamin B12 Supplement, Thiamine Mononitrate, Ascorbic Acid, Biotin, Riboflavin, d-Calcium Pantothenate, Niacinamide, Choline Chloride, Menadione Sodium, Bisulfite Complex, Folic Acid, Pyridoxine Hydrochloride, Calcium Iodate, Zinc Sulfate, Ferrous Sulfate, Manganese Sulfate, Copper Sulfate, Cobalt Sulfate, Sodium Silico Aluminate, Polyoxyethylene Glydcol (400) Mono and Dioleates, Artifical Flavor.	
Calculated analysis and ingredients of commercial calf starter (solid feed) fed during the experimental period	
Calculated analyses	
Crude Protein, min	12.00%
Crude fat, min	3.00%
Crude fiber	4.1%
Calcium, min	1.25%
Calcium, max	1.50%
Phosphorus, min	0.90%
Vitamin A, min	100,000 IU/LB
Vitamin D3, min	10,000 IU/LB
Vitamin E, min	200 IU/LB
Ingredients	
Corn, cracked/roll, Soybean hulls, Oats, Barley, Wheat millrun, Soybean meal, 48% , Molasses, Limestone, T. M. Salt , Dical. Phos, Dynamate, Vit ADE.	

Fig. 4. A separate feeding trough at the front of each pen

Behavioral Observations

Behavior was recorded by video camera (CB-HD39N-L; Nuvico Corp., Englewood, NJ) between 7 am to 7 pm. One camera was able to record behavior in 1 to 3 pens; so, there were 10 cameras in each room. Output from the cameras was recorded with digital video recorders (Easy Net DVRED-U1600; Nuvico Corp.) and (Geovision DVR; USA Vision Systems, Inc.,Irvine, CA; **Fig. 5**). Continuous observations were conducted by a single observer using an automated behavior recording program (Observer XT, Version 5; Noldus Information Technology, Wageningen, Netherlands). Observations were carried out using 2 sampling methods; instantaneous scan-sampling every 5 min within 30-min observation sessions from 0700 to 1900 h during the 1st, 2nd, 3th, 4th, and 5thmonth of the experiment to determine daily time budgets of calves using a complete ethogram **(Table 2)**. The percentage of calves involved in ingestive, activity, abnormal oral, and postural behaviors within the pen were calculated by dividing the number of calves in the act of the

behavior within each 30-min observation session by the total number of calves in the group within the 30-min session (each 30- min observation session contained 7 time points: 0, 5, 10, 15, 20, 25, 30 and 35,… for the 2nd 30-min), and data are presented as the mean percentage of calves performing each behavior within the day of observation. Day of observation was divided into 4 time periods (0700 to 0900, 0900 to 1300, 1300 to 1700, and 1700 to 1900 referred to as periods 1, 2, 3, and 4, respectively).These times were selected to include a time immediately after morning feeding, a time of inactivity after feeding, and a time of increased activities before and during evening feeding. Certain behaviors were combined to create behavior categories for posture, maintenance, oral, self-grooming, aggressive, walking, play, and other behaviors. The second method of recording behavior was by continuous focal sampling; on d 0, 1, 5, 14, 42, and 70 relative to day of group formation, one randomly selected calf from each pen was observed continuously around feeding time (30-min intervals before, during, and after feeding). Calves were observed during the evening feeding time from 1700 to 1900 h. Continuous behavior recordings focused on all instances of oral and aggressive behaviors to determine number of bouts, average bout length, and total time spent engaged in these behaviors **(Webb et al., 2012).**

Plate. 5. Nuvico CB-HD39N-L Bullet Camera with 24 IR LEDS and Easy Net DVRED-U1600

Table 2. Ethogram for instantaneous scan sampling and continuous focal observation of group-housed veal calves(certain behaviors were combined into a single category according to **Webb et al., 2012**)

Behavior	Description	Category
Standing	Standing with all four feet on the ground either active or inactive	Posture
Lying	Lying on sternum with head held in a raised positionor down	Posture
Eating	Head in trough accompanied by chewing movements	Ingestive behavior
Drinking	Mouth around drinker	Ingestive behavior
Chewing/ruminating	Irregular, repetitive chewing without discernible food in the mouth	Ingestive behavior
Manipulating object	Biting, sniffing, sucking or licking pen fixtures or fitting	Abnormal oral behavior
Manipulating prepuce	Biting, sniffing, sucking or licking other calf's prepuce, including urine drinking	Abnormal oral behavior
Conspecific contact	Biting, sniffing, sucking or licking other calf excluding prepuce	Social behavior
Self-licking	Movements with tongue overown body surface	Self-grooming
Scratching	Scratching themself using leg	Self-grooming
Rubbing	Moving body against walls, partitions or other calf	Self-grooming
Inactive	Carrying out no discernible behavior	Other
Aggression	Butting, pushing or displacing another calf	Aggression
Walking	Stepping and moving	Walking
Play	Vigorous jumping, kicking and running interrupted by fast stop and turn in new direction, mount other calf from any side	Play

Frequency (total number) of each behavior: calculated as the total number of occurrences of each behavior/per unit time (1.5 h).

Total duration: calculated as the total length of time for all occurrences of each behavior, second.

Bout duration: calculated as the length of time for a single occurrence, second.

Growth Parameters

Body weight was obtained at the day of grouping (initial BW) and when calves moved out of the facility for slaughter at 157 ± 3 d of age (final BW), and average daily gain (ADG) was calculated at the end of the experiment. Two mid-weight calves (based on visual body condition) from the 4- and 8-calf treatment groups were selected to represent the pen throughout the experiment. Hip height and heart girth were measured on these calf pairs monthly during the 5-month experiment. Hip height was measured as the distance from the floor beneath the calf to the top of the hip by using a measuring stick **(Fig. 6),** whereas chest girth was measured as the minimal circumference around the body immediately behind the front shoulder (**Fig. 7)** using measuring tapes for the same selected calves **(Wilson et al, 1997).**

Plate. 6. Measuring the hip height of calf

Plate. 7. Measuring chest girth of calf

Health Status

Health scores were evaluated by using the University of Wisconsin calf health scoring chart **(Fig. 8).** Fecal scores of calves were evaluated using a 3-point scale (0 = firm/dry; 1 = creamy; and 2 = loose/wet). A visual observation of calves' feces consistency was carried out once a month for 5 mo and used as an indicator of gastrointestinal diseases. Ear (0 = normal; 1 = ear flick or head shake; and 2 = slight unilateral droop), eye (0 = normal; 1 = small amount of ocular discharge; and 2 = moderate amount of bilateral discharge), cough (0 = none; 1 = induce single cough; and 2 = induced repeated coughs or occasional spontaneous coughing), and nasal discharge scores (0 = normal serous discharge; 1 = small amount of unilateral, cloudy discharge; and 2 = bilateral cloudy or excessive mucus discharge) were visually evaluated on all calves in the whole group every month for 5 mo.

Plasma Cortisol Assay

Blood samples (10-mL EDTA vacuum tube, Tyco Healthcare Group, Mansfield, MA) of two randomly selected calves from each pen were collected at 1000 h by jugular venipuncture on the day calves were moved into their assigned treatment groups, and then monthly for 4 mo at the same time of day. The samples were centrifuged at 1,500 × g for 10 min at room temperature, and plasma was harvested into 0.5 mL microcentrifuge tubes and stored at -20ºC until analyzed. Plasma cortisol concentrations were determined using a previously-validated (**Burdick et al., 2011),** commercially-available ELISA kit (Arbor Assays, Ann Arbor, MI). The inter- and intra-assay CV were 6.1% and 11.8%, respectively, and assay sensitivity was 17.3 pg/mL (results are presented as ng/mL).

Fig. 8. The University of Wisconsin calf health scoring chart

Hematological parameters

Blood hemoglobin concentration (Hb) was measured at the day of grouping and then at monthly interval. Packed cell volume (PCV), and red and white blood cell counts (RBC and WBC**,** respectively) were monitored at the beginning of experiment (41 to 43 d of age) for all experimental calves and repeated in the middle of the experiment (85 to 97 d of age) for one calf/pen **(Table 3)** to check the health of calves and to ensure that hemoglobin levels of calves at the beginning of the experiment were not below the normal level (7 to 8 gm/dL; **Schwartz, 1990**) as a part of normal farm management.Blood samples were collected by jugular venipuncture into 5-mL EDTA vacuum tubes (BD, Franklin Lakes, NJ), and analyzed using STKS hematology analyzer (Beckman Coulter, Indianapolis, IN).Calves that had low Hb and WBC levels were given 10-cc, whereas those with only low Hb level were given 5-cc, i.m. injection of iron dextrin (FerroDex 100,AgriLabs, St. Joseph, MO) in accordance with the standard operating procedures of the farm. The number of calves treated for iron deficiency at the onset of the experiment was 42, 13, and 10 for groups of 8, 4, and 2, respectively.

Differential blood cell count

For each EDTA blood sample a slide was prepared for differential leukocytes **(Fig. 9).** The slides were air-dried and stained using Hema 3 solutions (Fisher Scientific Company L.L.C. Kalamaza, MI). One hundred cells including neutrophils, lymphocytes, monocytes, eosinophils and basophils were counted under the microscope using oil immersion lens at 40× magnifications and the N: L ratio was calculated.

Table 3. Least-squares means (± SE) of various hematological measures in veal calves as affected by group size and age of calves.

	Group size (calves/pen)			Calf age, d[1]		*P*- value[2]		
Measures	2	4	8	41 to 43	85 - 97	GS	d	GS × d
Total white blood cell count, 10^6 cell/ml	8.7 ± 1.2	7.7 ± 1.1	9.0 ± 1.2	8.4 ± 1.1	8.4 ± 1.1	0.142	0.993	0.814
Total red blood cell count, 10^6 cell/ml	9.6 ± 0.4	9.5 ± 0.4	9.3 ± 0.4	9.6 ± 0.4	9.4 ± 0.41	0.266	0.266	0.941
Hemoglobin, g/dl	9.7 ± 0.2	9.2 ± 0.2	8.9 ± 0.2	9.6 ± 0.1	8.9 ± 0.2	0.019	0.008	0.460
Packed cell volume, %	28.3 ± 0.6	26.9 ± 0.7	26.0 ± 0.6	28.3 ± 0.4	25.8 ± 0.6	0.034	0.001	0.417

[1]Blood samples were collected from all calves at the beginning of experiment (41 to 43 d of age) in order to detect initial health status (calves with low blood hemoglobin and white blood cell counts were i.m. injected with either 5 or 10 cc of iron dextrin (FerroDex 1000, AgriLabs, St. Joseph, MO), and blood samples were collected from one calf/pen at the period from 85 to 97 d of age.
[2]Probability values for the main effects of group size (GS) and calf age (d), and the interactive effect of GS × d.

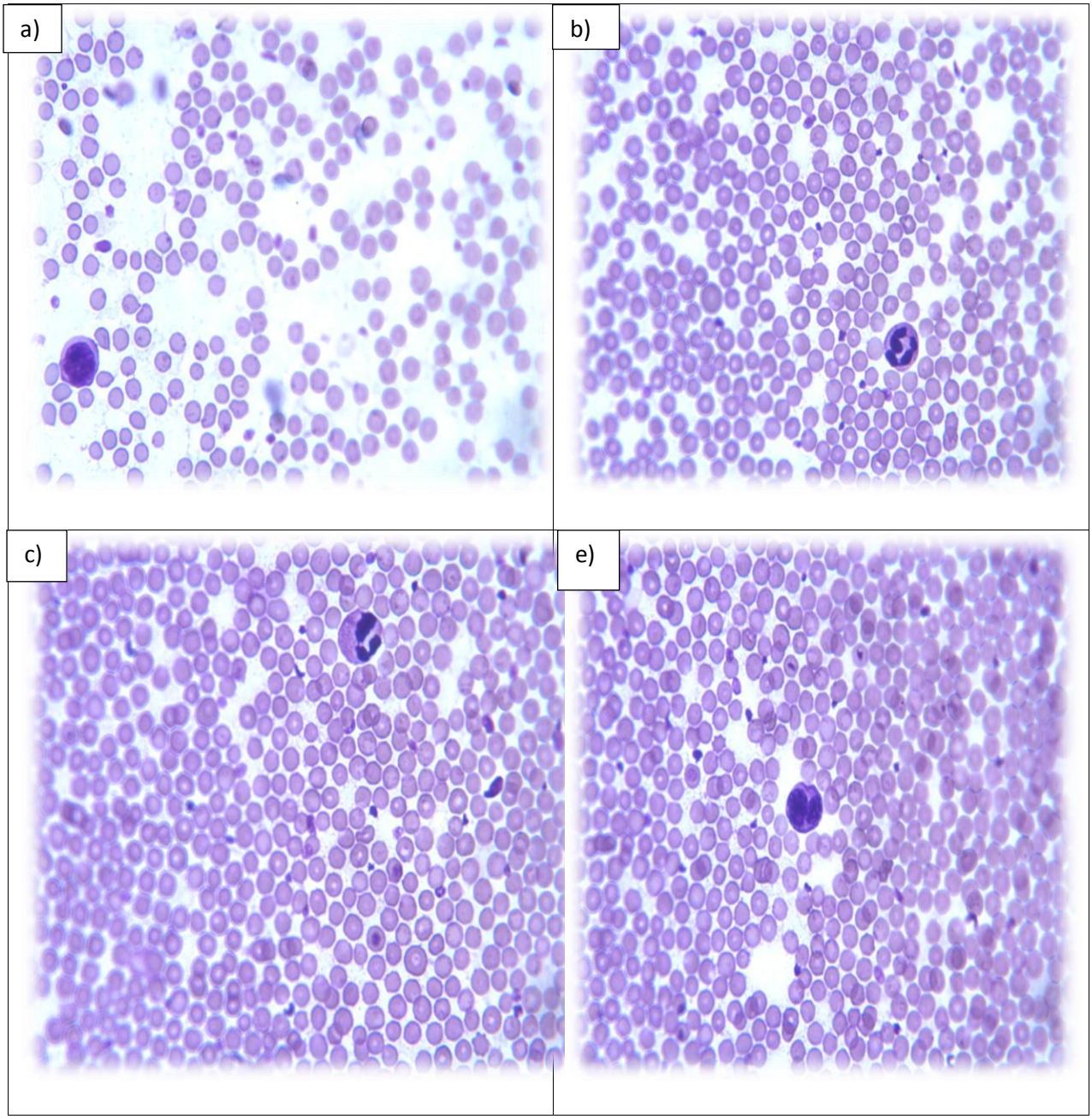


Fig. 9. Blood smears stained with Hema 3 solutions. Lymphocyte (Panel a) have a round centrally located nucleus with a small volume of clear to gray-blue cytoplasm. Segmented neutrophil (Panel b): nucleus with 2-8 lobes chromatin in dense coarse lumps. Eosinophil (Panel c): the nucleus usually bilobed with numerous large eosinophilic granules stained pale-pink in cytoplasm. Monocyte (Panel d): largest leukocyte cell with kidney bean nucleus.

RNA extraction

Extraction of mRNA was performed using LeukoLOCK Total RNA isolation system (Ambion, Austin, TX). Approximately 10 mL of blood was passed through a LeukoLOCK filter to capture leukocytes, after that each filter was flushed with 3 mL of PBS to remove residual RBCs and 3 mL of RNAlater to stabilize leukocyte mRNA (all steps of leukocyte separation were performed on the farm immediately after blood collection). The LeukoLOCK filters were stored at -20 for subsequent laboratory work. In the laboratory, LeukoLOCK filters were flushed with 2.5 mL pH-adjusted lysis/binding solution. The lysate was collected and treated with 25 µL Proteinase-K for 5 min. Leukocyte RNA was isolated through RNA binding beads and treated with TURBO DNase for removal of genomic DNA. After a series of washes, RNA was eluted in 50 µL Elution Solution and stored at -20 ºC. Extracted RNA was analyzed for quantity and purity using OD 260 nm/OD 280 nm spectrophotometer readings (GeneQuant pro, Biochrom Ltd., Cambridge, UK).

cDNA synthesis

Relative amount of RNA samples were combined with RNase-free water to achieve a concentration equal to 100 µL/mL. Reverse transcription was carried out using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). The reagent mixture contained 10 µL 10X TaqMan RT Buffer, 22 µL of 25mM Magnesium Chloride, 20 µL of dNTP, 5 µL of random hexamers, 2.5 µL of Multiscribe reverse transcriptase, and 2 µL of RNase inhibitor. Exactly 61.5 µL of the reagent mixture was added for each RNA sample and then centrifuged briefly to remove air bubbles. Samples were transferred to a thermal cycler (PCR Express, Hybaid Ltd., Ashford, UK), and amplified

for 60 min. After reverse transcription, samples were stored at -80 ºC until further analysis.

Primers and probes

Primers and probes sequences used for quantitative real time-PCR (qRT-PCR) were designed using Primer Express 1.1 software (Applied Biosystems, Foster City, CA) and synthesized by Applied Biosystems (**Table 4**). Probes were labeled with reporter (VIC) fluorescent dye.

Quantitative real-time RT-PCR

TaqMan Real-time PCR was used to determine leukocyte mRNA expression of IL-1β, IL-1Ra, TNF-α, TLR4, TAC 1, and 18S according to **O'Driscoll et al. (2009).** Briefly, the housekeeping gene (18S) and genes of interest were run in separate wells of a 96-well plate, a total of 2.5 µL of template cDNA was used for RT-PCR for each gene, each sample was combined with12.5 µL of Master Mix (TaqMan Universal PCR Master Mix, Applied Biosystems), 2.25 µL of forward and reverse primer, 1.625 µL of probe, 3.875 µL of nuclease free water in a final volume of 25 µL. All qRT-PCR reactions were performed in duplicate using template cDNA from individual animals in each reaction. A relative standard curve was used as the qRT-PCR quantification method. A single control sample of highest CT value for each separate gene was selected to be used as the template for the relative standard curve. The relative standard curve was designed using the following dilution of control sample cDNA: 1, 0.5, 0.25, 0.125, 0.0625, and 0.0315. All standards were run in triplicates. 18S rRNA was used as a control (house-keeping) gene and used for normalization (Pre-Developed TaqMan Assay Reagent for Human Euk 18S rRNA, Applied Biosystem, Grand Island, NY). Samples and standards were amplified using Step One Plus Real-Time PCR System and StepOne Plus software v2.3 (Applied Biosystems, Catalog No.

4376600) with initial incubation of 2 min at 50 ºC and then heated for 10 min at 95 ºC for activation of AmpliTaq Gold DNA Polymerase, followed by 45 cycles for annealing and extension. Data are expressed as relative abundance of the gene of interest to the house keeping gene (18S).

Calculation of qRT-PCR Data:

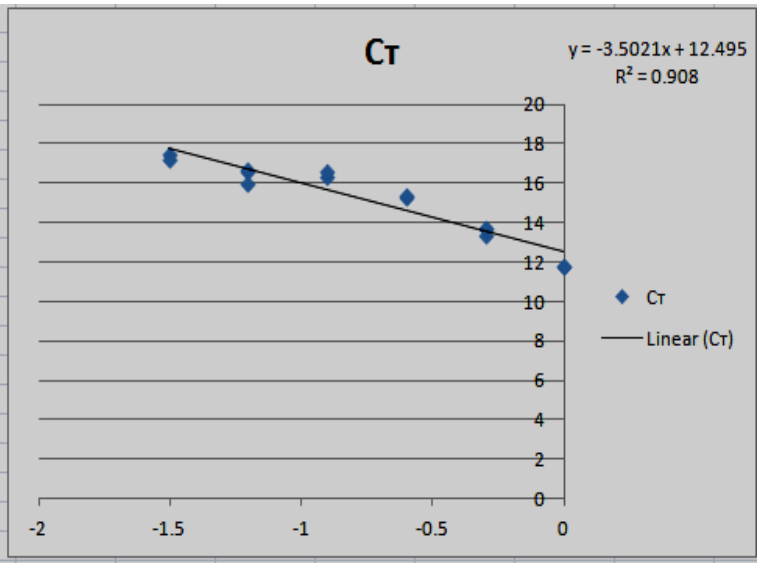
1- After obtaining the amplification curve and CT value for samples and standards.

2- Design linear regression chart between the log doses of following dilution of control sample cDNA: 1, 0.5, 0.25, 0.125, 0.0625, 0.0315 and Ct values.

3- Display the R-squared value and Equation on chart

Y=-3.5021x+120495

log dose	Ct
0	
0	11.75186157
0	11.77815151
-0.30103	13.38649368
-0.30103	13.73313046
-0.30103	13.69338703
-0.60206	15.29360199
-0.60206	15.35343933
-0.60206	
-0.90309	
-0.90309	16.341259
-0.90309	16.58296204
-1.20412	16.56783104
-1.20412	16.67172241
-1.20412	16.00777054
-1.50515	17.49225044
-1.50515	17.17890358
-1.50515	



4- Calculate the log dose of each sample by using the Equation

x = (Y-31.397)/-3.0205

Where X is log dose and Y is the Ct of sample

5- Calculate the dose of each sample by using X: **10^X**

6- Calculate the average dose of the duplicate of each sample

7- Calculate the relative abundance of each sample through dividing the average dose of the sample by the average dose of 18s for the same sample.

Table 4. Primer and probe sequences of genes used for real time RT-PCR.

Gene	Forward (f) and reverse (r) primer sequences	Probe sequence (5′ - 3′)	Reference/ Accession no.
IL-1β	(f[1]) TTCCTGTGGCCTTGGGTATC	CAAGAATCTATACCTGTCTTGT (VIC/MGB)	Ito and Kodama, 1996
	(r[2]) TGGGCGTATCACCTTTTTTCA		
IL-1Ra	(f) CCTCCTTTCTCACCCCAGATC	CAG GCGCTCACTTC (VIC/MGB)	Kirisawa et al., 1998
	(r) AGAAAATGGAAGCCGCTTAGG		
TNF-α	(f) TGGGAAGCT TACCTTTTCCTTTC	CCTCAAGTAACAAGCCG (VIC/MGB)	Bienhoff and Allen, 1995
	(r) CTTCTTCATGACCCAGATACATCCT		
TLR4	(f) CCGGATCCTAGACTGCAGCTT	CCGTATCATGGCCTCT (VIC/MGB)	AAG32061
	(r) TCCTTGGCAAATTCTGTAGTTCTTG		
TAC1	(f) GCCGTGGCAGTGATTTTTTT	TCTCCACTCAACTGTCTG (VIC/MGB)	O'Driscoll et al, 2009
	(r) CGTTGGCTCCGATTTCTTCT		

[1]Forward primer
[2]Reverse primer

Statistical Analysis

A randomized complete block design with repeated measures was adopted for the experiment. Distributions of responses in the study were tested for normality with PROC UNIVARIATE of SAS (version 9.2; SAS Inst., Inc., Cary, NC). All data were analyzed using the mixed model procedure of SAS, with pen as the experimental unit and barn as a random effect. For growth performance data (the mean of the 2 calves per pen), group size (2, 4, or 8 calves/pen) was the lone fixed effect in the model. Using the means of 2 calves/pen, month of trial (1^{st}, 2^{nd}, 3^{rd}, 4^{th}, or 5^{th}), and the 2-way interaction were the fixed effects in the ANOVA for growth indicators (hip height and heart girth), health status scores (ocular and nasal discharges, cough, ears, and fecal scores), and plasma cortisol concentration. Group size, month and observation period within day, as well as all 2- and 3-way interactions were fixed effects in the model for behavior data analysis, except for play and aggression. Least squares means were calculated (LSMEANS statement) and separated statistically ($P \leq 0.05$) using the PDIFF option of SAS. Behavioral data that were not normally distributed were transformed using log transformation prior to analysis, and least squares means and standard errors were back-transformed for presentation of results in tabular form. However, data which were not normally distributed even after transformation (play and aggression) were analyzed using Chi-square testing (PROC FREQ of SAS) within each observation month.

For the data of differential cell counts, blood hemoglobin, health scores, and mRNA expression of IL-1β, IL-1Ra, TNF-α, TLR4, TAC1 genes (the mean of 2 calves per pen),the model considered group size (2, 4, and 8), month, and their interactions as fixed effects and barn as a random effect. Least square means and standard errors were calculated using the LSMEANS statement in the PROC

MIXED procedure. The genes data presented in this paper show the non-transformed values of the data. All *P*- values were calculated using the transformed data. Statistical differences were reported when *P*-values were < 0.05 and tendencies toward significance ($0.05 < P < 0.1$) are also reported. Data are presented as least squares mean (LSM) ± standard error.

RESULTS

RESULTS

Table 5. Least square means and standard errors ($X \pm$ S.E) for the percentage of veal calves showed ingestive behaviors as affected by studied factors

Main effect	[3]Eating & drinking[2]	Chewing & ruminating[2]
Group size effect (Gs)		
2	9.5 ± 0.5^{a}	12.3 ± 0.7^{a}
4	6.2 ± 0.4^{b}	7.1 ± 0.5^{b}
8	4.1 ± 0.3^{c}	5.5 ± 0.5^{c}
P-value	< 0.001	< 0.001
Observation month effect (M)		
1st month (March)	5.8 ± 0.3^{a}	6.7 ± 0.5^{b}
2nd month (April)	5.8 ± 0.4^{a}	7.1 ± 0.5^{b}
3rd month (May)	5.7 ± 0.4^{a}	8.5 ± 0.6^{a}
4th month (June)	6.8 ± 0.5^{a}	8.7 ± 0.6^{a}
5th month (july)	6.7 ± 0.4^{a}	8.2 ± 0.5^{ab}
P-value	0.17	0.01
Observation period effect (P)		
0700 to 0900	6.7 ± 0.4^{b}	9.5 ± 0.6^{a}
0900 to 1300	4.5 ± 0.3^{c}	8.7 ± 0.6^{ab}
1300 to 1700	4.6 ± 0.1^{c}	6.9 ± 0.5^{c}
1700 to 1900	10.2 ± 0.5^{a}	6.6 ± 0.5^{c}
P-value	< 0.001	< 0.001
GS × M	0.08	0.77
GS × P	0.05	0.04
GS × M × P	0.97	0.26

Means within column with no common superscripts are significantly different, $P < 0.05$.

Table 6. Least square means and standard errors (X± SE) for the percentage of veal calves showed abnormal oral, social, inactivity and self-grooming behaviors as affected by studied factors.

Main effect	Object manipulation	Conspecific contact	Inactivity	Self-grooming
Group size effect (Gs)				
2	38.6 ± 0.8[a]	4.7 ± 0.3[b]	51.7 ± 2.1[b]	9.0 ± 0.4[a]
4	26.3 ± 0.6[b]	8.4 ± 0.4[a]	52.8 ± 2.1[b]	5.1 ± 0.3[b]
8	21.5 ± 0.5[c]	7.9 ± 0.4[a]	57.9 ± 1.9[a]	2.8 ± 0.2[c]
P-value	< 0.001	<0.001	<0.001	<0.001
Observation month effect (M)				
1st month (March)	30.3 ± 0.7	6.3 ± 0.3	56.4 ± 2.1[a]	4.8 ± 0.3
2nd month (April)	28.6 ± 0.7	6.5 ± 0.2	57.3 ± 2.1[a]	4.9 ± 0.3
3rd month (May)	26.2 ± 0.6	6.9 ± 0.3	55.3 ± 2.1[a]	4.7 ± 0.3
4th month (June)	29.6 ± 0.9	7.1 ± 0.5	46.8 ± 2.6[b]	5.5 ± 0.3
5th month (July)	25.5 ± 0.6	7.2 ± 0.4	55.0 ± 2.0[a]	5.3 ± 0.3
P-value	0.09	0.49	<0.001	0.13
Observation period effect (P)				
0700 to 0900	21.9 ± 0.6[c]	6.4 ± 0.4[b]	56.8 ± 2.3[b]	5.2 ± 0.2
0900 to 1300	26.6 ± 0.6[b]	6.7 ± 0.3[b]	57.7 ± 2.0[b]	5.1 ± 0.3
1300 to 1700	26.6 ± 0.6[b]	5.3 ± 0.3[c]	62.1 ± 1.9[a]	4.7 ± 0.3
1700 to 1900	39.3 ± 0.9[a]	9.3 ± 0.4[a]	40.1 ± 2.1[c]	5.1 ± 0.3
P-value	< 0.001	< 0.001	< 0.001	0.24
GS × M	**0.03**	0.80	0.17	0.16
GS × P	0.03	0.005	0.44	0.21
GS × M × P	0.24	0.68	0.80	0.54

Means within column with no common superscripts are significantly different, $P < 0.05$.

Table 7. Least square means and standard errors (X± SE) for the percentage of veal calves showed different postures and locomotion as affected by studied factors.

Main effect	Standing	Lying	Walking
Group size effect (Gs)			
2	40.2 ± 1.5[b]	48.6 ± 0.9[a]	1.1 ± 0.2[c]
4	43.1 ± 1.6[a]	42.0 ± 0.8[b]	1.2 ± 0.1[b]
8	43.1 ± 2.4[a]	41.5 ± 0.7[c]	1.4 ± 0.1[a]
P-value	<0.001	<0.001	<0.001
Observation month effect (M)			
1st month (March)	37.3 ± 1.9	48.1 ± 0.9	1.2 ± 0.2[b]
2nd month (April)	42.0 ± 1.6	41.4 ± 0.8	1.1 ± 0.2[b]
3rd month (May)	42.5 ± 1.6	43.9 ± 0.9	1.2 ± 0.2[b]
4th month (June)	43.1 ± 2.4	42.8 ± 1.1	1.4 ± 0.1[a]
5th month (July)	43.1 ± 1.6	43.7 ± 0.8	1.4 ± 0.1[a]
P-value	0.19	0.25	<0.001
Observation period effect (P)			
0700 to 0900	34.7 ± 2.3[b]	50.3 ± 1.1[b]	1.3 ± 0.2[a]
0900 to 1300	32.3 ± 1.2[b]	53.4 ± 0.9[b]	1.2 ± 0.1[b]
1300 to 1700	30.5 ± 1.2[b]	56.6 ± 0.9[a]	1.2 ± 0.1[b]
1700 to 1900	68.8 ± 1.7[a]	24.5 ± 0.6[c]	1.3 ± 0.1[a]
P-value	< 0.001	< 0.001	0.001
GS × M	0.53	0.001	0.003
GS × P	0.91	0.22	0.02
GS × M × P	0.68	< 0.001	0.003

Means within column with no common superscripts are significantly different, $P < 0.05$.

Table 8. Percentage of veal calves performing aggressive behavior as affected by group- size, and observation month

	Group size (GS), calves/pen			*P*- value[1]
Observation month	2	4	8	GS
1st month (March)	0.23 ± 0.40[a]	0.25 ± 0.40[a]	0.05 ± 0.37[a]	0.23
2nd month (April)	0.00 ± 0.40[a]	0.11 ± 0.40[a]	0.04 ± 0.37[a]	0.13
3rd month (May)	0.07 ± 0.41[a]	0.15 ± 0.41[a]	0.08 ± 0.39[a]	0.59
4th month (June)	0.52 ± 0.54[a]	0.83 ± 0.53[a]	0.35 ± 0.46[a]	0.10
5th month (July)	0.42 ± 0.39[a]	0.05 ± 0.39[a]	0.36 ± 0.38[a]	0.70

Means within row with no common superscripts are significantly different, $P < 0.05$.
[1]Aggressive behavior was analyzed using Chi-square testing (PROC FREQ of SAS) for each observation month

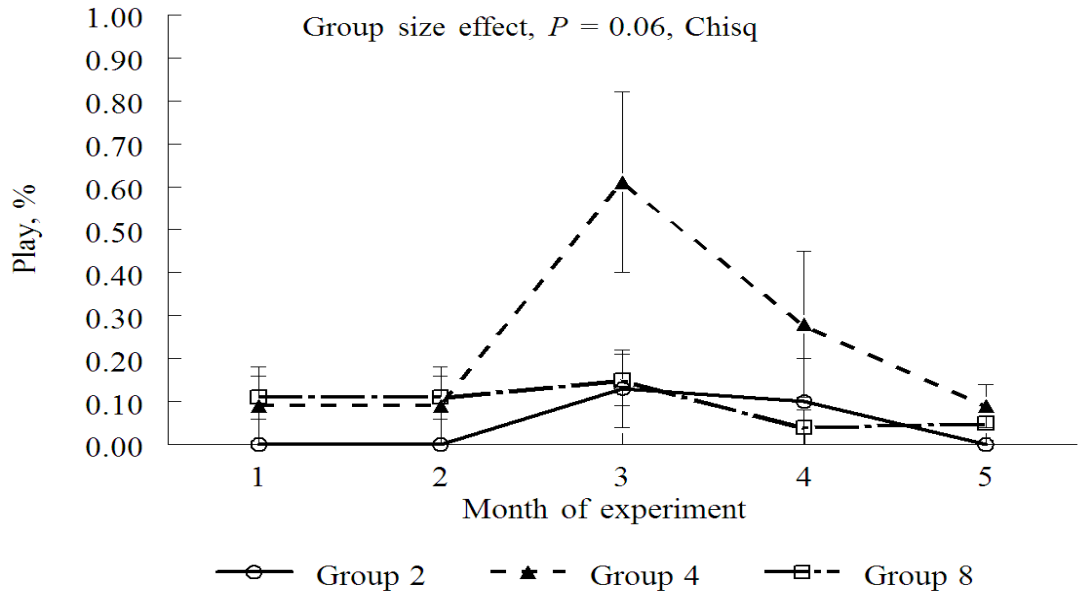


Fig. 10. The effect of group size within each month on the percentage of calves playing (P = 0.06 for mo 3). Play behavior was analyzed using chi-square testing (PROC FREQ of SAS) within each observation month.

Table 9. Frequency[1] (total numbers) of behaviors observed at 30-min intervals before, 30-min during and 30-min after feeding as affected by group- size and observation day after group formation

	Group size (GS)				Observation day (D)						
	2	4	8	*P-value*	0	1	5	14	42	70	*P*-value
Eating & drinking	5 ± 0.3[a]	6 ± 0.3[a]	6 ± 0.3[a]	0.21	4 ± 0.5	6 ± 0.4	6 ± 0.5	5 ± 0.5	6 ± 0.5	7 ± 0.5	0.40
Chewing & ruminating	1 ± 1.3 [a]	1 ± 0.8 [a]	1 ± 1.2 [a]	0.28	1 ± 1.0	1 ± 1.8	1 ± 2.7	1 ± 1.2	1 ± 1.2	1 ± 0.7	0.26
Object manipulation	10 ± 0.7 [a]	11 ±0.7 [a]	9 ± 0.6 [a]	0.09	10 ± 1.2	10 ± 0.8	11 ± 1.0	10 ± 0.9	9 ± 1.0	9 ± 0.9	0.58
Conspecific contact	7 ± 0.5 [a]	8 ± 0.5 [a]	7 ± 0.5 [a]	0.18	7 ± 0.9	8.1 ± 0.6	6 ± 0.7	7 ± 0.7	7 ± 0.7	7 ± 0.7	0.87
Aggression	2 ± 0.5 [a]	2 ± 0.3 [a]	2 ± 0.3 [a]	0.23	2 ± 0.9	2.0 ± 0.4	2 ± 0.5	2 ± 0.5	2 ± 0.7	2 ± 0.4	0.69

Means within row with no common superscripts are significantly different, $P < 0.05$.
[1]Frequency (total number) of each behavior: calculated as the total number of occurrences of each behavior/per unit time (1.5 h).

Table 10. Total duration[1] (s) Behaviors observed at 30-min intervals before, during, and after feeding as affected by group-housing size and observation day after group formation

	Group size (GS)				Observation day (D)						
	2	4	8	*P*	0	1	5	14	42	70	*P*-value
Eating & drinking	108 ± 9.8	136 ± 11.9	139 ± 12.1	0.49	81 ± 2.7	142 ±13	146 ±13	111±11	189 ± 16	188± 18	0.35
Rumination	4 ± 1.1	4 ± 1.1	3 ± 1.1	0.69	5 ± 1.4	3 ± 1.2	2 ± 1.4	4 ± 1.3	3 ± 1.1	7 ± 1	0.10
Object manipulation	171 ± 4.2	177 ± 4.4	162 ± 3.9	0.89	242 ± 9.5	181 ± 5.2	200±6.7	151±5.2	136 ± 4.8	132 ± 5	0.19
Conspecific contact	105 ± 2.9	94 ± 2.6	105 ± 2.8	0.78	128 ± 5.7	121 ± 3.7	97 ± 3.8	93 ± 3.6	80 ± 3.2	93 ± 3	0.43
Aggression	2 ± 2.0	4 ± 2.0	7 ± 2.4	0.07	2 ± 1.9^{b}	8 ± 3.1^{a}	2 ± 2.2^{b}	8 ± 3.2^{a}	2 ± 2.1^{b}	7 ± 3^{a}	0.01

Means within row with no common superscripts are significantly different, $P < 0.05$.
[1]Total duration: calculated as the total length of time for all occurrences of each behavior, second.

Table 11. Bout duration[1] (s) of behaviors observed at 30-min intervals before, during, and after feeding as affected by group size and observation day after group formation

	Group size (GS)				Observation day (D)						
	2	4	8	*P*	0	1	5	14	42	70	*P*
Eating & drinking	19 ± 1.6	20 ±1.7	22 ± 1.7	0.74	17 ± 1.6	22 ± 2.1	23 ± 2.8	18 ± 1.8	16 ± 1.5	26 ± 2.4	0.31
Chewing & ruminating	4 ± 0.7	3 ± 0.6	2 ± 0.7	0.11	3 ± 0.8	3 ± 0.8	2 ± 1.0	3 ± 0.8	3 ± 0.6	4 ± 0.8	0.21
Object manipulation	21 ± 1.8	18± 1.6	22 ± 1.8	0.25	26 ± 1.9	20 ± 2.2	20 ± 1.7	18 ± 2.3	20 ± 1.7	18 ± 1.9	0.45
Conspecific contact	20 ±0.6^{a}	13±0.4^{b}	16 ±0.4^{b}	0.01	19 ± 0.8	18 ± 0.5	16 ± 0.6	15 ± 0.5	14 ± 0.6	16 ± 0.6	0.47
Aggression	2 ± 1.8	2 ± 1.3	3 ± 1.2	0.11	2 ± 1.5^{b}	4 ± 1.6^{a}	1 ± 4.4^{b}	4 ± 1.6^{a}	1 ± 1.5^{b}	3 ± 1.5^{a}	0.00

Means within row with no common superscripts are significantly different, $P < 0.05$.
[1]Bout duration: calculated as the length of time for a single occurrence, second.

Table 12. Growth Performance of veal calves as affected by group size and observation month

	Group Size (GS), calves/pen			
Items	2	4	8	*P*- value
Initial BW	65.3 ± 3.7 [a]	66.5 ± 3.7 [a]	67.6 ± 3.7 [a]	0.50
Final BW	232.4 ± 15.7 [a]	241.4 ± 15.3 [a]	237.6 ± 15.3 [a]	0.50
BW gain[1], kg	167.1 ± 13.2 [a]	174.9 ± 12.7 [a]	170 ± 15.7 [a]	0.60
ADG[2], kg/d	1.08 ± 0.1 [a]	1.13 ± 0.1 [a]	1.10 ± 0.1 [a]	0.50

Means within row with no common superscripts are significantly different, $P < 0.05$.
[1]BW gain (Body weight gain) = Final BW- Initial BW
[2]ADG (Average daily gain) = BW gain/period of fattening (155 days)

Table 13. Hip height and chest girth of veal calves as affected by group size and observation month

Items	Group Size (GS), calves/pen			*P*- value		
	2	4	8	GS	Month	GS × Month
Hip height[1]	94.4 ± 2.5a	94.7 ± 2.5a	94.7 ± 2.5a	0.98	< 0.001	0.99
Hip height change, cm	21.6 ± 0.8a	23.3 ± 0.8a	22.4 ± 0.8a	0.38	0.001	0.35
GS × Month interaction						
1st month (March)	95.3 ± 1.2	94.6 ± 1.2	95.1 ± 1.2	-	-	0.99
2nd month (April)	101.6 ± 1.2	101.4 ± 1.2	101.4 ± 1.2	-	-	0.99
3rd month (May)	105.5 ± 1.2	108.3 ± 1.2	107.3 ± 1.2	-	-	0.99
4th month (June)	112.7 ± 1.2	111.5 ± 1.2	114.3 ± 1.2	-	-	0.99
5th month (July)	117.1 ± 1.2	117.9 ± 1.2	117.6 ± 1.2	-	-	0.99
Chest girth[2], cm	123± 1.2[a]	124±1.2 [a]	124±1.2 [a]	0.18	0.001	0.78
Chest girth change, cm	47.7 ± 5.3 [a]	48.2 ± 5.3 [a]	46.7 ± 5.3 [a]	0.82	0.001	0.78
GS × Month interaction						
1st month (March)	103.1 ± 1.6a	104.0 ± 1.6a	104.1 ± 1.6a	-	-	0.78
2nd month (A7pril)	111.8 ± 1.6a	113.1 ± 1.6a	113.2 ± 1.6a	-	-	0.78
3rd month (May)	120.8 ± 1.6a	120.1 ± 1.6a	123.4 ± 1.6a	-	-	0.78
4th month (June)	133.4 ± 1.6a	135.4 ± 1.6a	134.4 ± 1.6a	-	-	0.78
5th month (July)	148.3 ± 1.6a	150.8 ± 1.6a	149.3 ± 1.5a	-	-	0.78

Means within row with no common superscripts are significantly different, $P < 0.05$.
[1]Hip height= distance from the floor beneath the calf to the top of the hip
[2]Chest girth= minimal circumference around the body immediately behind the front shoulder

Table 14. Health status indicators of veal calves as affected by group-housing size and observation month

	Group size (GS), calves/pen			Observation month				*P*-values		
	2	4	8	1st	2nd	3rd	4th	GS	M	GS × M
Ocular discharge[1]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	0.2 ± 0.1[a]	0.45	0.003	0.16
Nasal discharge[2]	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.63	0.52	0.02
Cough[3]	0.2 ± 0.1[c]	0.3 ± 0.1[b]	0.4 ± 0.1[a]	0.4 ± 0.2[a]	0.3 ± 0.2[a]	0.2 ± 0.2[b]	0.2 ± 0.2[b]	0.02	0.02	0.03
Ears orentation[4]	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.06	0.04	0.39
Fecal score[5]	1.4 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.2 ± 0.1[b]	1.2 ± 0.1[b]	1.6 ± 0.1[a]	1.2 ± 0.1[b]	0.15	< 0.001	0.20

Means within row with no common superscripts are significantly different, $P < 0.05$.

[1]0 = normal to 2 = moderate amount of bilateral discharge.

[2]0 = normal serous discharge to 2 = bilateral cloudy to excessive mucus discharge.

[3]0 = none to 2 = induced repeated coughs or occasional spontaneous coughing.

[4]0 = normal to 2 = slight unilateral droop.

[1]0 = firm/dry to 3 = loose/wet.

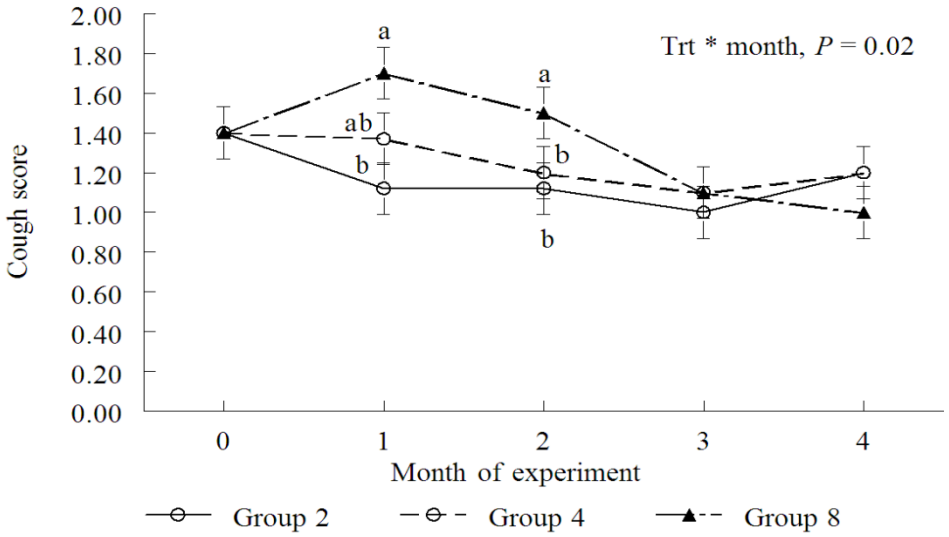


Fig. 11. Cough score (0 = none; 1 = induce single cough; and 2 = induced repeated coughs or occasional spontaneous coughing, $P = 0.03$); and b) for calves in groups of 2, 4, or 8. $^{a\text{-}c}$ within a month, least squares means lacking similar superscripts differ, $P < 0.05$.

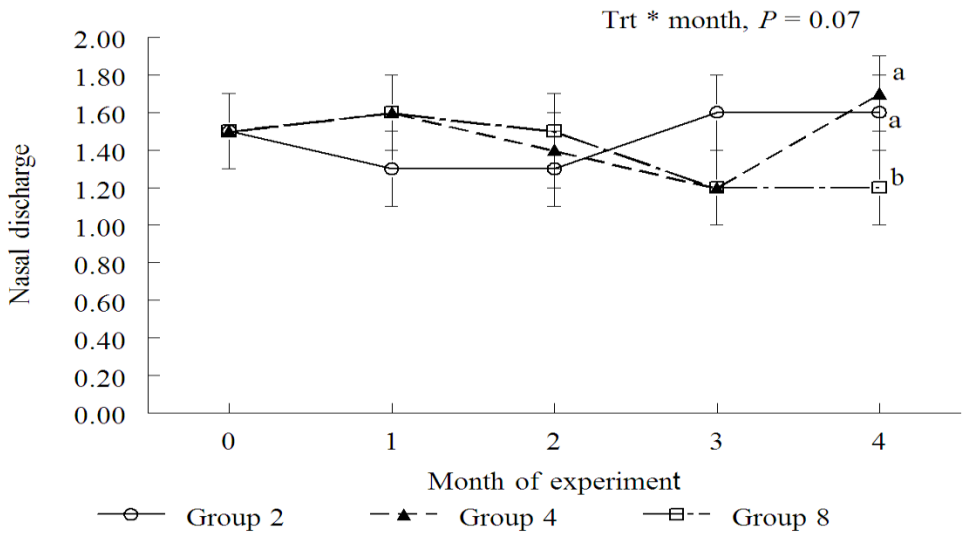


Fig. 12. Nasal discharges score (0 = normal serous discharge; 1 = small amount of unilateral, cloudy discharge; and 2 = bilateral cloudy or excessive mucus discharge, $P = 0.02$) for calves in groups of 2, 4, or 8. $^{a\text{-}c}$ within a month, least squares means lacking similar superscripts differ, $P < 0.05$.

Table 15. Plasma cortisol and blood hemoglobin (HB) concentrations of veal calves housed in groups of 2, 4, and 8 calves /pen.

Items	Cortisol, ng/mL	Blood hemoglobin , g/dL
2	18.3 ± 5.3	9.0 ± 0.1
4	16.1 ± 5.5	8.5 ± 0.2
8	14.4 ± 5.5	8.6 ± 0.2
Group Size effect	0.37	0.13
P-value		
Mo 2	10.8 ± 5.8[b]	9.6 ± 0.1[a]
Mo 3	15.5 ± 5.7[a]	8.9 ± 0.2[b]
Mo 4	18.4 ± 5.5[a]	8.9 ± 0.2[b]
Mo 5	20.3 ± 5.3[a]	7.6 ± 0.2[c]
Month effect	0.03	<0.001
P-value		
Group Size × Month effect	0.42	0.12
P-value		

Means within column with no common superscripts are significantly different, $P < 0.05$.

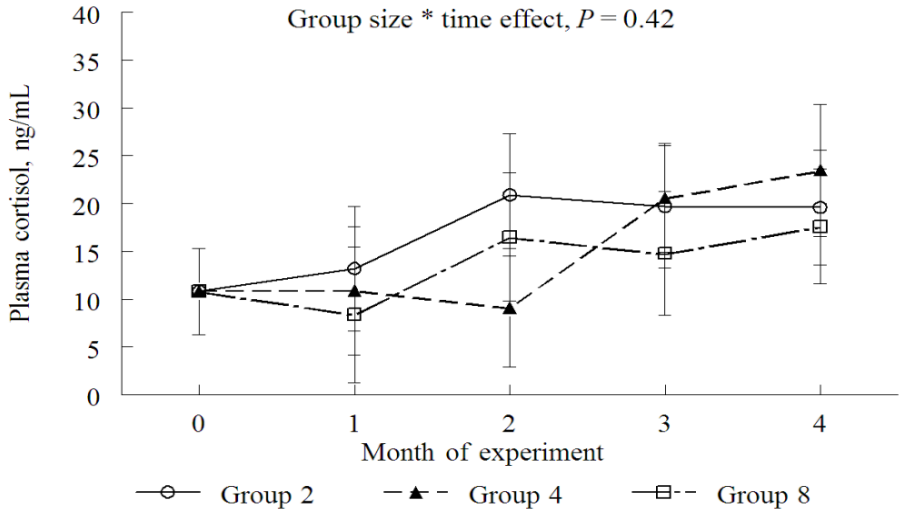


Fig.13. The interactive effect of group size and observation month on plasma cortisol concentrations (P = 0.42) of veal calves housed in groups of 2, 4, 8 calves/pen over 5-month finishing period.

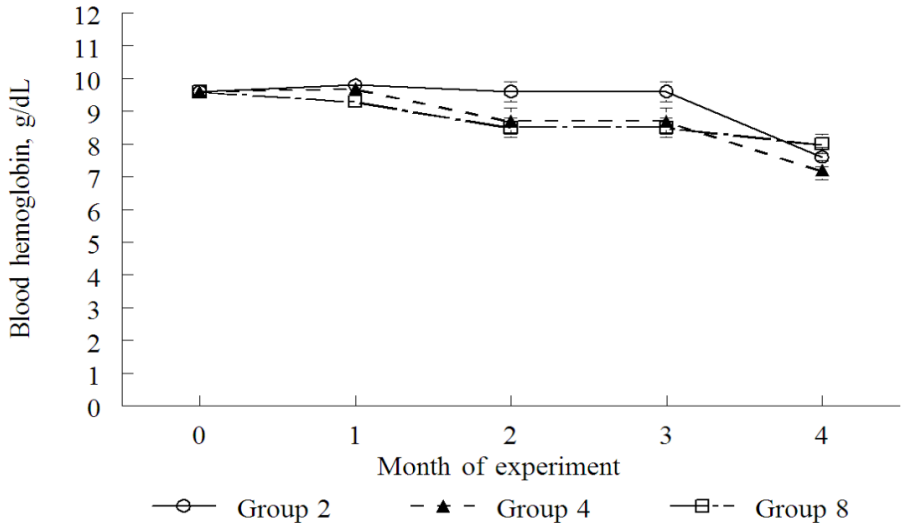


Fig.14. Blood hemoglobin concentrations (P = 0.14) of veal calves housed in groups of 2, 4, 8 calves/pen over 5-mo finishing period.

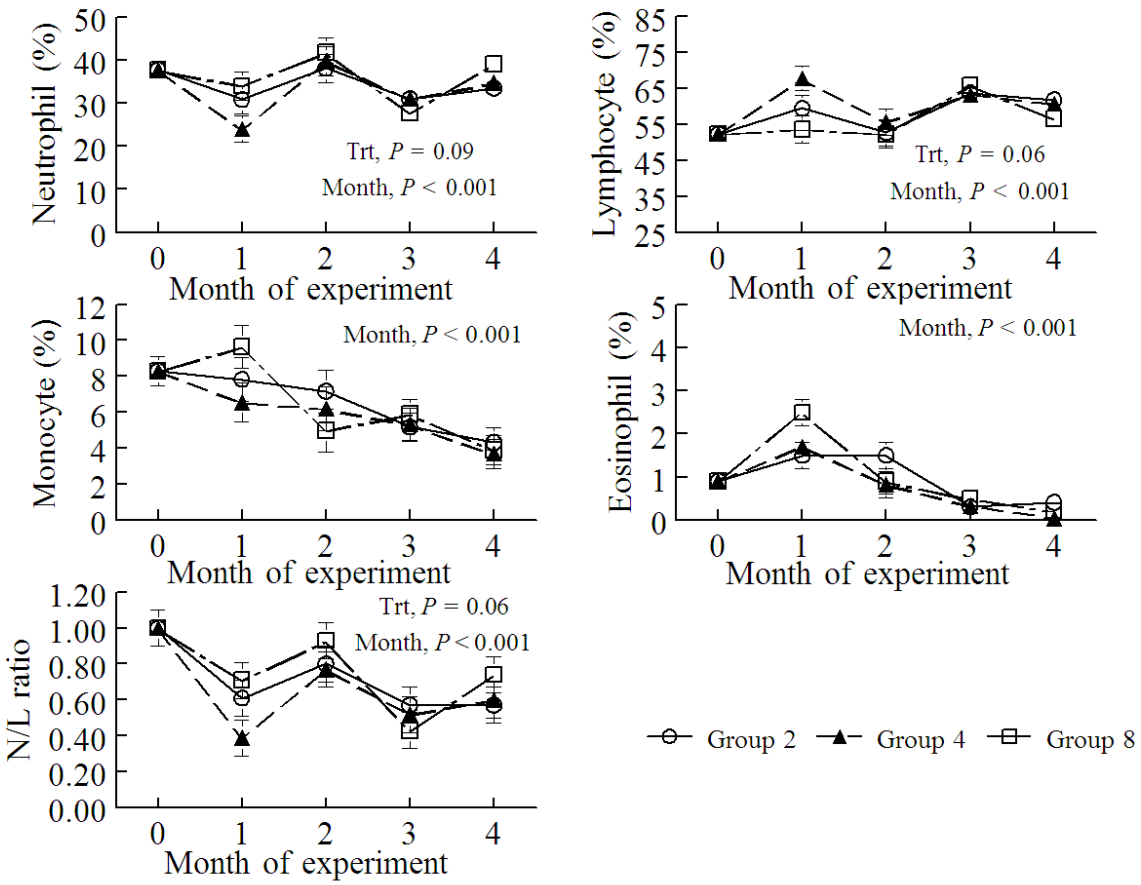


Fig.15. Differential leukocyte cell percentages (LSM ± SE) of veal calves housed in groups of 2, 4, 8 calves/pen over 5-months finishing period. Segmented neutrophil % (Panel a), lymphocyte % (Panel b), monocyte % (Panel c), eosinophil % (Panel d), and neutrophil to lymphocyte ratio (Panel e). Different superscripts indicate significant difference at (P< 0.05).

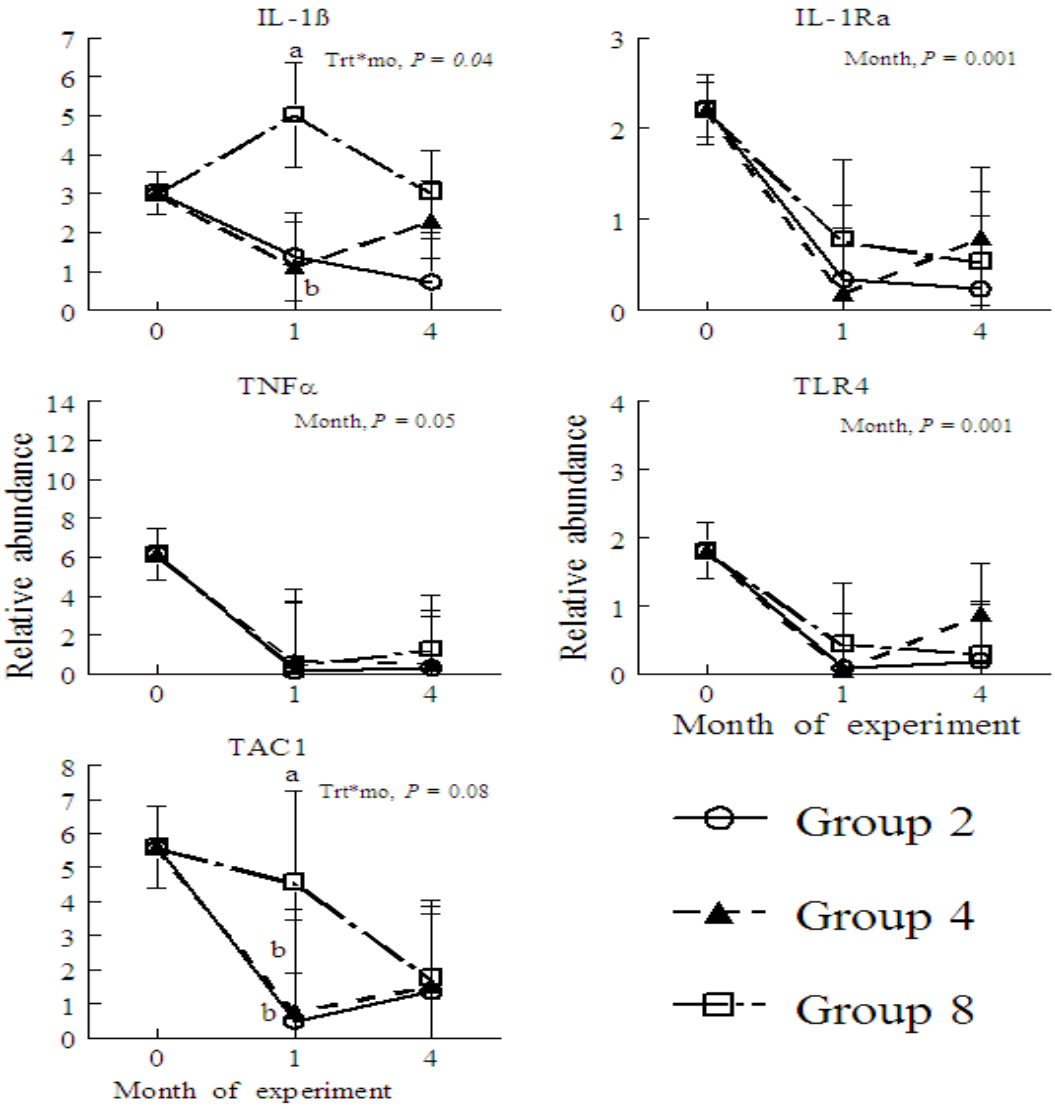


Fig. 16. Relative expression (ratio of gene of interest to 18S) of interleukin-1 (IL-1β mRNA; Panel a), interleukin-1receptor antagonist (IL-1Ra mRNA; Panel b), Tumor necrosis factor (TNF-α mRNA; Panel c), tachykinin1 (TAC1 mRNA; Panel d), and toll-like receptor4 (TLR4 mRNA; Panel e) in peripheral blood leukocytes from veal calves housed in groups of 2, 4, or 8 calves/pen inmo: 0 (41 ± 3 d of age), 1 (72 ± 3 d of age), and 4 (157 ± 3 d of age) relative to day of grouping. Data were shown as least squares means ± SEM. Data were log transformed for analysis but the non-transformed values of the data were presented. Different superscripts indicate significant difference at (P< 0.05).

Discussion

Discussion

The aim of this study was to investigate the effect of number of veal calves/group (stocking density) with equal space per calf and equal age on behavioral, productive, and physiological indicators of well-being during the entire finishing period. Previous studies have tested the effect of regrouping or group size in different spaces. Housing veal calves in large groups while maintaining calf floor space allowance offers more total space and increases options for calves to select their preferred areas in the pen. However, there are increased concerns about housing veal calves in large groups; thus, integrating physiological, behavioral, hematological, and productive indicators form the best basis for assessing the impact of increasing group size on the welfare of veal calves **(Rushen and de Passillé, 1992).**

II- Effect of group size on behavior and performance of veal calves

Effect of Group Size on Behavior

1.1. Instantaneous, 5-min Scan Sampling.

Ingestive Behaviors.

The results of the effect of group size, month, and periods of day on ingestive behavior of veal calves are presented in **Tables (5).** Analysis of 12-h scan sampling revealed that more ($P = 0.001$) calves ate and drank in groups of 2 than calves in the groups of 4 or 8 (9.5 ± 0.5^{a} vs. 6.2 ± 0.4^{b} and 4.1 ± 0.3^{c}, respectively). Furthermore, more ($P < 0.001$) calves ate and drank from 5 pm to 7 pm than during the other periods. Similarly, more ($P < 0.001$) calves in pens of 2 were observed chewing and ruminating than calves in groups of 4 or 8 (12.3 ± 0.7^{a} vs. 7.1 ± 0.5^{b}

and 5.5 ± 0.5c, respectively). As expected, calves chewed and ruminated more ($P < 0.001$) between 0700 and 0900 than in other time periods. In addition, more ($P = 0.012$) calves were observed chewing and ruminating during the 3rd and 4th month than during the first 2 months. In the current study, group size affected eating and drinking behavior, Fewer percentages of calves eating and drinking throughout the day in the groups of 4 and, especially, 8 may be caused by the increased social interaction among calves in these groups, which could have increased the speed of diet consumption, thereby reducing eating and drinking behavior in comparison to calves in groups of 2**. Jensen and Budde (2006)** showed that calves housed in groups of 6 were ingested milk faster than calves housed in groups of 2. Furthermore, the greatest percentage of calves observed eating and drinking were in groups of 2 and is likely attributed to increased number of visits to feeder by these calves throughout the day. **De Paula et al. (2010)** found that paired calves spent more time at the feeder, visited the feeder more often, and started ingesting concentrate more rapidly than did individually housed calves. The greatest periods of chewing and rumination were observed after morning feeding, when most calves, regardless of group size, were lying and engaged in rumination. Calves spent more time chewing and ruminating in periods from 10 am to 2 pm, similar to **Viessier et al. (1998),** and the greatest percentage of calves chewing and ruminating was observed during the last 3 months of our experiment, indicating, as expected, that rumination increased with age.

Non-nutritive oral behaviors.

The results of the effect of group size, month, and periods of day on abnormal oral behaviors of veal calves are presented in **Tables (6).** Non-nutritive oral behaviors observed in this study was objects manipulation and prepuce sucking. An interaction between group size and month was observed for objects'

manipulation (**Table. 6**). Pens with only 2 calves manipulated objects more ($P < 0.05$) throughout the day than calves in the groups of 4 or 8 during the 3rd observation month, whereas calves in groups of 2 and 4 manipulated objects more ($P < 0.05$) than calves in groups of 8 during the 4thmo of the experiment (treatment × month, $P = 0.029$). Moreover, calves manipulated objects more ($P < 0.001$) from 5 pm to 7 pm than in other time periods.

Prepuce sucking occurrences were rare and typically short in duration and not subjected to statistical analysis. In our study, the percentage of calves performing prepuce sucking was low (mean 0.86);only one pen of 8 excessively exhibited prepuce sucking during the last month of experiment and they were treated with anti-sucking devices and excluded from behavior observation during that month. The feeding regime used in this study (giving calves solid food with an adjusted fiber concentration of 4% directly after milk feeding) may have contributed to the low percentage of prepuce sucking. Several studies suggested that cross-sucking occurs less frequently when calves have free access to food **(Chua et al., 2002)** and are given roughage or food with high fiber content **(Bøe and Havrevoll, 1993).**

Social deprivation enhanced the non-nutritive oral activities in dairy calves **(Veissier et al., 1997; Chua et al., 2002).** Furthermore, **Veissier et al. (1998)** mentioned that calves housed in individual stalls spent more time licking the objects than those housed in groups of 4/pen. **Bokkers and Koene (2001)** mentioned that the low level of social contact in dairy calves is an important factor in directing oral behavior to objects. In our study oral behaviors were performed usually around the feeding time and most object manipulation was seen close to feeding times. **Bokkers and Koene (2001)** reported that most calves, regardless of group size, engaged in manipulation of objects before and after milk feeding.

Social interaction (conspecific contact).

Data of conspecific contact was represented in (**Table 6**). More ($P < 0.001$) calves in groups of 4 and 8 participated in conspecific contact than calves in groups of 2 (8.4 ± 0.4^{a} and 7.9 ± 0.4^{a} versus 4.7 ± 0.3^{b}). However, conspecific contact was often observed around feeding time (from 1700 to 1900; $P = 0.01$), in particular licking and sucking on the areas of face and ears. The mean number of calves showed social interaction during the periods 0700 to 0900, 0900 to 1300, 1300 to 1700, and 1700 to 1900 was 6.4 ± 0.4b, 6.7 ± 0.3b, 5.3 ± 0.3c, and 9.3 ± 0.4a, respectively. More calves interacted with each other socially in groups of 4 and 8 when compared with calves housed in groups of 2. These results were strongly supported by previous research **(Babu et al., 2004; Færevik et al., 2007),** which concluded that group housing affords calves an opportunity to interact with each other and perform normal social behavior. Moreover, the period of greatest conspecific contact was observed directly after drinking milk, when calves sucked and licked on areas around the mouth and ears in order to obtain remnants of milk in those areas. **Jensen and Budde (2006)** did 30-min behavioral recording immediately after milk feeding, and found that most cross-sucking was directed to the head and around the muzzle, which was covered with milk. However, in our study bout duration of conspecific contact during feeding time was greatest for calves in groups of 2 than those in groups of 4 and 8, which may indicate that calves in groups of 2 had no disturbance from pen-mates.

Inactivity.

More calves in groups of 8 were inactive ($P < 0.001$, **Table 6**) compared to calves in groups of 2 or 4 (57.9 ± 1.9a versus, 51.7 ± 2.1b and 52.8 ± 2.1b, respectively). Whereas, calves were more (P = 0.001) active in the ^{4th} month than in other months. In addition, the greatest ($P < 0.001$) proportion of inactivity was

in period 3 (1pm to 5 pm), and the lowest ($P < 0.001$) percentage of inactivity was observed in period 4 (5 pm to 7pm). Our study revealed that more calves in groups of 8 were inactive than calves in groups of 2 or 4 which could be a result of displaced calves waiting for access to the feed **(Færevik et al., 2007)**. However, the greatest period of inactivity was from 1300 to 1700 h, where most calves showed a typical afternoon lying bout, whereas most activity was from 1700 to 1900 h, which corresponded with feeding time. Calves spent 38% of their day inactive and the most inactive period occurred between milk meals **(Veissier et al., 1998).**

Self-grooming.

More ($P < 0.001$, **Table 6**) calves in groups of 2 were observed self-grooming than calves in groups of 4 and 8 (9.0 ± 0.4a versus 5.1 ± 0.3b and 2.8 ± 0.2c, respectively). Period of day did not ($P = 0.241$) affect self-grooming. Group size changed how oral needs of veal calves were manifested; calves housed in groups of 2 had less social contact. So, oral behavior appeared to be directed to either objects (greater objects manipulation) or to itself (greater self-grooming).

Posture.

(Table. 7) revealed that calves in groups of 4 and 8 stood more ($P = 0.001$) throughout the day compared to calves in groups of 2 (43.1 ± 1.6a and 43.1 ± 2.4a versus 40.2 ± 1.5b). Calves stood the most ($P = 0.001$) during period 4 (5 pm to 7 pm) than during any other observation period. Calves in the groups of 4 and 8 stood more when compared with calves in groups of 2, which may be attributed to available free space in these groups which gives an opportunity for calves to stand and walk more. Although calves in the present study had the same space allowance, there was greater usable space as group size increased. Space allowance seems to be an important factor for expression of all types of postures. **Veissier et**

al. (1997) found that calves moved more easily when they were housed together in a pen than when they were housed in individual stalls because they can walk together around the pen and lie down close to other calves leaving space for others to remain standing. In addition, most standing behavior was observed around the time of feeding, which includes standing while engaged in eating and drinking.

An interaction between group size and month was reported for lying behavior **(Table. 7).** Calves housed in pens of 2 were observed lying more ($P < 0.05$) in the 2^{nd} mo than calves from groups of 4 or 8, whereas calves in groups of 2 and 4 lay more ($P < 0.05$) than calves in groups of 8 in mo 3 and 5 (treatment × month, $P < 0.001$). Most calves were observed lying in period 3(1 pm to 5pm), and the fewest ($P < 0.001$) calves were observed lying in period 4 (5 pm to 7 pm). On the other hand, the reduced lying behavior in groups of 8 and 4 could be attributed to greater social interaction between calves and disturbance from pen-mates, which prevent lying in those groups. **Færevik et al. (2007)** concluded that time spent lying decreased with increasing group size. In addition, most lying behavior occurred from 1300 to 1700 h, which was typical of afternoon lying bouts between morning and evening feeding times. **Babu et al. (2004)** noted that calves ruminated more while lying than standing, and most rumination occurred during the post-milk feeding period.

Walking.

An interaction between group size and month was reported for locomotion behavior **(Table. 7).** In the 4^{th} and 5^{th}mo, calves housed in groups of 4 and 8 walked more ($P < 0.05$) than calves from groups of 2 (treatment × month, $P = 0.003$). Moreover, calves walked more ($P = 0.001$) from 0700 to 0900 and 1700 to 1900 than between 0900 and 1700.

Calves housed in groups of 8 and 4 walked more than calves housed in small groups of 2, suggesting that increased group size is accompanied with increased locomotion. The increased locomotion in larger groups may be attributed to the higher social interaction which force calves to move away to escape competitors **(Færevik et al., 2007),** moving away to avoid prepuce sucking and aggression between calves **(Bøe and Færevik, 2003),** or may be attributed to increased usable space in pens of these groups **(Chua et al. 2002). Telezhenko et al. (2012)** found that group size had no effect on movement of cows. They concluded that pen size may be more important than density or group size because group-housed dairy cows were able to share the space in the pen, and even with the same number of cows/m^2, larger pens provided more free space for cows to move about. More walking was observed at the time of feeding from 0700 to 0900 h and 1700 to 1900 h which corresponded to greater activity in these periods **(Bokkers and Koene, 2001; Webb et al., 2012).**

Aggressive Behaviors.

Chi-square analysis of aggressive behaviors throughout the day for each observation month revealed that group size had no effect on aggressive (**Table 8**). In agreement with **Grasso et al. (1999),** the insignificant amount of aggressive interactions observed in this study may be attributed to use of instantaneous scan sampling, which is considered to be insensitive to short-lasting behaviors, such as aggression in calves. Results of the present study agree with those of **Telezhenko et al. (2012),** who found that number of displacements at the feed bunk was not affected by group size (6 vs. 12 calves/group). **Kondo et al. (1989)** reported that sufficient space was of greater importance than group size in order to reduce the occurrence of agonistic behavior in calves. In the current study, the space allowance of 1.82 m^2 /calf for all groups exceeded the European Community's

minimum space allowance recommendation (**European Council, 2008**), which resulted in similar degree of aggression among treatments.

Play Behavior.

Chi-square analysis of play behavior throughout the day for each observation month revealed that group size had no effect on play behavior. (**Figure 10**) However, in the 3rd month of the experiment, calves in groups of 4 tended (P = 0.06,) to play more than calves in groups of 2 and 8. **Jensen and Kyhn (2000)** noted that keeping calves in groups while providing space above the minimum requirements of 1.5 m^2 /calf afforded them a greater opportunity to play, and play behavior may be a good indicator of well-being in veal calves. In our study, the similarity in occurrence of play behavior in all treatments may be attributed to similarity in space allowance (1.82 m^2 /calf).

1.2. Continuous Focal Observations.

Frequency. The results revealed that group size had no ($P \geq 0.09$) effect on frequency of eating and drinking, chewing and ruminating, manipulation of objects, conspecific contact, and aggression during feeding times (**Table 9**). The most common aggression encounter subjectively observed during feeding time was displacement from the feeding trough to gain access to milk or solid feed, whereas, butting and mounting were rarely seen.

Total duration. Continuous observations around feeding time revealed that the total duration of eating and drinking, chewing, objects manipulation, conspecific contact, and aggression were similar ($P > 0.05$) among treatments (**Table 10**). Even though more ($P = 0.01$) aggression occurred at the feed trough on d 1, 14, and 70 than on d 5 and 42.

Bout duration. Group size had no ($P \geq 0.11$) effect on bout duration of eating and drinking, chewing and ruminating, objects manipulation, and aggressive behaviors during feeding times (**Table 11**). Conversely, calves in groups of 2 had greater ($P = 0.01$) bout duration of conspecific contact than calves in groups of 4 and 8, and bout duration of aggression was greater ($P < 0.01$) on d 1, 14, and 70 than d 5 and 42 of behavior observation.

Our results concluded that the frequency, total duration, and bout duration of ingestive behavior during the feeding time were similar among group sizes, which corresponds to growth performance. Also, frequency, total duration, and bout duration of object manipulation around feeding times were similar among group sizes, which is consistent with the finding of **Jensen and Budde (2006)**, who found that group size had no effect on time spent licking fixtures around milk feeding time.

When continuous observations were used, the most common aggression encounter observed subjectively during feeding time was displacement from the feeding trough. Frequency of aggression at the feed trough was not affected by treatment, which may be attributed to allowing each calf an ample feeder space allowance by portioning the trough. Greater aggression, as evidenced by greater total and bout durations of aggression during feeding, was observed on d 1, 14, and 70 after grouping, with no particular pattern, in contrast, **Færevik et al. (2010)** reported that the number of displacements from the feed manger increased over time.

Effect of Group Size on Growth Performance

Veal producers are concerned that group housing of calves will affect performance and, consequently, their profits. In the current study, grouping of calves had no adverse effect on growth performance of veal calves. Throughout the

5-mo study, no ($P \geq 0.50$) differences among group sizes were found regarding initial and final BW, BW gain, and ADG **(Table 12).** As expected, hip height and heart girth increased ($P = 0.001$) as calf age increased; however, neither hip height ($P = 0.38$) nor heart girth ($P = 0.82$) were affected by the number of calves in a pen **(Table 13).** These results are in agreement with **Fæverik et al. (2007) and De Paula Vieira et al. (2010),** who reported similar growth performance among different group sizes, but contradict previous studies that reported increased weight gains for group-housed calves **(Chua et al., 2002; Xicatto et al., 2002; Tapki, 2007).** These conflicting results may be attributed to the difference the focus of the investigation. Proper management of the housing system, like feeding, ventilation, cleanliness, and calves' immunity, may play more important roles in growth and performance of calves than group size.

Effect of Group Size on Health Status

(Table 14) revealed that there were no ($P = 0.15$) differences among group sizes regarding the incidence of diarrhea, but fecal scores were greater ($P < 0.001$) during the 3rdmo than during mo 1, 2, or 4. In the first mo of the trial, calves in groups of 8 coughed more ($P < 0.05$) than calves in groups of 2 and coughed more than calves in groups of 4 and 2 during the 2ndmo (treatment × month, $P = 0.03$; **Fig. 11**) in response to an induced cough. In addition, nasal discharge scores were similar ($P > 0.05$) among group sizes during mo 1, 2, and 3, but calves in groups of 2 and 4 had more ($P < 0.05$) nasal discharge than calves in groups of 8 in the 4thmo (treatment × month, $P = 0.02$; **Fig.12**). Ocular discharge and ears scores did not ($P \geq 0.06$) differ among group sizes; however, calves had more ocular discharge ($P = 0.003$) and drooped ears ($P = 0.04$) in mo 1 than mo 2, 3, and 4.

Greater coughing observed in groups of 4 and 8 during the first 2 months after grouping may be a response to the close physical contact between many

calves and increased social contacts in large groups which increased susceptibility to infection; however, visual observation is not a definitive measure of the health status of calves. Detecting sick calves and treating them are more difficult in large groups. The risk of respiratory disease was 2.8 times greater in calves housed in large groups than in individual pens **(Svensson et al., 2003).** Our results demonstrated that group housing of young calves in groups up to 8 calves/pen did not inevitably lead to increased health problems. The incidence of diarrhea was similar among treatments, which agrees with results of **Svensson and Liberg (2006),** who found no difference in diarrhea between calves kept in the small- vs. large-sized groups.

Effect of Group Size on Plasma Cortisol Concentrations

(Fig. 13) revealed that plasma cortisol concentrations were similar ($P = 0.42$) among calves in groups of 2, 4, and 8; however, calves had greater ($P = 0.03$) cortisol levels during mo 3, 4, and 5 (15.5 ± 5.7, 18.4 ± 5.5, and 20.3 ± 5.3 ng/mL, respectively) than the first 2 months of the experiment (10.8 ± 5.8, and 10.8 ± 5.8 ng/mL for mo 1 and 2, respectively).

Group housing of veal calves may represent a source of chronic stress for the calf **(Veissier et al., 1998),** and plasma cortisol levels have been used in cattle to detect activation of the hypothalamo-pituitary-adrenocortical axis due to external stressors (**Friend et al., 1987; Munksgaard et al., 1999; Burdick et al., 2011).** It was hypothesized that calves housed in larger groups would have greater plasma cortisol levels, but results of the present study revealed that group size did not alter circulating cortisol concentrations, which may be attributed to housing of calves in the same space area per calf; yet, at the same time, calves housed in groups of 8 were subjectively observed to be difficult to handle during data collection and blood sampling. This may not be calf reactivity per se, but calves can escape more

easily from the handler due to increased floor space and increased interference because of the number of calves. **Veissier et al. (1998)** observed no effect of group housing on circulating ACTH level in response to a corticotrophin releasing factor challenges or on cortisol level during dexamethasone and ACTH challenges. However, they found that calves housed in groups had grater basal cortisol concentrations and those calves were more reactive to weighing than calves housed individually. The increased plasma cortisol level with advancing age may be attributed to changes because of increased growth. **Stull and McMartin (1992) and Wilson et al. (1999)** noted that cortisol concentrations increased over time during the production cycle of Holstein veal calves.

Effect of Group Size on Blood Hemoglobin Concentrations

(Table 15 and Fig. 14 and) revealed that no differences (P = 0.14) were found in Hb concentrations due to housing of veal calves in groups of 2, 4, or 8 (9.0 ± 0.1^{a}, 8.5 ± 0.2^{a}, and 8.6 ± 0.2^{a} g/dl, respectively). However, Hb was greatest ($P < 0.001$) in all calves during the 1^{st} and 2^{nd}mo and least in mo 5. In the present study, Number of calves per pen had no effect on blood hemoglobin concentrations which were used as indicator of anemia when calves provided with the same floor space allowance (1.8 m^2/calf). The Hb concentrations at the end of fattening period were above the minimum threshold of 7.2 g/dL set by the European Union **(EFSA, 2006).**

III- <u>Acute phase cytokines, TAC1, and Toll-like receptor 4 mRNA expression association with housing and health in veal calves</u>

This study utilized the group size as a model to investigate the effect of group housing on innate immunity of veal calves. Very little is known about how grouping calves will influence the immune response at both physiological and molecular levels. Previous studies of calves' group housing have focused on

behavior **(Veissier et al., 1997 and Færevik et al., 2007), growth (Xiccato et al., 2002),** health status **(Svensson and liberg, 2006),** traditional indicators of stress such as cortisol **(Friend et al.1985 and Veissier et al., 1998).** It thought that grouping veal calves after they have been housed individually for 6 weeks may be stressful. Group housing may introduce additional factors that may result in stress and suppression of innate immunity by increase in glucocorticoids and introduction of novel pathogens through cross-sucking and calf-to-calf interaction **(Hulbert and Balloul, 2012).** For this study, we used mRNA to monitor the gene expression in blood leukocytes, physiological measures and health to examine effect of group size over 5 months finishing period. Stress can impair the immune, endocrine, and nervous systems. The lack of change in plasma cortisol concentrations between treatments indicated that calves had already adapted to group housing. Friend (1980) reported that response to ACTH decrease after some weeks of exposure to a chronic stress. Traditionally, assessment of physiological stress has been estimated by measuring levels of sympathoadrenal and HPA hormones, such as cortisol. However, there are associated drawbacks, such as the potential for rapid changes in hormone levels as a response to short–term acute stressors. Handling alone can stimulate a major cortisol response. These factors substantially diminish the use of plasma cortisol measurements in assessing animal immune status **(Coetzee et al., 2008).**Thus, an alternative method of assessing physical stress with potential to reflect a more chronic state for the animal, is investigation of relative white blood cell counts **(Davis et al., 2008).** Cattle blood immune parameters like white blood cells are sensitive indicators of the physiological or patho-physiological responses of animals to stress **(Gupta et al., 2007).**

1. ***Differential leukocyte count***

The results of the effect of group size and month on differential leukocyte count of veal calves are presented in **Figure (15).** Calves housed in groups of 8 tended ($P = 0.09$; **Panel a**) to have greater neutrophil percentage than those housed in groups of 4 or 2. Moreover, there was significant effect of month ($P< 0.001$) on neutrophil percentages. Calves had greater neutrophil percentage during months 2 and 4 (39.8 ± 1.9 and 35.7 ± 1.3 %, respectively) than months 1 and 3 relative to the day of grouping (29.6 ± 1.9 and 29.8 ± 1.4 %, respectively). Similar to neutrophil percentage, there was also an effect of group size on lymphocyte percentage. Calves housed in groups of 8 tended to have lower lymphocyte percentage ($P = 0.06$; **Panel b**) than those kept in groups of 4 or 2. A month effect was found for lymphocyte percentages ($P < 0.001$). Calves had lower lymphocyte percentage during months 2 and 4 (53.6 ± 2.1 and 59.7 ± 1.5 %, respectively) than mo 1 and 3 after grouping (60.3 ± 2.1 and 64.2 ± 1.6 %, respectively). Although month ($P < 0.001$) had an effect on monocyte and eosinophil percentages, no effect of group size or interactive effects were detected **(Panel c and d).** Monocyte and eosinophil percentages decreased with advancing time. In contrast, group size tended to have an effect on neutrophil to lymphocyte ratio ($P = 0.06$; **Panel e**) with calves housed in groups of 8 tending to have greater neutrophil to lymphocyte ratio than calves kept in groups of 4 and 2. Also a month effect was found for neutrophil to lymphocyte ratio ($P < 0.001$). Calves had greater neutrophil to lymphocyte ratios during months 2 and 4 (0.83 ± 0.09 and 0.63 ± 0.06, respectively) than months 1 and 3 after grouping (0.57 ± 0.09 and 0.51 ± 0.07, respectively). The basophil percentages ranged from 0 to 2 in all treatments.

The significant increase in neutrophil and decrease in lymphocyte that were observed in calves housed in groups of 8 indicated that these calves suffered from

stress. Neutrophilia and lymphopenia are common findings in stressed animals and are associated with change in the WBC trafficking and release from the bone marrow by elevated concentration of glucocorticoids **(Dunn, 1989).** Neutrophilia, lymphopenia and eosinopenia that were observed in this study on mo 2 and 4 are associated with increased respiratory infection in these months. It has been previously reported that increased neutrophil and decreased lymphocyte and eosinophil percentage occurred following a variety of environmental stressors of cattle including diseases of bacterial infection **(Radostist et al., 1994).** The tendency for N/L ratio for calves in groups of 8 to be higher suggests that calves housed in groups of 8 experienced profound stress response compared to those housed in groups of 4 or. **Svensson and libeng (2006)** found that calves housed in pens of 12 to 18 calves had a higher incidence of respiratory illness than calves housed in groups of 6 to 9 calves. They demonstrated that reducing group size is associated with a reduced risk of respiratory illness. In the same line, **Davis et al. (2008)** mentioned that physiological stress causes a concurrent rise in neutrophil (N) number, and drop in lymphocyte (L) number, and this ratio has also been used as a welfare indicator in several studies of veal calves **(Friend et al., 1987; McFarlane et al., 1988; Stull and McDonough, 1994)**. In the present study, the N:L ratio was within the normal range reported in the most veal calves studies which ranged from 0.51 to 0.83 **(Wilson et al., 1999).** However, the N:L ratio was elevated in mo 2 and 4 in all treatments which corresponded to neutrophilia and lymphopenia detected in the same months. **Mattiello et al., (2002)** mentioned that N: L ratios greater than the range of 0.35 to 1.10 between 2 wk of age and market weight were indicative of stress in special-fed veal calves.

2. *Leukocyte mRNA gene expression*

The data of mRNA gene expression was reported in **Figure 16.** An interaction between group size and month of experiment was observed for expression of IL-1β (*P* = 0.04, **Panel a**) and TAC1 mRNA (*P* = 0.08; **Panel e**). On the 1st month after grouping, veal calves housed in groups of 8 had greater expression of IL-1β mRNA and tended to have greater TAC1 mRNA expression than calves housed in groups of 4 and 2. There was no effect of group size on expression of IL-1Ra (*P* = 0.93; **Panel b**), TNF-α (*P* = 0.66; **Panel c**) and TLR4 mRNA (*P* = 0.97; **Panel d**). However, a day effect was found for expression of IL-1Ra (*P* = 0.001), TNF-α (*P* = 0.05) and TLR4 mRNA (*P* = 0.001); expression of these genes were greater at day of grouping than at the 1st and 4th month after grouping (*P* = 0.001).

In the current study, the relative gene expression of a number of pro-inflammatory (IL-1β, TNF-α) and anti-inflammatory (IL-1Ra) cytokines was measured to assess the inflammatory stress response following group housing. Interleukin-1β is a cytokine produced by monocytes and macrophages and mediates a variety of immune-regulatory and inflammatory activities including propagation of activated T-helper cells, induction of fever, stimulation of prostaglandin and collagenase production, enhancement of B-cell responses, neutrophil mobilization and chemotaxis, and induction of acute phase proteins. Interleukin 1 expression is rapidly up-regulated following immune stimulation (Takashi and Kodama, 1994). Up-regulation of IL-1β in calves housed in groups of 8 on the first month after grouping could indicate immune stimulation due to respiratory infection. We observed that calves in groups of 8 had greater coughing during the first 2 mo after grouping than calves in groups of 4 and 2. As the immune response for any infection, the innate immune response of the lung leads to release of pro-inflammatory cytokines such as IL-1β, TNF-α, and expression of

receptors such as TLR4. Interleukin-1β has been shown to be among the most biologically active cytokines in the lung early after the onset of infection. **Malazdrewich et al. (2001)** showed that expression of IL-1β was significantly increased in the airways and lung lesions of calves infected with bovine pneumonic pasteurellosis (the common and economically important disease of cattle) compared with mock-infected controls. Also, they found a correlation between the pulmonary expression of the inflammatory cytokines TNF-α, and acute pathological changes in the lung. Expression of IL-1β in peripheral leukocytes supports the fact that expression of IL-1β was more generalized and not localized to alveolar macrophages. The increased transcriptional response of IL-1β in leukocyte on the first month shows an enhanced response of the pro-inflammatory cytokines to respiratory infection. The pro-inflammatory effect of IL-1β can be inhibited by IL-1 receptor antagonist (IL-1Ra). IL-1Ra is produced by immune complex- or IL-4-stimulated macrophages and by TNF-stimulated neutrophils. IL-1Ra inhibits IL-1 action by competing with IL-1 for binding to the IL-1 receptor. The significant day effect, characterized by lower (P = 0.001) IL-1Ra expression on mo 1 and 4 after grouping, may reflect immunological adaptation of the rapidly developing calf to pathogen exposure during the first months of life. Failure to find any significant effect of group size on expression of TNF-α mRNA agrees with results obtained by **Malazdrewich (2001)** who mentioned that TNF-α was the least upregulated cytokine in case of respiratory infection in calves than expression of IL-1β and IL-8. Northern blots and ELISAs suggested that TNF-α gene and protein expression occurred predominantly within lung airways, and In Situ hybridization studies confirmed that TNF-α mRNA expression was localized to alveolar macrophages. Expression of IL-1β and IL-8 genes and proteins, by contrast, was more generalized. Tumor necrosis factor-α produced by monocytes and macrophages, is a key mediator of the inflammatory response and it promotes T-

and B-lymphocytes proliferation. Blood TNF-α concentration is frequently elevated during acute and chronic inflammation associated with the body's response to infection and is considered a marker for immune activation **(Nonnecke et al., 2009).** The significant decrease in expression of TNF-α on mo 1 and 4 after grouping compared with d 0, could be related to the significant decrease of monocytes ($P < 0.001$) with advancing age in the present study.

To date, 10 TLRs have been identified. TLR-4 is responsible for gram negative lipopolysaccharide (LPS) recognition and cell-signaling. TLR4 induces the transcription of inflammatory cytokines, chemokines, and establish the first line of defense against injury or disease (**Uematsu and Akira, 2007**). Toll-like receptor 4 leads to increased interferon (IFN)-β, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-13 (**Eicher et al., 2004**). The greater expression of TLR-4 mRNA on the day of grouping as compared to mo 1 and 4 after grouping could be related to the start of respiratory bacterial infection on that day especially in groups of 8. The down-regulated TLR4 which is essential in recognition of invasive pathogens in mo 1 and 4 after grouping could be the cause of higher coughing groups of 8. The lipopolysaccharide of Gram-negative bacteria is recognized by a complex of TLR-4 and cluster of differentiation (CD14) at the surface of macrophages and neutrophils, and subsequently activated macrophages produce and secrete cytokines. Important cytokines produced by activated macrophages in response to bacterial infection include TNF-α, IL-12, and IL-8 **(Pan, 2011).**

Greater expression of TAC-1 mRNA observed in groups of 8 during first mo after grouping could be an immune response to respiratory infection or indicator of social stress in those large groups.The neuroendocrine stress response especially peripheral nervous system amplifies the local immune responses, with many

neuromediator display a wide variety of immunomodulatory actions (**Spika et al., 2010**). The gene TAC-1 is a precursor gene for substance P, a neuropeptide that is involved in pain perception. Substance P (SP) is a member of tachykinin (TAC) family neuropeptides which are small molecules secreted from the peripheral terminals of sensory nerve fibers and act as neurotransmitter or hormone (**Mapp et al., 2000**). Also immunologic active cells have been shown to release SP after activation including macrophages, eosinophils, lymphocytes, and dendritic cells. SP enhances lymphocyte proliferation and chemotaxis (**Schratzberger et al., 1997).** Substance P stimulates macrophage and eosinophil to secrete the pro-inflammatory cytokines TNF-α, IL-1β, IL-2 and IL-6 in vitro (**Bardelli et al., 2005**). Additionally, the expression of SP receptors was demonstrated in bovine alveolar macrophages (**Rogers et al., 2006**). Up-regulation of tachykinin was observed in lung cells of calves infected with bovine respiratory syncytial virus (BRSV) which are the major causes of respiratory diseases in young calves. Several symptoms associated with severe BRSV infection, such as bronchoconstriction, excessive mucus production and oedema, are induced by substance P and other tachykinins (**Valarcher et al., 2006**). Housing calves in large groups increased the number of direct contact between calves which can lead to increase spread of viral respiratory tract diseases. Respiratory-tract disease is mostly caused by viral infections and virus transmission by aerosol is well documented (**Svensson and liberg, 2006**). Our results reflected that expression of TAC1 mRNA may be an up-regulation associated with inflammation and pain. **Spika et al. (2010)** mentioned that substance P has a strong influence on up-regulation of IL-1β. This study showed that SP up-regulated in the same time with IL-1β up-regulation which may suggests that there is a link between the neurotransmitter SP and cytokines production.

Summary

Summary

The study was conducted at a Strauss Veal Feeds Inc. (North Manchester, IN, USA) finisher barn, which provided facilities, calves, and feed in cooperation with Benha University. The objectives of the present study were:

1) To determine the effect of group size on behavior, health, growth, and welfare of veal calves,
2) To investigate the effect of group size on immune system
3) To evaluate leukocyte gene expression of major pro- and anti-inflammatory cytokines mRNA of veal calves housed in different group size during the finishing period.

The first part of the study:

The study was undertaken in spring and summer of 2012 from March 21 to July 21, 2012 on one commercial veal farm. Holstein-Friesian bull calves (n = 168; 44 ± 3 d of age) were assigned randomly to 1 of 3 treatments of group housing with 2, 4, or 8 calves/pen. The pens used for housing were 3 × 1.20 m (2 calves/pen), 3 × 2.40 m (4 calves/pen), and 3 × 4.80 m (8 calves/pen), supplying a total pen space allowance of 1.82 m^2 /calf, regardless of pen size. Behavior was recorded from video data throughout the day from 0700 to 1900 h, during a single day each month for 5 mo using scan sampling every 5 min within 30-min observation sessions. On d 0, 1, 5, 14, 42, and 70 after grouping, continuous focal sampling around feeding time (30-min intervals before, during, and after feeding) focused on oral and aggressive behaviors. Plasma cortisol, blood hemoglobin concentrations and differential leukocyte counts were determined.

The first part of the study:

mRNA expression of interleukine-1β (IL-1β), IL-1 receptor antagonist (IL-1Ra), tumor necrosis factor (TNF)- α, toll-like receptor 4 (TLR4) and tachykinin 1 (TAC1) was determined using real-time RT-PCR in calves blood leukocytes. Health was evaluated monthly.

The obtained results summarized in:

1) In the present study, housing veal calves in large groups (4 or 8 calves/pen) resulted in more conspecific contact, walking, standing, and less objects manipulation, self-licking and less lying when compared with calves housed in small groups (2 calves/pen), although calves were provided with the same housing space.
2) Housing of veal calves in large groups changed how oral needs were manifested; calves housed in groups of 8 had more social contact. So, oral behavior appeared to be directed to pen-mates.
3) The number of calves in a group with the same space (1.82 m2 per head) did not affect growth performance of veal calves.
4) Neither plasma cortisol nor blood hemoglobin were ($P \geq 0.14$) affected by group size.
5) Housing of veal calves in groups of 8 was associated with greater percentage of neutrophil, lower percentage of lymphocytes, and greater neutrophil to lymphocyte ratios.
6) In agreement with our hypothesis, we observed that increasing the number of animals per group from 2 to 8 was associated with greater coughing during the first 2 months after grouping

7) In agreement with our anticipation, expression of IL-1β and TAC1 mRNA was up-regulated in peripheral leukocytes of veal calves housed in groups of 8 compared to those kept in groups of 4 or 2.
8) Our results suggest that there is a link between the neurotransmitter SP and cytokines production.
9) These results may have important implication for the assessment of social stress in farm animals and the development of novel science-based variable used to assess animal well-being in livestock production systems. Measuring the expression of some marker genes in leukocytes in addition to use of traditional methods including total leukocyte count and N:L ratio is a good approach for assessment of stress in young calves. Further investigation needed to know how shifting of veal industry from individual to group housing will modulate the innate immunity of calves.

We concluded that:

1- When provided with the identical space allowances, the number of veal calves in a group did not affect production and physiological indicators of welfare and had transient effects on health during the finishing period.
2- Housing of veal calves in groups of 4 and 8 after 6 wk of age had no detrimental effects on growth, and performance, and provided calves with a greater opportunity to interact socially and utilize available space when compared with calves housed in groups of 2. However, calves housed in groups of 2 had the benefits of more eating and drinking, self-grooming, and lying behaviors and less inactive periods.
3- There is immunological evidence of stress from group housing of calves in larger groups even when the space allowance per calf was the same (1.8 m2/head).

4- Housing of veal calves in groups of 8 was associated with neutrophilia, lymphopenia, and greater neutrophil to lymphocyte ratios and greater coughing during the first 2 months after grouping. Therefore, these data suggest that housing of veal calves in larger groups during the finishing period may lead to greater incidence of respiratory disease.

Conclusion

Conclusion

When provided with the identical space allowances, the number of veal calves in a group did not affect production and physiological indicators of welfare and had transient effects on health during the fattening period. Housing of veal calves in groups of 4 and 8 after 6 weeks of age had no detrimental effects on growth, and performance, and provided calves with a greater opportunity to interact socially and utilize available space when compared with calves housed in groups of 2. However, calves housed in groups of 2 had the benefits of more eating and drinking, self-grooming, and lying behaviors and less inactivity. There is immunological evidence of stress from group housing of calves in larger groups even when the space allowance per calf was the same (1.8 m^2/head). Housing of veal calves in groups of 8 was associated with neutrophilia, lymphopenia, and greater neutrophil to lymphocyte ratios and greater coughing during the first 2 months after grouping. Therefore, these data suggest that housing of veal calves in larger groups during the fattening period may lead to greater incidence of respiratory disease.

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Arabic summary

الملخص العربى

سلوكيات وكفاءة العجول البتلو وعلاقتها بالاسكان الجماعى

أجريت هذه الدراسة فى قسم علوم الحيوان بجامعه بردو، ولايه انديانا ، الولايات المتحده الامريكيه كجزء من بعثة الاشراف المشترك بين جامعة بنها فى مصر وجامعه بردو بالولايات المتحده الامريكيه وكان الهدف منها:

١- دراسة تأثير حجم المجموعة فى الاسكان الجماعى على سلوكيات وصحة، ونمو ورفاهية العجول البتلو خلال فتره التسمين.

٢- دراسة تاثير حجم المجموعه على الجهاز المناعى لعجول البتلو خلال فتره التسمين.

٣- معرفة تاثير الاسكان الجماعى على التعبير الجينى لبعض الجينات المرتبطه بالضغط النفسى.

الجزء الاول من الرسالة :

وقد أجريت هذه الدراسة في ربيع وصيف عام ٢٠١٢ في الفترة من ٢١ مارس وحتى ٢١ يوليو فى مزرعة تجارية لتربيه العجول البتلومن نوع الفريزيان هولشتاين، استخدم فى هذه التجربه عدد ١٦٨عجل عمر(٤٤ ±٣ يوم) واستمرت حتى الذبح عند عمر ٥ شهور.قسمت العجول عشوائيا إلى ثلاث معاملات من الاسكان الجماعى هى ٢، ٤ و ٨ عجول /حظيره ابعادها كالاتى ٣ × ١.٢٠ م (٢ عجل / حظيره)، ٣ × ٢.٤٠ م (٤ عجول / حظيرة)، و ٣ × ٤.٨٠ م (٨ عجول / حظيرة) بما يعنى ثبات المساحة الارضيه لكل عجل فى جميع المعاملات (١.٨٢ م٢ لكل عجل). وذلك لقياس الصفات التالية: بعض الانماط السلوكية وكذا وزن الجسم والزيادة المكتسبة فى وزن الجسم وبعض العلامات الصحية على الحيوان وبعض قياسات الدم والتعبير الجينى لبعض الجينات المرتبطه بالضغط النفسى.

تم تسجيل سلوكيات الحيوانات خلال يوم واحد من كل شهر لمده ٥ شهور باستخدام طريقه المسح كل خمس دقائق داخل دورات الملاحظة ٣٠ دقيقة. تم تقسيم اليوم الى ٢٤ دورة ملاحظة (٣٠ دقيقة/دورة). وتم تسجيل سلوكيات العجول كل خمس دقائق. كما تم تسجيل سلوكيات العجول بطريقة الملاحظه المستمرة اثناء فترة التغذية المسائيةخلال الايام (يوم الاسكان، ١، ٥، ١٤، ٤٢، و ٧٠ يوممن بدايه الاسكان الجماعى)، تم قياس بعض الانماط السلوكيه اثناء فترة التغذية (قبل الاكل وخلاله وبعده بمعدل ٣٠ دقيقه لكل فترة) وركزت الملاحظات على السلوكيات العدوانيه، التغذية،الشرب، تناول الاجسام المحيطه واللحس المتبادل).

تم قياس تركيز الهيموجلوبين فى الدم وكرات الدم البيضاء بانواعها المختلفه وكذا قياس هرمون الكورتيزول فى بلازما الدم وذلك بمعدل مره شهريا طوال فترات التجربة.

الجزء الثانى من الرسالة :

خصص هذا الجزء لمعرفه العلاقة بين الاسكان الجماعى للعجول والتغير الجينى لبعض الجينات المصاحبه للضغط الاجتماعى.

تم قياس معدل التغير الجينى لكلا منالجينات (TLR-4, TAC1, IL-1βRa, IL-1β) فى كرات الدم البيضاء باستخدام اختبار تفاعل البلمره المتسلسل الوقتى(RT-PCR) ، حيث تم عزل الماده الجينيه من كرات الدم البيضاء المتواجده فى اطراف الجسم (خلال يوم الاسكان، الشهر الاول بعد الاسكان، وفى الشهر الاخير من التجربه).تم تقيم الحالة الصحيه لجميع حيوانات التجربة شهريا لمده خمس شهور.

كانت اهم النتائج على النحو التالى:

١- كان لحجم المجموعة تأثير عال المعنوية على سلوكيات العجول حيث اظهرت العجول الموجودة في مجاميع كبيرة (٤ أو ٨ عجول / حظيرة) تفاعل اجتماعى وحركة ووقوف أكثر، واقل معدل لتطمير الجسم الذاتى وتعامل مع الاشياء المحيطه، واقل رقود مقارنه بالعجول المرباه فى مجاميع صغيره (٢ عجل / حظيره).

٢- لم يكن لحجم المجموعة اى تاثير معنوي على سلوك اللعب والسلوك العدواني عند تناول الغذاء.كما لم يؤثر حجم المجموعة على الأنماط السلوكية المختلفة اثناءالتغذية باستثناء مدة التفاعل الاجتماعى والتى اظهرت اعلى معدل لها فى المعامله الثانية (٢ عجل / حظيرة).

٣- لم تتاثر الكفاة الانتاجيه بتغير حجم مجموعة الاسكان الجماعى. وكان معدل التغير فى طول الحيوان ومحيط الصدر متماثل فى المجاميع المختلفة خلال فترة التجربة. ومع تقدم الحيوانات فى العمر حدثتزيادة معنوية فى كلا من طول الحيوان ومحيط الصدر.

٤- عانت العجول المرباه فى المجموعات المكونة من (٤ او ٨ عجول/حظيرة) من الاعراض التنفسية بمعدل أكبر من العجول المرباة في مجموعات صغيرة (٢عجل/ حظيرة)، كما اظهرت العجول المرباه في مجاميع(٨ عجول/حظيره)اعراض تنفسيه أكثر وأقل افرازات من الأنف خلال الشهر الثانى والاخير على الترتيب وذلك مقارنة بالعجول المرباة في مجاميع (٢ او ٤ عجل/حظيرة).

٥- لم يكن لحجم المجموعه تأثيراً معنوياً على معدل الاسهال ومعدل الافرازات من العين خلال فترة التجربة.

٦- لم يكن لحجم المجموعه اى تاثير معنوى على محتوى بلازما الدم من الكورتيزول ومحتوى الدم من الهيموجلوبين.

٧- كان لحجم المجموعه تأثير عالي المعنوية علي نسبه خلايا الدم البيضاء حيث وجد ان اسكان العجول فى مجموعات كبيرة (٨ عجول / حظيرة) ادى الى ارتفاع نسبة خلايا النتروفيل، وانخفاض نسبة الخلايا الليمفاوية.

٨- ارتفع معدل التعبير الجينى لكلا من الانترليوكين١ والاتاك١ فى العجول التى تم تربيتها فىالمجموعات المكونه من٨ عجول بالمقارنه بالعجول الموجودة في مجموعات مكونه من ٤ و٢ عجل/حظيرة وذلك بعد الشهر الاول من الاسكان الجماعى.

٩- هناك أدلة مناعية عن وجود الإجهاد او الضغط النفسى في المجموعات المكونه من ٨ عجول / حظيرةبالمقارنة بتلك المرباة في مجموعات مكونة من ٤ أو٢عجل/ حظيرة على الترتيب.

١٠- تشير البيانات السابقة أن ايواء العجول البتلو في مجموعات كبيره تتكون من ٨ عجول خلال الفترة الأخيرة من التسمين قد تؤدي إلى زيادة معدل انتشار أمراض الجهاز التنفسي.

١١- عندما تعطى العجول البتلو المرباه فى مجاميع نفس المساحة الارضية، لم يكن هناك تاثيرا على معدلات النمو ومؤشرات الرفاهيه. بينما يكون لها تأثير عابر على صحة العجول.

وقد خلص البحث الى ما يلى:

١- أظهرت العجول البتلو المرباه في مجموعات كبيرة (٤ أو ٨ عجول / حظيرة) تفاعل اجتماعى وحركة ووقوف اكبر،وأقل لعق للاشياء المحيطة واللعق الذاتى،بالمقارنة بالعجول المرباه في مجموعات صغيرة (٢ عجل/ حظيرة)،على الرغم من توفير نفس المساحه الارضيه لكل عجل .

٢- ادى اسكان العجول البتلوفي مجموعات كبيرة الى تغير فى التعبير عن السلوك الفمى والذى ظهر بوضوح فى المجاميع الكبيرة (٨ عجول / حظيرة) حيث ان معظم سلوكيات الفم تتجه الى العجول المرافقة فى نفس الحظيره.

٣- ادى اسكان العجول البتلو فى مجموعات كبيرة (٨ عجول / حظيرة) الى زيادة غير معنوية فى الوزن خلال فترة التسمين.

٤- لم تتغيرنسبه الكورتيزول فى بلازما الدم وكذا الهيموجلوبين في الدم بتغير حجم المجموعة فى الاسكان الجماعى.

٥- اظهرت العجول البتلو المرباة في المجموعات المكونة من ٨ عجول/ حظيرة نسبة أكبرمن خلايا النتروفيل، نسبة أقل من الخلاياالليمفاوية وزيادة النسبه بين خلايا النتروفيل والخلاياالليمفاويه.

٦- ادى زيادة عددالحيوانات من ٢الى٨ عجول لكل مجموعة الى زيادة معدل الاعراض التنفسية خلال الأشهر الأولى بعدالايواء.

٧- كان معدل التعبير عن جينات السيتوكينIL-1β و TAC1 في كرات الدم البيضاءلعجول البتلو المرباة في المجموعات المكونه من ٨ عجول/حظيرة اكبر بالمقارنة مع العجول المرباة في مجموعات مكونة من ٤ أو ٢ عجل/مجموعة على الترتيب.

٨- ونظرا لندرة الدراسات السابقة التى اجريت على دراسة تاثير نظام الايواء على مؤشرات الرفاهيه التقليديه والغير تقليديه فى عجول البتلو خلال فتره التسمين. لذا تعتبر هذه النتائج مدخلا جديدا لقياس الضغط النفسى فى حيوانات المزرعة باستخدام وسائل دقيقة غير تقليديه من اجل تقييم مستوى الرفاهية التى لها عائد ايجابى على انتاجية الحيوانات.

٩- توصى هذه الدراسة اصحاب مزارع العجول البتلو بالانتقال من نظام الايواء الفردى الى نظام الايواء الجماعى بدون تحفظ على انتاجية العجول لما له من تاثير ايجابى على سلوكيات ورفاهية العجول مع مراعاة حجم المجموعة.

١٠- يوصى باتباع نظام الاسكان الجماعى بمعدل (٤او٢ عجل/حظيره) وذلك عند تربية العجول البتلو للحصول على اعلى معدلات رفاهية تفاعل اجتماعى واقل معدل للاصابة بالامراض والاجهاد.

جـامـعـة بنـــها

كلية الطب البيطري بمشتهر

قسم الصحة وسلوكيات ورعاية الحيوان

سلوكيات وكفاءة العجول البتلو وعلاقتها بالاسكان الجماعى

رسـالة مقـدمـــة إلـــى

كلية الطب البيطري بمشتهر

جـامـعـــة بنـــــها

مـــــن

ط.بـ / عصام محمود ميزار عبد الفتاح

بكالوريوس العلوم الطبية البيطرية مشتهر جامعة بنها (٢٠٠٥)

ماجستير العلوم الطبية البيطرية – كلية الطب البيطري مشتهر جامعة بنها (٢٠٠٨)

للحصول علي درجة دكتوراه الفلسفة

في العلوم الطبية البيطرية

(سلوكيات الحيوان والدواجن ورعايتها)

تحت إشراف

ا.د / محمد مرسي رمضان قاروصه

أستاذ سلوكيات الحيوان والدواجن ورعايتها – ووكيل الكلية لشئون خدمة المجتمع وتنمية البيئة

كلية الطب البيطري بمشتهر

جـامـعة بنـــها

ا.د / جعفر محمود الجندي

أستاذ رعاية الدواجن بقسم الإنتاج الحيواني – كلية الزراعة بمشتهر – جامعة بنها

ا.د / سوازن أيكير

استاذ فسيولوجيا الحيوان في وحدة أبحاث سلوكيات الحيوانات بوزارة الزراعة الأمريكية – جامعة بردو- الولايات المتحدة الامريكية

د./ عصام على أحمد

أستاذ مساعد سلوكيات الحيوان والدواجن ورعايتها

كلية الطب البيطري بمشتهر

جـامـعة بنـــها

٢٠١٤